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FAUNISTIC AND BIOLOGICAL NOTES ON MARINE INVERTEBRATES III.

The Reproduction and Larval Development of some Polychaetes from the Isefjord, with some Faunistic Notes.

BY

ERIK RASMUSSEN

(Report from the Isefjord Laboratory No. 3)

København 1956
i kommission hos Ejnar Munksgaard

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CONTENTS

Synopsis.

The reproduction and larval development of ten polychaete annelids from the Isefjord (Zealand, Denmark) are described.

The species are: *Harmothoë imbricata* (L.), *Harmothoë impar* Johnston, *Eteone longa* (Fabr.), *Magalia perarmata* Marion and Bobretzky, *Microphthalmus sczelkowi* Mecznikow, *Nereis pelagica* L., *Scolecolepis fuliginosa* (Claparède), *Heteromastus filiformis* (Claparède)?, *Capitella capitata* (Fabr.), and *Metatrochophora* I of an unidentifiable species.

The material used was collected during a number of years. The collecting methods employed were simple, consisting of both dredgings and plankton samples; quantitative vertical hauls were only taken in 1950 and 1951. Rearing in the laboratory of the larval stages in question was only occasionally carried out.

The hydrography of the area is mentioned and the importance of the temperature to spawning is shown.

The polychaete fauna of the Isefjord is briefly discussed, and the possible relation of the southern faunal element to climatic changes is touched on.

Printed in Denmark.
Bianco Lunos Bogtrykkeri A-S.

Introduction.

Like the preceding two reports—from 1944 and 1951—the present paper, dealing with the breeding habits of some polychaete annelids, is to be considered a continuation of the series of marine biological investigations started by me in the Isefjord from a laboratory on the small creek of Vellerup Vig (Zealand, Denmark).

The knowledge of the reproduction of the bottom animals with the settling of their larvae and the growth of the young individuals is of fundamental importance to the study of the production of bottom invertebrates and thus to the ecology of the sea area in question.

The results now published were obtained from the early beginning of the research work and were gathered throughout the years often together with other work. However, during the last few years the work has been intensified, especially after the acquisition in 1950 of the present house, which now yields good conditions for marine biological investigations. To a rather great extent this is due to the "Statens almindelige Videnskabsfond", which in 1953 has contributed to the installation of electricity with a grant. In this connection it may seem natural to point out that the laboratory is a private institution, established and run by the author since 1942.

My thanks are due to Prof. Math. Thomsen, Ph. D., and Lektor Gunnar Thorson, Ph. D., for their valuable advice during the preparation of the manuscript.

All the figures of animals in the paper have been drawn from living specimens by the author with the camera lucida of Abbe.

The investigations were carried out with support from the "Japetus Steenstrups Legat".

Methods and Technique.

As already stated in the introduction, the material used in this paper was collected during a number of years. The methods employed have always been very simple, especially during the first years, and the direct observation in nature has played a dominant rôle as far as conditions would allow. However, it has always been tried to follow the whole life cycle of the single species, from egg to the full-finished bottom stage. So not only numerous plankton samples have been taken, but the results obtained by this technique have as far as possible been supplemented with collections of adult individuals by dredgings, digging up followed by sifting, and by the use of other collecting apparatus.

Quantitative vertical plankton samples were collected for the first time in 1950 and I am indebted to Cand. mag. Preben Metz for submitting to me the results of his studies of these samples, which have supplemented my own observations in a very valuable manner. The samples were collected regularly, every fortnight from November 1950 to June 1951 in Vellerup Vig. They were taken with a conical net with a diameter at the mouth of 30 cm, about 60 cm deep and ending in a small plankton pail. The gauze of the net was No. 12, i. e. 12 meshes per mm. Each sample included two hauls from the bottom to the surface with an average depth of about eight metres. After the transferring of the samples to glass vessels in the laboratory the total contents in both hauls of bottom invertebrate larvae were counted and as far as possibly roughly determined as to species or genus. The purpose of this work was to obtain material for a later calculation of the production of the most important bottom invertebrate groups similar to the results published in 1946 by Thorson from the Sound (Øresund). Unfortunately the investigations had to stop in July 1951, and this important work is still unfinished, but forms a prominent part of the future programme of the laboratory.

Up to the renewal of the laboratory building in 1950 most of the study on the larval development of the single species could only be carried through by continuous procuring of fresh material since the aquarium conditions were too poor and incomplete.

However, during the last year it was possible to rear some of the larval forms here described, especially *Nereis pelagica*, cf. p. 58.

Hydrography.

As to hydrographical conditions the Isefjord is exceptional in several respects.

The salinity of the water has been discussed by me in a previous paper (Rasmussen, 1951). The average annual salinity seems to be rather constant about 20 $^{0}/_{00}$, and in the innermost areas it is a little higher than at the opening of the fjord into the open sea.

To a very high degree these conditions are due to the small supply of freshwater to the Isefjord from rivers in connexion with a rather high evaporation from the surface of the area. For further information see E. Steemann Nielsen (1951).

From an ecological point of view the rather stable salinity can only be of very slight or no importance to the breeding habits of the polychaete species described here.

Conditions of the temperature are quite different. As pointed out in several cases in the following descriptions of the single species, the temperature is the direct stimulus to the start of the spawning by the mature individuals. In order to clarify this correlation, I have tried to compare the measurements of the annual temperature with the spawning periods of practically all the polychaete species dealt with in this paper; cf. fig. 1 (p. 6). I am indebted to Mrs. E. Hagelund, who in 1949 to 1950 rendered valuable assistance by taking daily measurements of the temperature of the surface water in Vellerup Vig. In fig. 1 the temperature (degrees centigrade) is calculated on the basis of one diurnal measurement, but in the graph, the average of only every third of each month is marked. Even if the temperature graph published represents one year's measurements only, while the spawning periods are based on several years' observations, I have nevertheless ventured to compare these results, since in broad outlines this one-year temperature graph represents an average picture of the temperature conditions of the area, apart from the very severe winters, which of course will have a dominating and often very fatal influence on the fauna of lower bottom animals.

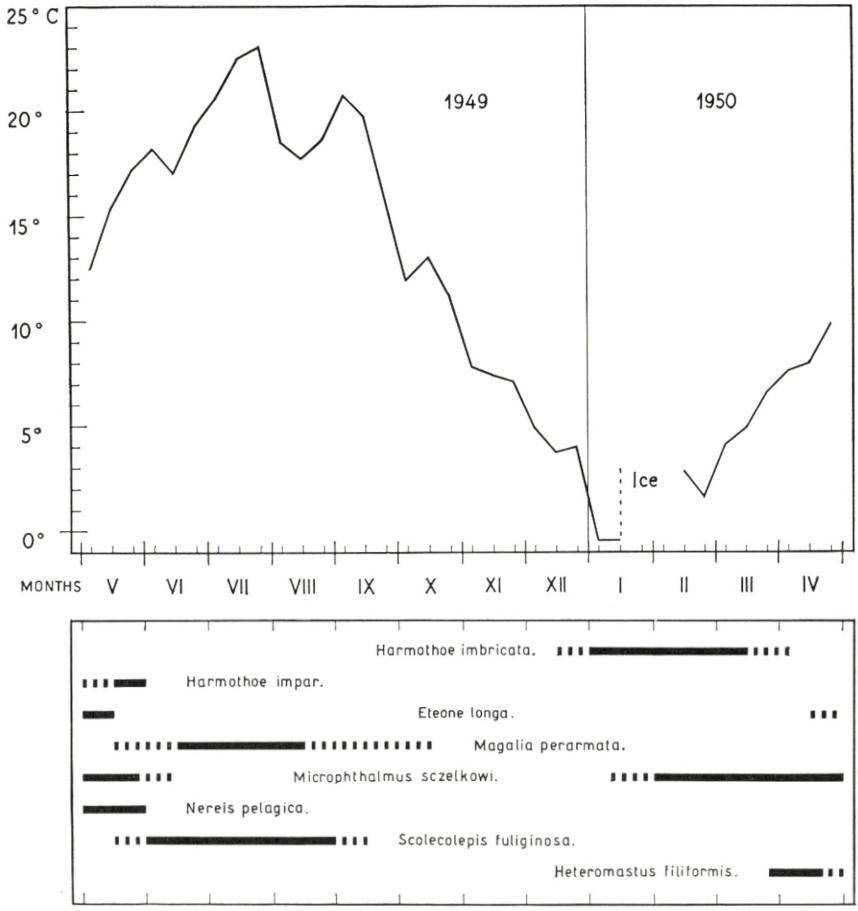


Fig. 1. Spawning periods in the Isefjord of most of the polychaete species dealt with in this paper (continuous lines = main spawning, dotted lines = beginning or decreasing spawning activity) in relation to the water temperature.
The recording of the spawning periods is based on results gained during several years, whereas the temperature curve, calculated from daily samples, represents one year's measurements only, from 1949 to 1950, cf. the text on p. 5.
As to the spawning activity the species can be divided into three groups; those with their main spawning in winter, comprising *H. imbricata* and *Microphthalmus sczelkowi*. Another group comprising the species which breed in the spring, *H. impar*, *Eteone longa*, *Nereis pelagica*, and *Heteromastus filiformis*; as appears from the figure *M. sczelkowi* may also be included in this group as its spawning period extends to June. The third group comprises the summer-spawning species, *Magalia perarmata* and *Scolecolepis fuliginosa*, representing the heat-loving faunal element of the Isefjord polychaete fauna. Both are true southern (Mediterranean) species hitherto unrecorded in the Danish fauna. Cf. the text on p. 48.

In this connection it is well worth remembering the character of the Isefjord area, which is small, not only in extension but also in the depths, which again means that the temperature of the water masses is highly influenced by the air temperature.

The species of the fam. *Aphroditidae* found in Vellerup Vig.

Since the beginning in 1942 of my research work in the Isefjord, regular collecting of plankton samples has played a prominent part in the work of the laboratory. Among the quantitatively important groups, the pelagic larvae of scale worm species have always played a dominating rôle in that part of the zooplankton which consists of bottom invertebrate larvae.

However, not until in the last few years the species of the family have been studied in so much detail by me that the breeding habits of two of the most common species have now been cleared up. The species are the two closely related ones *Harmothoë imbricata* (L.) and *Harmothoë impar* Johnston, both of which are very common in Vellerup Vig.

In all, four scale worm species have so far been reported from the innermost part of the Isefjord; the other two are *Lepidonotus squamatus* (L.) and *Pholoë minuta* Fabricius. All species are extremely common, especially *Pholoë minuta*, which is found, often in great numbers, from the shallow sandy areas along the shore to the deepest parts where the bottom consists of mud (maximum depth about 10 metres). Also hard stony bottom is inhabited by this small delicate sigalionid worm, which, like *L. squamatus*, does not enter into the present investigations.

The three polynoid species of the area are more restricted in their occurrence. They are true epifauna-animals, which live among the huge masses of *Mytilus edulis* often found in the littoral and sublittoral zone of Vellerup Vig. The mild winter climate during the last few years, practically without any ice layer in the low water, has increased the *Mytilus* banks so that even pure sand flats are now covered by a thick layer of mussels. Again, this means that the polynoid species have had optimal conditions of life and have offered the investigator an excellent material for study.

1. *Harmothoë imbricata* (L.).

A survey of the literature reveals the rather surprising fact that the reproduction and larval development of the common scale worm *Harmothoë imbricata* (L.), which is one of the commonest polychaetes in the northern hemisphere (cf. E. Wesenberg-Lund, 1953) are by no means fully known.

In his small and remarkable paper about the development of annelid worms Sars (1845) as the first author describes and figures the spawning and early larval stages of the species. According to this description *H. imbricata* off the Norwegian coast is spawning in February to March. The opaque eggs—50 μ across—are rosy in colour and, when just spawned, they are found under the elytra of the female. There the early development takes place and about a fortnight after the spawning the young larvae will leave the mother animal as very simple, almost spherical trochophores, which now have a light greyish-green colour.

McIntosh (1900) states the egg size to be from 56 to 78 μ and mentions the first week of March as the period of the swarming of the young trochophores, which were observed to be positively phototactic. His observations as well only deal with the early pelagic stages.

From the bay of Kiel Leschke (1903) mentions the larvae of two polynoid species. One of these taken from January to February in the Trochophora stage might be the larva of *H. imbricata*. Unfortunately no descriptions nor figures accompany his statement. The other, referred by Leschke to *Lepidonotus squamatus*, is probably a *Harmothoë* larva. Its occurrence in the Kiel plankton from June to October makes it unlikely that it could be *H. imbricata*, as claimed by Thorson (1946), nor can it be *H. impar* (see below). The number of chaetigerous segments, eight, of Leschke's old Nectochaeta (Pl. VII, fig. 2) and the warty condition of tentacles, tentacular cirri, and the dorsal cirri of the segments also exclude *H. imbricata* as a possibility.

Izuka (1912) and Sæmundsson (1918) confirm the observation by Sars concerning the presence of brood protection, and the latter found the eggs attached to the back of the female in April-May off Iceland.

The extensive survey by NOLTE (1936) discusses the identity of a large number of *Harmothoë* larvae described by earlier authors; efforts which, however, have not been crowned with success, since the confusion about these incomplete old descriptions is even worse after than before NOLTE's activities.

Larvae of a *Harmothoë* species which presumably belong to *H. imbricata*, are briefly described and pictured by E. SMIDT (1944, p. 253), who as to the determinations of his stages from the harbour of Copenhagen only writes that they are "based on observations at Ven." The occurrence of his larvae in the plankton is limited to "the cold part of the year (Nov., Jan., Feb., March)."

The most detailed account of the larvae of *H. imbricata* is found in THORSON's paper (1946). His descriptions, however, are all based on material from plankton samples; results which have not been verified by observations on the breeding habits of the adults. Thus the larvae from the Sound cannot with complete certainty be determined as *H. imbricata*, even if it seems highly probably that they do belong to this species (cf. THORSON, p. 48). His larval descriptions, exclusively based on observations from the Sound, deal with the metatrochophores I—II and the Nectochaeta. The young bottom stage (THORSON, fig. 18, A, p. 47), the smallest one found, seems to be somewhat defective, since the scales and probably also the tentacles and the tentacular cirri are missing. That the appendages of this stage should be undeveloped, as supposed by the author, seems to me to be unlikely owing to the broken appearance of for instance the basal parts of the lateral tentacles. Furthermore, the hindmost segment with the pygidium is lacking. It is hardly to be believed that THORSON's bottom stage represents the newly settled and metamorphosed bottom animal, since its body length, 1.2 mm, is double the length of the largest free-swimming Nectochaeta (fig. 17, C—D) about 600 μ. According to THORSON (*l. c.* p. 47) "the larvae have their maximum occurrence in spring, summer and autumn, while they are rather rare in the winter, but they may occur in any month throughout the year." This is a very interesting fact, because it is in clear contrast with the literature, which with a few exceptions says that *H. imbricata* propagates in the cold parts of the year. Also the present results show a well-defined winter breeding of the same species in the Isefjord (cf. diagram fig. 2). The

explanation of this seasonal difference is maybe to be sought in a different biology of *H. imbricata* in relation to the exceptional hydrographical conditions of the Sound, which has a distinct stratification both as to temperature and salinity. Another possibility might be that the material of *Harmothoë* larvae from the Sound comprises not one species, *H. imbricata*, but several, closely related species, only distinguishable with great difficulty, and having their spawning maxima spread over the year so that this obscure picture will appear.

The more detailed discussion of the single stages from the Sound will be given in the later description.

M. Pettibone (1953) has found egg-carrying females in Puget Sound in June, July, and August, but says that "it does not necessarily exclude the other months as very little collecting was done other than in the summer months." In mature females the eggs were found in the body segments 8—30. The egg-size is stated to be 136 μ; Furthermore, a short description with figures of the early larval development is given.

In 1954 Davenport, in his paper about the early stages of the commensal polynoid *Acholoë astericola* (Delle Chiaje), gives a brief summary of what is known in the literature about the development of polynoids. Newell (1954, p. 333) has observed *H. imbricata* carrying the young beneath the elytra in January.

In the Isefjord *Harmothoë imbricata* seems to be a widespread and in the right type of locality (mussel-banks, cf. above) very common polychaete worm. However, owing to the difficulty in the determination of the species in connection with great variation as to pigmentation and shape of the scales I am only able to give reliable information about its occurrence in the neighbourhood of the laboratory, where adult individuals have been collected in great numbers and determined with certainty to *H. imbricata*.

Very often the species is found in specimens of considerable size, up to more than four cm, and most of the individuals used for the following description were large animals with an average length of about three cm.

No distinct difference in the size or in other external morphological characters was found between the males and females.

Spawning season. The first considerable number of spawning

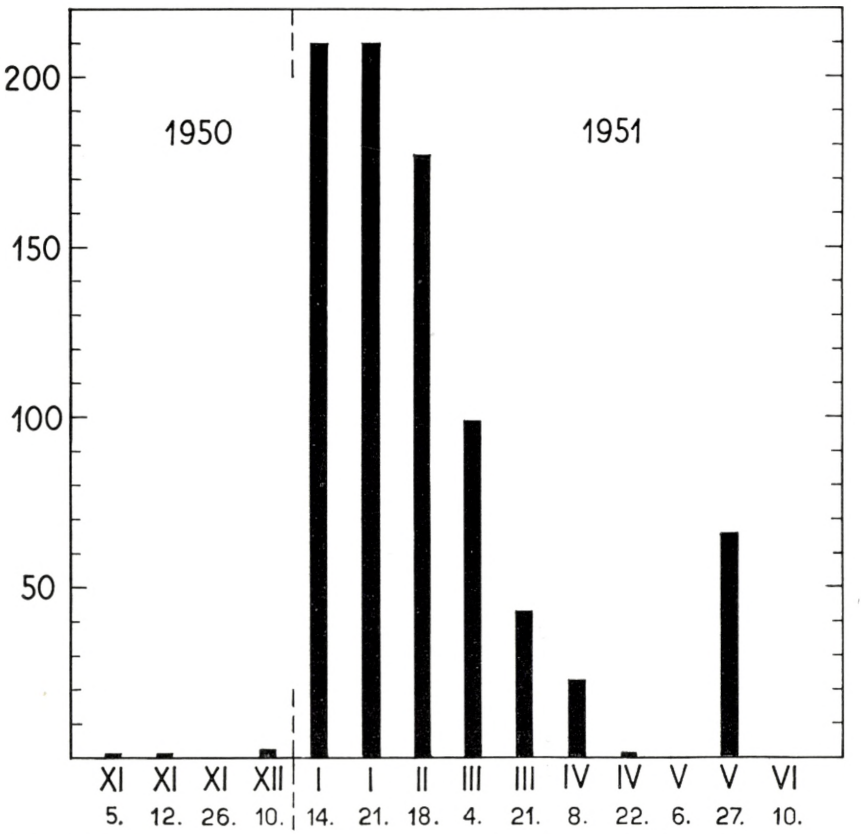


Fig. 2. The occurrence of polynoid larvae in the plankton in the Isefjord, Vellerup Vig, from November 1950 to June 1951.
The results are based on quantitative plankton samples from this period, each column showing the total number of larvae from two vertical hauls from the bottom to the surface, at a depth of about eight metres. The abscissa gives the dates of collection. The large winter maximum is exclusively made up by the larvae of *Harmothoë imbricata* (L.), while the column in May represents the larval maximum of *Harmothoë impar* Johnston. Cf. the text p. 13 and 27.

females were recorded in the second half of December, an observation which I have made in 1950, 1953, and 1954. The main spawning happens to come very soon after the first appearance of females with eggs beneath their scales, and in January, when the water temperature in Vellerup Vig has reached the lowest level of the year, *the spawning culminates* (cf. fig. 2). In February the intensity decreases and early in April the last few pelagic larvae are found in the plankton; cf. fig. 1, which shows the

spawning period in relation to the temperature. On January the 5th (1954) about 50 % of the females collected had eggs or young trochophores under their scales, while the rest contained large eggs — 130 to 150 μ — ready for spawning. Only a very few were spent. On April the 5th (1953) about 50 % of both males and females were quite empty and only a few per cent. contained ripe sexual products.

All eggs just spawned measured 150 μ across and were whitish in colour. According to M. Sars (1845), the new-laid egg is rosy, and Sæmundsson has found the egg to be pink. A distinct egg membrane can be seen round the spherical yolk. This comparatively large egg-diameter is rather noteworthy as compared with the diameters given for the same species by Sars (50 μ) and McIntosh (1900) (from 56 to 76 μ). If the determination to species by these two authors is correct, this means that *H. imbricata* has *a most varying egg size*. Unfortunately no descriptions of the larval development accompany the egg-size statements of Sars and McIntosh so that the possible deviations which may be present, cannot be compared with the present results.

When lying under the elytra of the female the eggs are placed in lumps on the dorsal part of each parapodium. Generally these lumps will fuse so that one coherent mass of white eggs can be found. The eggs are protected and hold together by a clear and sticky, very elastic mucus, especially developed as two broad longitudinal walls on the sides of the egg mass. M. Sars has also observed this mucus (1845, see p. 13). As a rule any segment from the 8th up to the 34th chaetigerous segment is carrying eggs.

A female, 3.3 cm in length, with about 150 eggs on each of the egg-carrying segments had in all about 3750 eggs. Another female, only 3.0 cm long, was estimated to have a total of about 5000 eggs beneath its scales.

When the females carry new-laid eggs and especially when having young trochophores on their backs, they have a tendency to leave the shelter between the mussels and seek the opener places with turbulent water. This phenomenon was observed several times by me in 1953—1954 during the main spawning period.

The larval development. As already noticed by several authors, Sars, McIntosh and others, *H. imbricata* has a sort of

brood protection, which ends when the quite young Trochophora is ready to hatch. The same condition was found in the Isefjord material, but the duration of the protected larval period was not recorded. According to SARS two weeks elapse from the hatching of the eggs to the beginning of the free-swimming stage.

The young Trochophora has—just as the egg—a whitish hue. There are two distinct dark-brown eye-spots with lenses. Round the almost spherical larval body there is a girdle of cilia together with an apical tuft.

The pelagic stages. During the period, from November 1950 to June 1951, when regular quantitative samples were taken in Vellerup Vig (compare p. 4), no distinction in species was made between the scale worm larvae, and in our lists these were only indicated as "polynoid larvae". When taking up the closer examination in 1953 of the breeding biology of the two *Harmothoë* species here described, I took test samples in the same months as in 1950—51 in order to determine the number of species of the two maxima found (fig. 2). As expected, and in good accordance with the results published above, the large maximum of larvae, beginning in January, decreasing during the early spring, and ceasing in April, exclusively belonged to one species only, which through transitional stages to the young, newly settled bottom individuals could rather easily be determined as belonging to *H. imbricata*. The short and rather small maximum in May, however, was only composed of larvae which without doubt belonged to *Harmothoë impar*. The columns (fig. 2, see text below) show the total number of larvae comprising all stages of development in two vertical hauls from bottom to surface; the average height of the water column was eight metres.

It appears from the figure that the number of *H. imbricata* larvae of one single haul in Vellerup Vig is remarkably high. Thus in January 1951, when the largest number was found, more than 210 specimens of all stages were collected. Compared with the records from the Sound (cf. THORSON, p. 47), off Elsinore, the largest number being 45 in one main series, i. e. 10 vertical hauls 18 m high = the number of larvae in a water column over $^1/_2$ sq.m of bottom (THORSON, p. 369), the density is much larger. If the number from the Isefjord is converted to the Sound scale, *thus maximally almost* 1000 *H. imbricata larvae*

against 45 *from the Sound are present in a water column above* $^1/_2$ *sq. m* of bottom; that is on an average about 250 individuals per one cubic metre water or one larva in about four litres of water!

From January to March (1951) the larvae of *H. imbricata* play a dominating part in the plankton of Vellerup Vig. Thus in January they constitued *about* 80 $^0/_0$ *of the total number of bottom animal larvae.*

The older Trochophora (fig. 3) has a size varying between 250—400 μ. The individual pictured was about 370 μ long and is a transitional stage between the Trochophora and the Metatrochophora, since a beginning segmentation is visible. Its shape is like a double cone as usually present in the annelid trochophores.

The segmentation of the larval body has just begun, and seven segments in all can be distinguished. Almost every segment has both the notopodial (no.) and the neuropodial section (neu.), the latter being the most advanced by far. A small refractive dot in each setigerous sac represents the first developing larval seta (se.). On the ventral side of the body, in the furrows between the fine superficial segmentation a rather clear, bluish green pigmentation is found (b. g. pi.), being most evident posteriorly. Pigmentation is also present round the mouth; thus the upper lip has a conspicuous reddish brown colour (r. b. pi.). Apart from these two groups of pigment cells no other pigment is present. The whole larva has a clear, slightly yellowish colour. On both sides of the prototroch girdle, this yellowish colour is more concentrated, almost lemon-coloured (c. l.), and apparently this thickened cell-layer contains a circular girdle of muscular tissue. At any rate this region of the larval body is very contractile and often during swimming, the Trochophora will be seen changing its shape to a more elongate one.

The ciliation on the body surface mainly concentrates on a prototroch (pr.) round the larva, but a small anterior row of cilia, the akrotroch (ak.) is found (see a. o. GRAVELY, 1909, p. 11, pl. I and NOLTE, 1936). There is not yet any apical tuft nor telotroch, the latter being totally absent during the whole larval development. The prototroch consists of two rows of long cilia, the anterior of which is forming one unbroken ring passing above the mouth (m.). The posterior row stops just before the

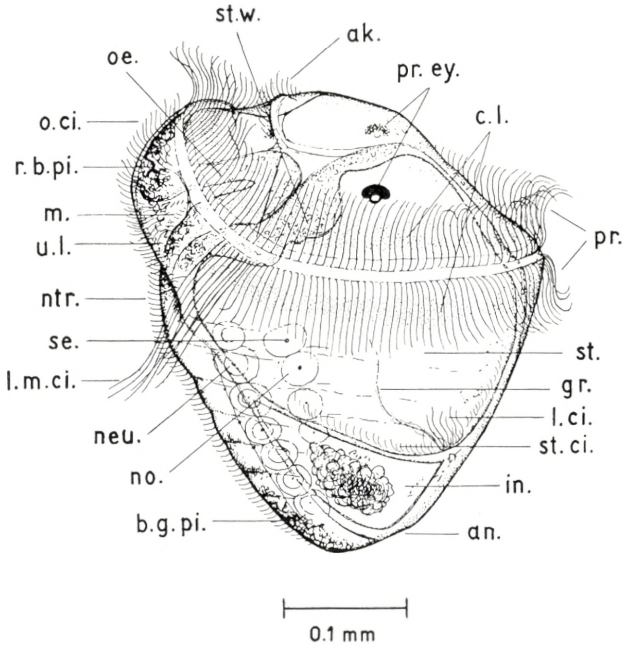
upper lip, ending on each side in one group of exceptionally long cilia, which are motionless and always held straight (l. m. ci.). Round the mouth aperture there is a dense ciliation of fine short cilia (o. ci.). The narrow longitudinal neurotroch is well-developed (ntr.).

In front of the prototroch one pair of black (in transmitted light) eyes with distinct lenses is present. The eyes, being of an oblong shape, are the first developed of the three later pairs. These primary eyes (pr. ey.) are later totally reduced when the metamorphosis occurs and only two pairs are left.

The mouth opens below a very prominent upper-lip (u. l.), and leads to the oesophagus (oe.), a rather thick-walled duct, which at the stage pictured does not yet seem to be in open connection with the stomach cavity. Close behind the oesophagus there is a double thickening, which seems to represent part of the stomach wall (st. w.). However, this tissue may be identical with the region which GRAVELY on his polynoid Trochophora from Port Erin (see GRAVELY, 1909, p. 11) characterizes as a "rudiment of the supra-oesophageal ganglion". The same region is correspondingly developed in the polynoid Trochophora pictured by LESCHKE (1903, p. 129 as *Lepidonotus squamatus*, Pl. VI, fig. 14), but no importance is attached to this detail by the author.

As to volume the larval stomach (st.) is far the most dominating section of the alimentary system. It is clear and quite empty. The colour is faintly greenish against the yellowish body colour. The stomach ciliation consists of fine short cilia (st. ci.), apart from one small part of the hindmost wall, which has a bundle of long cilia (l. ci.). There is a fine groove (gr.) in the stomach wall. The intestine (in.) is very small and leads directly to the dorsally placed anus, which has no opening so far. A rather interesting thing is the contents of the intestine. Owing to the above-mentioned lacking passage through the alimentary system this indefinable mass cannot be food remnants, true excrements. I have examined a number of larvae of this species from the plankton which were at the same stage of development, and they all had the same dark-brown body in the intestine and always of about the same size and appearance.

The Trochophora of *Harmothoë imbricata* is not described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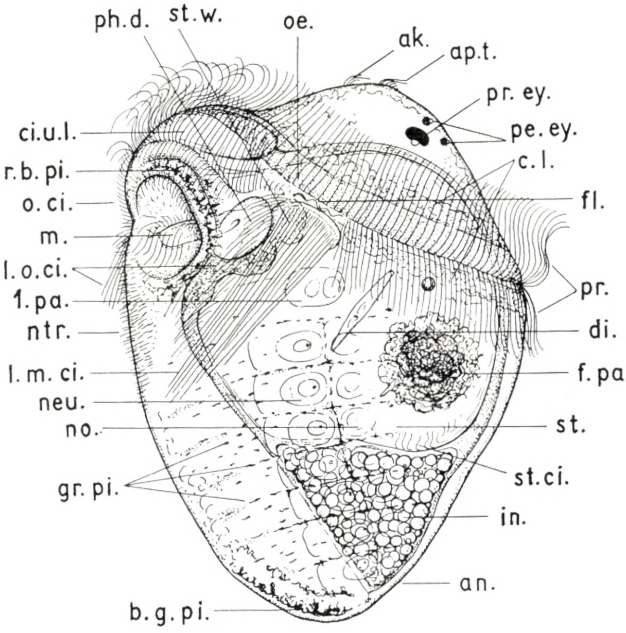
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ak.
ap.t.
pr. ey.
ci.u.l.
pe. ey.
r.b. pi.
c. l.
o. ci.
fl.
m.
l. o.ci.
pr.
1. pa.
ntr.
di.
l. m. ci.
f. pa.
neu.
no.
st.
st.ci.
gr. pi.
in.
an.
b. g. pi.

Fig. 4.

by THORSON in his larval descriptions from the Sound; his youngest stage being the early Metatrochophora.

Metatrochophora I (fig. 4). The full length of this stage is varying between 430 and 450 μ, or about the same as THORSON (1946) states for the same stage from the Sound.

The shape is now more elongate and roundish, as the region posterior to the prototroch is stretched and the segmentation more advanced. Eight setigerous segments in all can now be distinguished. The first one (1. pa.) is more distinct from the others and represents the first modified setiger of the adult individual wearing among others the tentacular cirri. The noto- and neuropodial parts (no. and neu.), the latter of which is still the best developed, are more clearly separated. The budding parapodia and setigerous sacs are lying closely beneath the surface and their outlines are marked by small green pigment granules (gr. pi.).

Besides, chromatophores are found as a border of reddish-brown pigmentation round the mouth opening (r. b. pi.). On the ventral side of the hind part there is a bluish-green pigmentation (b. g. pi.) just as at the preceding larval stage. The lemon-

Fig. 3. *Harmothoë imbricata* (L.).
Older Trochophora with developing segmentation as seen from its left side and a little from above, Vellerup Vig, Isefjord, 17.1.54.
ak., akrotroch; an., developing anus; b. g. pi., bluish green pigment; c. l., lemon-coloured, thickened cell-layer on both sides of the prototroch; gr., stomach groove; in., intestine; l. ci., small bundle of longer cilia in the stomach wall; l. m. ci., bundle of long motionless cilia on each side of the mouth; m., mouth; neu., neuropodial section of developing parapodia; no., notopodial section of developing parapodia; ntr., ventral ciliary groove, neurotroch; oe., oesophagus; o. ci., oral ciliation; pr., prototroch; pr. ey., primary eyes with lenses; r. b. pi., reddish brown pigment; se., notopodial setae; st., stomach; st. ci., stomach ciliation; st. w., a bilobed thickening of the stomach wall?; u. l., upper-lip.

Fig. 4. *Harmothoë imbricata* (L.).
Metatrochophora I in left-side view, seen a little from the ventral side; the Isefjord, Vellerup Vig, 31.1.54.
an., anus placed dorsally; ak., akrotroch; ap. t., rudimentary apical tuft?; b. g. pi., bluish green pigmentation; ci. u. l., border of cilia on the upper lip; c. l., lemon-coloured, thickened cell-layer on both sides of the prototroch; di., a diatom in the stomach; fl., larval nephridium with flagella; f. pa., food particles?; gr. pi., small, green pigment granules marking the furrows in the segmentation; in., intestine; l. o. ci., fringe of long oral cilia on each side of the mouth; l. m. ci., bundle of long, motionless cilia; m., mouth; neu., neuropodial section of the parapodia; no., notopodial section of the parapodia; ntr., ventral ciliary groove, neurotroch, shortened and reduced in comparison with the Trochophora stage; o. ci., oral ciliation; oe., the oesophagus; 1. pa., first pair of parapodia; pe. ey., permanent eyes in development (lenses); ph. d., left pharyngeal diverticle; pr., prototroch; pr. ey., left primary eye; r. b. pi., reddish brown pigment round the mouth aperture; st., stomach; st. ci., stomach ciliation; st. w., thickened stomach wall.

coloured cell-layer on each side of the prototroch girdle (c. l.) is even more conspicuous than before and is more granular.

The ciliation is now at its highest and rather complicated. The prototroch is exactly as before: two rows of long vibrating cilia, the anterior of which passes before the mouth, forming one unbroken ring. The other ends on each side before the mouth in the long, motionless cilia (l. m. ci.). Furthermore a border of long cilia is developed on the upper lip just beneath the prototroch (ci. u. l.). The dense layer of fine short oral cilia is still found to be continued in the mouth and oesophageal ciliation. On both sides of the mouth before the long motionless cilia a fringe, a "whisker", of long vibratile cilia has been formed (l. o. ci.). The neurotroch on the ventral body surface is much reduced, being now only a patch or "bib" of fine cilia just beneath the mouth opening (ntr.). Besides the akrotroch (ak.) also a small and short-ciliated apical tuft (ap. t.) is formed.

The eyes are now present in their maximum number, three pairs. However, the two pairs most dorsally placed, are not full-sized even if distinct lenses are visible. These make the permanent eyes of the adult animal (pe. ey.), the larger pair being the primary eyes, which later during metamorphosis will disappear (pr. ey.).

On each side within the mouth opening (m.) there are two thick-walled pharyngeal diverticles (ph. d.), which are rudiments of the later well-developed muscular pharynx. The oesophagus (oe.) is now connected with the stomach cavity, which often may contain different sorts of food particles (st.). Thus the larva pictured has among other things the siliceous skeleton of a diatom in its stomach (di.). The intestine still makes a very small part of the alimentary system (in.), but contains a dense mass of rather large oil globules. The anus (an.) has free connection with the surrounding water.

The previously mentioned thickening of the stomach wall (st. w.) or possibly the supra-oesophageal ganglion is more distinctly bilobed and has a position close to the pharyngeal diverticles.

During the closer examination of the living larval stages under the microscope a very interesting detail was found on Metatrochophora I. Close to the base of the bundle of long, motionless

cilia (l. m. ci.) and immediately beneath the anterior row of cilia of the prototroch a fine bundle of undulating flagella was observed (fl. in fig. 4). In spite of a very thorough search for more details only these flagella lying in a fine membranous tube could be seen. The flagella were fixed ventrally and the undulating movements ran against the dorsal part of the body. Without doubt the tissue surrounding this organ is so hyaline in the living larva that only a fixation with a following staining of the region will show the true structure of this larval organ. However, it is a reasonable supposition to regard the flagella as a larval protonephridium. This idea is highly supported by observations on other annelid worms, e. g. the well-known results of Wolterreck (1902) on the *Polygordius* Trochophora, which has a protonephridium of exactly the same structure.

The Metatrochophora I of Thorson (1946, pp. 46—47, fig. 17 A) differs in several details from the description just given. Thus the ciliation is limited to the prototroch, which only has one row of uniform and rather short cilia. Also the pigmentation is different. The whole larva has "a purplish tint, which seems to be characteristic of eggs as well as of larvae of all stages of this species." Anteriorly to the prototroch above the mouth, spots of black pigment are present. The bluish-green pigment of the segments is totally absent.

The next stage (fig. 5) cannot be considered the true Nectochaeta, since the primary ciliation persists and together with the now well-defined parapodia with setae constitutes the swimming apparatus. Thus it is more like Metatrochophora II. The Nectochaeta, which I never found, seems to be of very short duration and the metamorphosis very soon follows.

The total length of Metatrochophora II is about 520 μ. The head part of the body before the prototroch is still Trochophora-like while the rest of the larva is developed as a young scale worm. There are seven chaetigerous segments with bundles of long setae, the ventral ones. The setae of the first setiger, which can be ascertained by the dorsal tentacular cirri (d. t. c.), are not yet observable, and the dorsal setae of the following segments are only very poorly developed (d. se.). Close ventrally to the dorsal tentacular cirri, which are always the first to appear, the palps (pl.) are developing. If the segment with the tentacular

cirri is considered the first chaetigerous segment, dorsal cirri (d. c.) are present on the following segments: nos. 3, 6, and 8, the latter being only small buds. The chaetigerous segments are not yet biramous, but the dorsal and ventral bundles of setae are separated. Two anal cirri (a. c.) end the body. All cirri are of the same type, a simple rounded-oblong appendage. There are four pairs of plain scales, elytra (el.), with almost smooth surfaces. They are attached to the chaetigerous segments nos. 2, 4, 5, and 7, or quite as in the adult individuals (cf. FAUVEL, 1923, p. 40).

At this larval stage the pigmentation is found to vary very much. The type pictured here has two groups of chromatophores. First, the bluish-green pigment on the posterior ventral side, which also is present as a ring round the pygidium (b. g. pi.); this group was seen in all larvae of this stage examined. Furthermore, distinct brownish pigment cells are visible in the intestinal wall (b. pi.). However, the variations can be seen round the head. Here there can be two almost parallel rings of brownish pigmentation of about the same appearance as sketched by THORSON (1946, p. 47, fig. 17 B—C). The larva pictured represents a type with quite colourless head.

This variation of the pigmentation is also a common detail of Metratrochophora II of *Harmothoë impar* (see text below, fig. 7 and 8).

The prototroch (pr.) is still developed, even if some reduction has taken place. Thus only one row—apparently the anterior one—of long vibrating cilia is present and nearly all the patches of cilia round the mouth have disappeared. This holds good also of the bundles of long, motionless cilia of the younger stages. On the head (p.) the apical ciliation, which at the previous stage was a tuft of cilia, is now formed as a dense cover of fine, short cilia (ap. ci.). The akrotroch (ak.) is still found.

The prostomium has all six eyes fully developed (pr. ey. and pe. ey.) and of equal size, being black in transmitted light. Of the head appendages, besides the palps, only the developing median tentacle can be seen (m. t.). The two lateral tentacles will not appear until later.

As to the alimentary system the pharynx cannot be seen in dorsal view. The larval stomach seems to be quite reduced and instead the intestine (in.) has gained a more adult appearance,

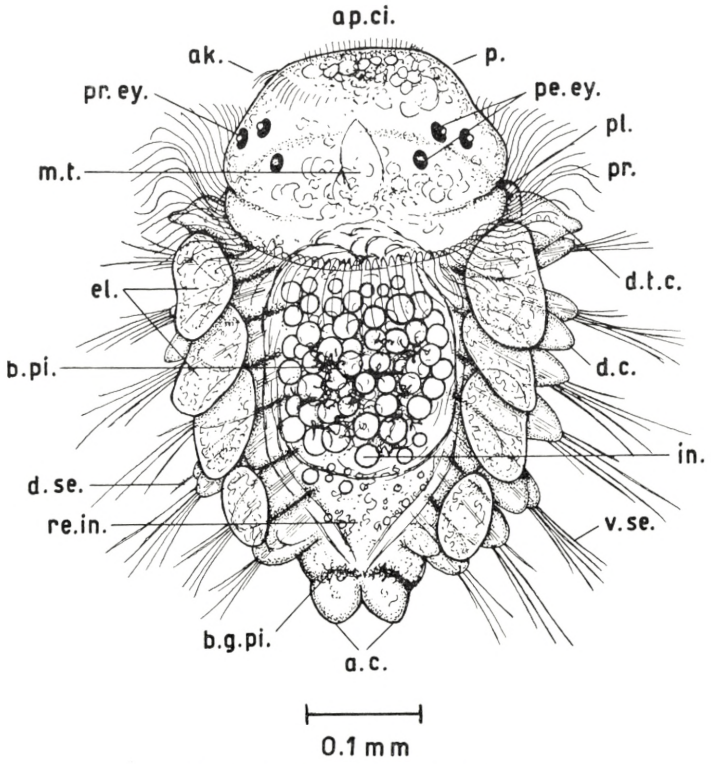

Fig. 5. *Harmothoë imbricata* (L.).
Metatrochophora II, in dorsal view, the Isefjord, Vellerup Vig, 4.4.54.
a. c., anal cirri; ak., rudimentary akrotroch?; ap. ci., apical cilia; b. g. pi., bluish-green pigment; b. pi., brownish pigment in the intestinal wall; d. c., dorsal cirrus of a cirrigerous segment; d. se., dorsal setae; d. t. c., right developing dorsal tentacular cirrus; el., elytra; in., intestine full of oil globules; m. t., developing median tentacle; p., prostomium; pe. ey., permanent eyes; pl., right developing palp; pr., prototroch; pr. ey., left primary eye; re. in., rectal part of the intestine; v. se., bundle of ventral setae.

being divided into a mid-part (in.) in which numerous oil globules are embedded in the wall, and a rectal part (re. in.).

THORSON (1946, p. 47, fig. 17 B) has a drawing and a short description of what he considers the transitional stage between Metatrochophora II, and the Nectochaeta of the same species. Considering that THORSON's transitional type is somewhat more advanced than the Isefjord larva (cf. fig. 5) its size is nearly the same, 480 μ as against 450 μ. Furthermore, the full numbers of chaetigerous segments and of the scales are the same. As to the pigmentation there is some difference, since the bluish-green

anal pigment and the intestinal colour is completely absent on the Sound larva. However, on account of the great variation in my material, this detail does not invalidate the probability of both belonging to *H. imbricata*. Also the true Nectochaeta stage from the Sound (fig. 17 C—D) seems to show great resemblance with my larvae.

The newly metamorphosed bottom stage (fig. 6). It seems remarkable that the large pelagic larvae of *Harmothoë imbricata* in the Isefjord are normally found to metamorphose in the water far above the bottom. Thus the samples from the beginning of April 1954 (i. e. near the end of the larval occurrence in the plankton, cf. fig. 2) contained almost exclusively large stages either in metamorphosis or having just metamorphosed.

The quite young bottom stages from the plankton were thoroughly compared with equal-sized individuals of the same species at the same stages, taken in bottom samples also from April 1954, and both were of exactly the same appearance and structure. So the metamorphosed specimens in the plankton represent the young adult bottom individuals.

It also seems beyond doubt that larger individuals (up to about 7—9 mm in length) live not only as true bottom animals, but for some time—before they are full-grown—*may live a semi-pelagic life* swimming briskly in the water at some distance above the bottom.

When all the larval attributes have disappeared and the young animal is ready for adult life, the full body size without

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Fig. 6. *Harmothoë imbricata* (L.).
Young bottom stage, only a few hours after metamorphosis; the Isefjord, Vellerup Vig, 4.4.54.
a. c., anal cirri; b. g. pi., girdle of bluish-green pigment round the pygidium; c. se., strong curved setae of the first setiger (actually the ventral setae of the reduced first parapodia); d. c., dorsal cirrus of a cirrigerous segment (setiger no. 3); d. c. 6, developing dorsal cirrus of the 6. setiger, a cirrigerous segment; d. el. ph., developing elytrophore of the 9. setiger; d. l. v., pulsating dorsal longitudinal vessel with an endothelial wall; d. se., dorsal setae of a parapodium (setiger no. 7); d. t. c. and v. t. c., first pair of cirri, dorsal and ventral tentacular cirrus; el., elytra; el. ph., elytrophore of the 2. setiger; in., intestine with a dense layer of oil globules in the wall; j., jaws; l. t., two lateral tentacles; m. c., muscle cylinder in the palps; m. t., median tentacle; pa., parapodium of the 2. setiger (the parapodia are not yet biramous as in the adult individual); pe. ey., permanent eyes with lenses; ph., muscular pharynx; pl., palps; pp., small blunt papillae with fine sensory hairs on the marginal surfaces of the elytra and on the tentacular cirri; re. in., rectal part of the intestine; v. c., small ventral cirrus of an elytrigerous segment (no. 4); v. se., bundle of ventral setae of a parapodium (setiger no. 7).

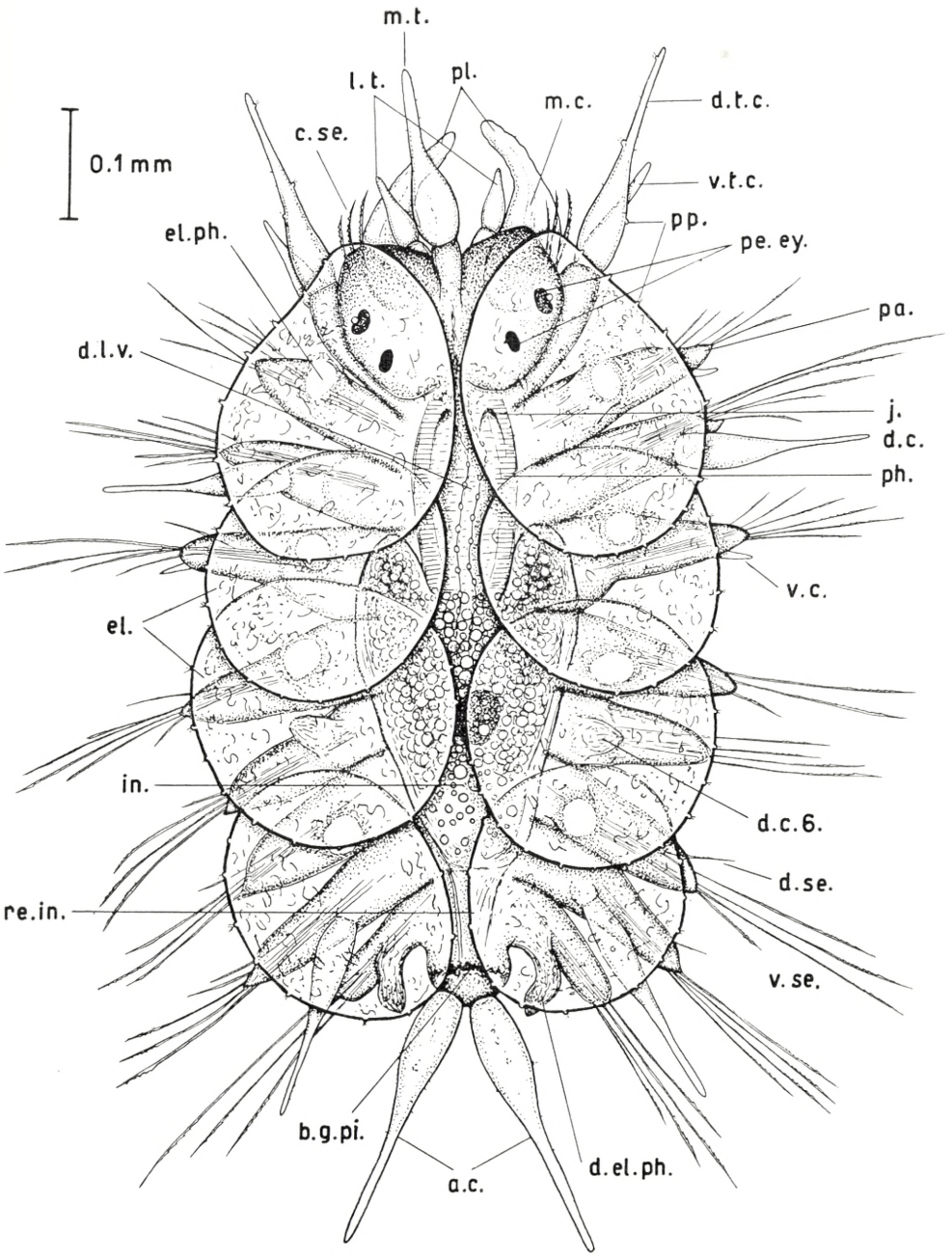
m.t.
l.t.
pl.
m.c.
d.t.c.
c.se.
0.1 mm
v.t.c.
pp.
el.ph.
pe. ey.
pa.
d.l.v.
j.
d.c.
ph.
v.c.
el.
in.
d.c.6.
d.se.
re.in.
v. se.
b.g.pi.
d.el.ph.
a.c.

Fig. 6.

the head and anal appendages is 700 μ. With the tentacles (the median tentacle) and the anal cirri included the length is about 950 μ. A number of metamorphosed specimens were measured, and the above sizes were found almost invariably. It is worth while stressing the importance of this detail, since the size is a decisive distinctive factor between the newly settled bottom stages of *H. imbricata* and *H. impar* (see below).

Including the first modified chaetigerous segment the total number of setigers immediately after the metamorphosis is always nine.

The first segment has two pairs of tentacular cirri, the dorsal ones being the largest (as in the adult animal) and first developed (d. t. c. and v. t. c.). The parapodia of this segment are highly reduced and only the ventral section has a few strong and curved setae (c. se.). Apart from aciculae the other setae are lacking. As to the supply of setae the following eight segments are more typical, having both short dorsal setae (d. se.) (few in number) and long numerous finely dentate ventral setae (v. se.). It is rather remarkable that the two types are not yet divided into two distinct bundles; similarly the parapodia (pa.) are not yet biramous.

All true body segments have small, slender ventral cirri (v. c.). The dorsal cirri (d. c.) are long and slender, about 200 μ in length and only present in two fully developed pairs on the body: on the chaetigerous segments no. 3 and 8. The dorsal cirrus of setiger no. 6 is only visible as a small bud on each side of the segment (d. c. 6). There are two long slender anal cirri (a. c.). All the body appendages are not quite smooth, but have some few scattered, blunt papillae (pp.) with fine sensory hairs.

There are four pairs of elytra (el.) as in the larvae, situated on the segments in the same succession as on the adult, on segment no. 2, 4, 5, and 7. Number 9 is not developed and is only a small bud (d. el. ph.). The single scales, except the first pair, which are tapering in front, have an almost circular shape. Like the body appendages the elytra have a scattered supply of tiny blunt papillae (pp.) with fine hairs. The elytra which are colourless and translucent, have well-defined elytrophores (el. ph.), appearing as clear, round dots about 30 μ across. The first pair has a

central position, the following are connected with the anterior part of their elytra (see fig.).

Pigmentation is practically absent on the body. Only the girdle of bluish-green pigment round the pygidium (b. g. pi.) is preserved.

Also the ciliation is highly reduced. All the larval bands of cilia, first of all the prototroch, have disappeared. Instead transverse bands of fine cilia are now seen on the dorsal surfaces of the segments. Since they were only recognizable and their extensions not visible on the living individuals even at rather high magnifications, I have chosen to omit the ciliation completely in my sketch (fig. 6).

The prostomium is now almost quite like the head-lobe of the adult. The larval eyes are lacking and the permanent eyes (pe. ey.) have changed their position towards the adult condition. *This point is very important for the identification of this stage of H. imbricata.* According to several authors the eye position of this species is very characteristic. Thus E. Wesenberg-Lund (1953, p. 20) in her description of *H. impar* writes: ". . . in *H. imbricata* the anterior pair of eyes is situated partly wholly on the ventral side of the prostomium, whereas in *H. impar* both pairs are visible from the dorsal side." Furthermore the *anterior pair of eyes is situated nearer to the tips of the frontal peaks of the head than is the case with H. impar.* This important adult character is also described in the literature, thus E. Berkeley and C. Berkeley (1948, p. 11, fig. 9) give a good figure illustrating this detail.

On the bottom stage from the Isefjord both characters can be seen. The anterior pair of eyes is far more ventrally situated than pair no. 2, and in comparison with the same stage of *H. impar* (fig. 9) the position is closer to the frontal peaks, a condition which on the larger specimens will prove still more pronounced.

The front margin of the head has now attained a very remarkable reddish-brown colour, a feature also present in the adult animal, where the head contrasts against the paler body (N.B. when all the scales have been removed!). All the head appendages are developed; the median tentacle (m. t.) is longer than the two lateral tentacles (l. t.). All three are distinctly separated from their basal part. Ventrally the two large and very mucous palps (pl.), each containing a muscular cylinder (m. c.), are developed.

The alimentary canal also shows its full adult structure. The muscular pharynx (ph.), about 170 μ in length, has two jaws in front (j.). In the following section, the intestine proper (in.), the cells have a dense content of rather large oil-globules. It has a characteristic bilobed shape, as a lobe protrudes on each side of the pharynx. The rectal section (re. in.) is narrow and very transparent.

Also the vascular system is partly visible, especially along the dorsal side of the body, where the pulsating, longitudinal vessel is distinguishable (d. l. v.). The endothelial wall of the vessel can also be seen.

The colour of the young bottom stage is faintly yellowish-green except for the head-lobe (see above).

The early bottom stage will mostly use the parapodia for locomotion, whether it crawls or swims. However, sometimes when observing the young specimens through the binocular microscope, I saw another mode of locomotion. The body with the parapodia was kept completely quiet and instead the dense cover of fine cilia of the body surface produced a slowly advancing movement.

It seems that it takes some time before the younger bottom individuals of *H. imbricata* are morphologically fully developed. On May the 16th, 1954, I collected several bottom specimens which in spite of their sizes, from 7 to 10 mm, were still lacking some of the more important systematic characters. Thus the smallest individuals found had only ten elytra and the largest, up to about one cm, had only 13 scales. Also the number of chaetigerous segments were smaller than normally in the adult; from 21 to 28 as against 37, the full normal number according to Fauvel (1923, p. 55). However, they all had the typical position of the eyes, as emphasized above.

2. *Harmothoë impar* Johnston.

The contributions to our knowledge of the reproduction and larval development of *H. impar* are remarkably scanty. McIntosh (1900 and 1927) is the only one who renders some details about the breeding time and early development, but nothing is

stated about the larval stages. Thus up till now these are undescribed.

As is known from other species of the genus, *H. impar* according to McIntosh has brood protection under the scales. The eggs are held together by a transparent mucus. Females carrying ripe eggs in this manner were recorded in February.

From the Sound, where the species is not very common, Thorson (1946, p. 48) is unable to give further information about the reproduction. However, the larvae of the two species may have been mixed up, in particular because *H. impar* is not so common as *H. imbricata*. From Dutch seas, where the species is common, Korringa (1951, p. 64) states that ". . . *Harmothoë impar* breeds in summer, and that young individuals settle down in June and July, sometimes again in September." In the plankton Korringa has taken "very young individuals" and larvae "in the last ten days of June and July, sometimes again in September." No description and figures of the larval development are given.

In the Isefjord, more especially in Vellerup Vig, *H. impar* is about as common as *H. imbricata*, both inhabiting areas where *Mytilus edulis* is distributed. Since the number of polynoid species has not been cleared up until the last few years, as the taxonomy has not been thoroughly studied, I have no doubt mistaken *H. impar* for young *H. imbricata*. This also means that the biology of the former has not been treated so completely. Thus only the larval stages in the plankton were dealt with, while the spawning and early development of *H. impar* is still unknown.

During the period when quantitative plankton samples were regularly taken, i. e. from November 1950 to June 1951, large larvae, which undoubtedly belong to *H. impar*, were only recorded within a short interval in the spring (see fig. 2).

Regular collecting from January to April 1953 of adult bottom specimens revealed the fact that mature individuals could only be found near the time of the occurrence of the pelagic larvae just mentioned. The size of the mature adults from the Isefjord is smaller than the mature *H. imbricata*, ranging from 1.2 to a maximum of 2.3 cm. Fauvel (1923, p. 60) states almost the same order of magnitude. In January 1953 about 20 % of the examined material of males and females contained sexual

products (females with only very small eggs). The rest had no eggs or sperm at all. On April the 6th the same year 50 % of the individuals examined were fully mature, while only about 12 % were quite empty. In the mature females the eggs were always present from the 7th chaetigerous segment on, while the number of egg-containing segments was varying with the total number of segments. Thus a female with 35 setigers and 1.2 cm long had eggs in the segments nos. 7—32; and another comprising only 30 setigers, but of the same length, had eggs from 7. to 28. segment.

Among the animals from April a female, 1.2 cm long, had a 1.5 mm long *Corophium* in its intestine.

The diameter of the largest eggs dissected out of the segments was about 70 μ across. The yolk, which was spherical in shape, was whitish and somewhat transparent.

So it seems beyond doubt that the spawning of *H. impar* in the Isefjord will take place, not in the winter months as in the case of *H. imbricata,* but in the spring. Besides supporting this view, the plankton samples will help to define the spawning time, which is restricted to the month of May.

The youngest pelagic stage collected is Metatrochophora II (fig. 7 and 8).

The full length of this stage, which resembles the corresponding stage of *H. imbricata,* varies between 600 and 650 μ.

The number of chaetigerous segments, i. e. the true body segments (the setae of the first modified setiger are not yet visible), is 6. The bundles of setae consist only of the ventral setae (v. se.), and the parapodia (pa.) are not yet biramous. Most of the body appendages are present. Thus the two dorsal tentacular cirri (d. t. c.) can be seen while the corresponding ventral ones are still lacking. Dorsal cirri (d. c.), only as bluntly conical appendages, are developed on body segments no. 3 and no. 6. The anal cirri (a. c.) are distinctly dorsally placed. Similar to Metatrochophora II of the preceding species there are four circular elytra (el.) situated on conspicuous elytrophores (el. ph.) on segments nos. 2, 4, 5, and 7; this is in good agreement with the adults of this and other species of the genus *Harmothoë.*

Just as the larvae of *H. imbricata* the transverse bands of reddish-brown pigment at the anterior and posterior margins of

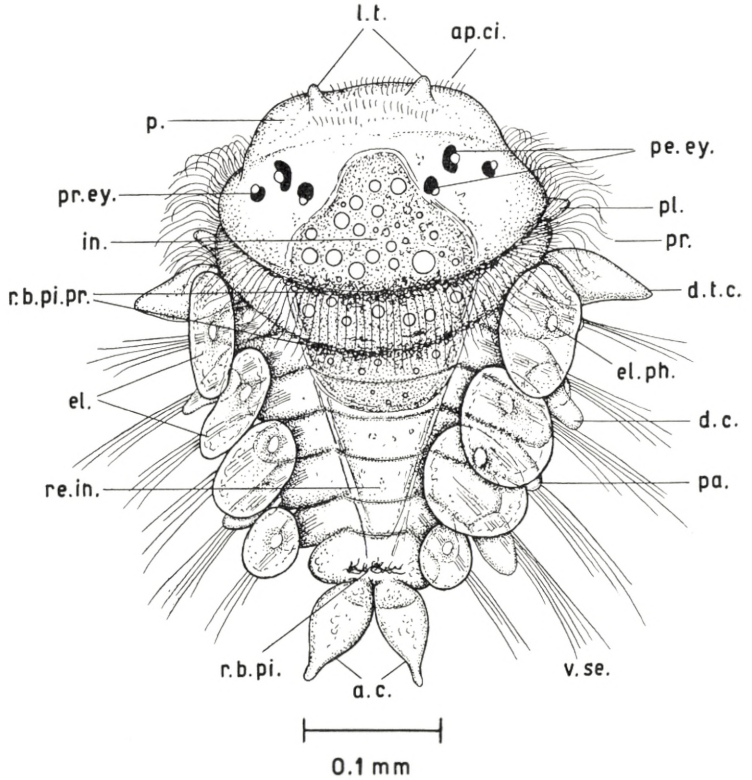

Fig. 7. *Harmothoë impar* Johnston.
Metatrochophora II, in dorsal view; the Isefjord, Vellerup Vig, 10.5.53.
a. c., anal cirri; ap. ci., apical cilia; d. c., dorsal cirrus of a cirrigerous segment (setiger no. 3); d. t. c., right developing dorsal tentacular cirrus, which appears before the ventral one; el., elytra, still without papillae; el. ph., an elytrophore (setiger no. 2); in., intestine with oil globules; l. t., developing lateral tentacles; p., prostomium; pa., parapodium of an elytrigerous segment (setiger no. 5); pe. ey., permanent eyes with lenses; pl., one of the developing palps (the right one); pr., long cilia in the anterior part of the prototroch; pr. ey., left primary eye with distinct lens; r. b. pi., reddish brown pigmentation at the bases of the anal cirri; r. b. pi. pr., two reddish brown pigment bands at the anterior and the posterior margins of the prototroch; re. in., rectal part of the intestine; v. se., bundle of long ventral setae of a parapodium; dorsal setae not yet visible (setiger no. 6).

the prototroch girdle can be fully developed as shown in fig. 7, or they may be totally absent as seen in fig. 8. In one single small plankton sample any type of pigmentation including all transitions may be present on the larvae of *H. impar*. It should be the right place here to emphasize that *the pigmentation as a systematic character in my experience must always be regarded with some*

caution. Not only the two *Harmothoë* species but several other species described later in this paper show similar variations during their larval stages. Besides the transverse pigment bands chromatophores of the same colour are also present as a small patch on the dorsal side of the pygidium (r. b. pi.). The total colour of the larva is faintly greenish to greenish-yellowish.

The ciliation (see fig. 7) consists of two groups, the apical one and the prototroch. The apical ciliation (ap. ci.) is a dense cover of short fine cilia on the frontal part of the head. While on Metatrochophora I of *H. imbricata* (cf. fig. 4) the apical ciliation is a tuft of cilia, the same stage of *H. impar* has a ring of cilia. The prototroch has two rows of cilia. The most pronounced one, composed of long cilia, is attached to the anterior margin of the prototroch girdle. The smaller and more short-ciliated row posteriorly is disappearing, and at the older stages only one row, the anterior one, is left. These stages correspond to the older Metatrochophora II of *H. imbricata* (fig. 5). On the colourless larva figured (fig. 8), which was drawn on the basis of a previous sketch, only the anterior row is figured.

On the prostomium (p.) all six eyes are visible, the two primary eyes (pr. ey.) and the four permanent eyes (pe. ey.), all with lenses. In front the two lateral tentacles can be seen as two small buds (l. t.). Owing to its transparency the developing median tentacle cannot be seen when the larva is viewed dorsally. Also the palps (pl.) are appearing, in the beginning as lateral buds.

When compared with larvae of *H. imbricata* of the same developmental stage it is interesting to note that the three tentacles of the head are appearing somewhat earlier.

As to the digestive system, only the intestine (in.), finely granulated and opaque and with oil globules in the wall, is observable. The rectal region (re. in.) is clear and transparent.

The newly metamorphosed bottom stage (fig. 9). Like *H. imbricata* the Nectochaeta stage of *H. impar* is of rather short duration. Large metatrochophores of the above type were brought in from the fjord plankton and later in the evening placed in vessels with clean sea-water. Early the next morning most of them were fully metamorphosed bottom stages as figured in fig. 9. There is every reason to believe that the young bottom individuals of *H. impar* behave like the corresponding stages of *H. imbricata*,

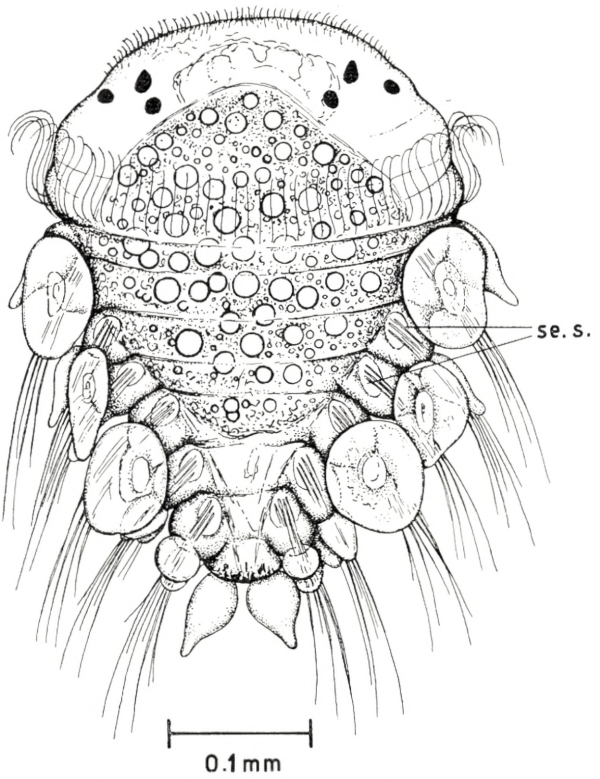

Fig. 8. *Harmothoë impar* Johnston.
Metatrochophora II, in dorsal view, the Isefjord, Vellerup Vig, 23.5.44.
As compared with fig. 7, which is a little more developed, this individual represents an almost non-pigmented larva of the same species.
se. s., seta sacs; these were not visible in the older stages.

being for some time semi-pelagic. Thus most of the specimens contained in the plankton sample of 27-5-1951 (fig. 2) were young metamorphosed worms without larval ciliation.

The few hours old metamorphosed individual measures 435 μ in body size and 670 μ in total length. In comparison with *H. imbricata* this is a considerable difference which is important when the aim is to separate the quite young specimens of the two species.

The complete number of chaetigerous segments immediately after the metamorphosis is *always seven*.

The first modified setiger has the same appearance as in *H. imbricata*. Only the ventral sections of the parapodia are

developed with a bundle of few strong and curved setae (c. se.). Both tentacular cirri are present (d. t. c. and v. t. c.), the dorsal ones being the larger. In the parapodia of the other six segments, which are not yet biramous, distinct dorsal setae (d. se.) and large bundles of long ventral setae (v. se.) can be found.

Dorsal cirri (d.c.) are found on the setigers nos. 3 and 6. They are from 80 to 100 μ long, but not so slender as in *H. imbricata* and more claviform in shape. The anal cirri (a. c.) are about 130 μ long. Compared with the previous species the body appendages are more papillated.

There are always four pairs of elytra (el.) fixed by distinct elytrophores on the segments nos. 2, 4, 5, and 7. Except for the first pair, which are somewhat oblong, the other scales are almost circular in shape. In contrast to *H. imbricata* the surfaces of the elytra are characteristic by having numerous conspicuous papillae (pp.) or tubercles with fine hairs. Considering that this structure is among the most characteristic ones of the adults of the species, its value in the larval determination is obvious. The papillae are largest on the last pair of scales.

Pigmentation is found not only at the basis of the two anal cirri, which have two groups of reddish-brown chromatophores (r. b. pi.), but on the posterior margin of the prostomium a patch of dark-green pigment can be seen (d. g. pi.). The colour of the young bottom stage is greenish-yellowish all over.

The shape of the prostomium is different from that seen in *H. imbricata*. There are no tapering frontal peaks and the breadth is greater than the length. Also the position of the eyes is different, since all four eyes (with lenses) are situated on a transverse line and on the same level (p. ey.). This feature is also very important in the determination of the bottom stage of *H. impar*. In transmitted light the eye colour is an intense black. The colour of the prostomium is more brownish than that of the body.

All the head appendages: the median tentacle (m. t.) and the two smaller lateral tentacles (l. t.) are present. The tips of the two ventrally based, large palps (pl.) are only just observable when the animal is viewed dorsally.

The strong muscular pharynx (ph.) is about 125 μ long, but no jaws could be seen; however, the patch of dark green pigment (d. g. pi.) may hide them. The intestine (in.) has the same bilobed

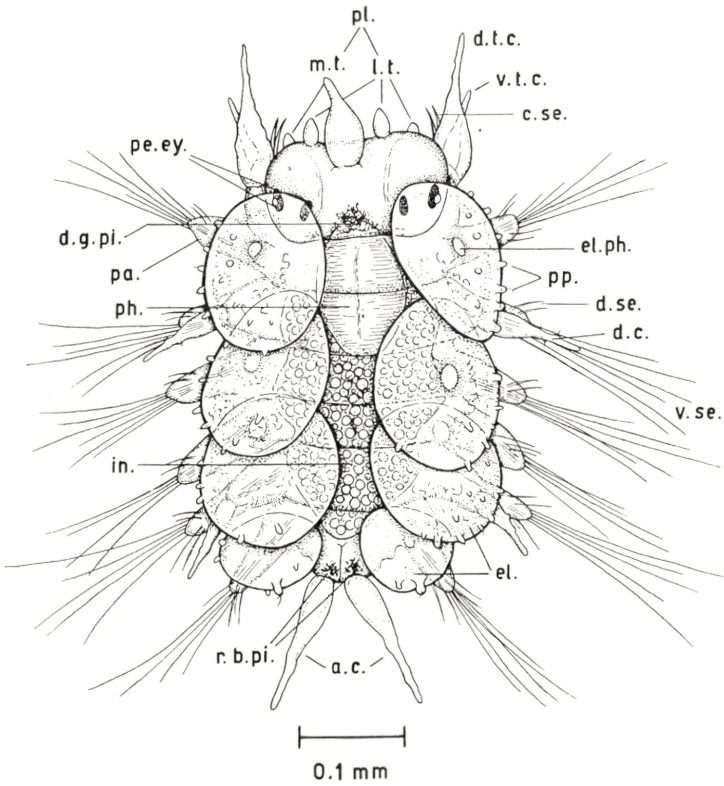

Fig. 9. *Harmothoë impar* Johnston.
Young bottom stage, drawn immediately after metamorphosis, the Isefjord, Vellerup Vig, 10.5.53.
a. c., anal cirri; c. se., strong curved setae of the first setiger (actually the ventral setae of the reduced first parapodia); d. c., dorsal cirrus of a cirrigerous segment (setiger no. 3); d. g. pi., dark-green pigmentation on the posterior margin of the prostomium; d. se., dorsal setae of a parapodium (setiger no. 3); d. t. c. and v. t. c., first pair of cirri, the dorsal and the ventral tentacular cirrus; el., elytra, all covered with rather prominent papillae especially on their posterior margins; el. ph., an elytrophore (setiger no. 2); in., intestine with numerous oil globules; l. t., two lateral tentacles; m. t., median tentacle; pa., parapodium of an elytrigerous segment (setiger no. 2); parapodia not yet biramous; pe. ey., permanent eyes with lenses; ph., muscular pharynx (the jaws are not yet visible); pl., tips of the palps; pp., small tubercular papillae with fine sensory hairs especially on the posterior marginal surfaces of the elytra; r. b. pi., reddish brown pigmentation at the bases of the anal cirri; v. se., bundle of long ventral setae of a parapodium (setiger no. 3).

structure as in *H. imbricata* and its wall has numerous, densely situated oil-globules. The rectal part is very short.

Before finishing the description of the development of *H. impar* it should be well worth *summarizing the main characters separating the young bottom stages of H. imbricata from those of H. impar.*

H. imbricata.—body size: 700 μ.—full length: 950 μ.—number of chaetigerous segments: always 9.—tentacles, tentacular cirri, dorsal cirri and surfaces of the elytra: with few scattered, small papillae.—the 6th dorsal cirri only present as a bud.—prostomium: breadth and length about the same; frontal peaks in development. The first pair of eyes ventral near the frontal peaks.

H. impar.—body size: 400—450 μ.—full length: 600—650 μ.—number of chaetigerous segments: always 7.—tentacles, tentacular cirri, dorsal cirri, and elytra: with numerous conspicuous tubercles. —the 6th dorsal cirri developed;—prostomium: the breadth is larger than the length; no frontal peaks present. The eyes on a transverse line on the same level.

3. *Eteone longa* (Fabr.).

The spawning habits of *Eteone longa* have never been observed under natural conditions. Meek and Storrow (1924) have observed spawning in aquaria. The eggs were shed freely in the water without any surrounding mucus.

The pelagic larvae of the species have been described and figured several times. In 1871 R. v. Willemoes-Suhm from the bay of Kiel published descriptions and rather rough drawings of the larval development of what he called *Eteone pusilla* Ørsted. From the same area Leschke in 1903 gave a new illustrated description of larvae, which he referred to the same species in spite of slight differences. In both cases Trochophora, Metatrochophora and Nectochaeta are described and figured, however, without any statement why they are referred to *E. pusilla*. Nolte (1938) emphasizes that the larvae described by both authors, owing to the presence of bristles on the second segment, i. e. the segment caudal to the buccal segment with the tentacular cirri, must belong to either *Eteone longa* or *Eteone flava*, which according to Fauvel (1923) are the only northern species of the genus *Eteone* with this character. Because of the small differences between the descriptions of Willemoes-Suhm and Leschke, Nolte concludes that their larvae cannot be referred to the same species, but must belong to two separate species, *Eteone longa*, which name Nolte gives to the type of larva of Willemoes-Suhm, and

Eteone flava, which then according to NOLTE should be the name of the larva described by LESCHKE.

The characters mentioned by NOLTE, according to which the larvae should be considered two distinct species, are: the time for the appearance of the mouth; the predominance of the umbrella; the pigmentation; the eyes with or without lenses; the larval segments; the presence or absence of cilia on the tentacles and tentacular cirri; shape and destiny of the apical tuft and the presence or absence of segmental organs.

The most important objection to the division into species made by NOLTE is to be found in the condition in which the figures of WILLEMOES-SUHM are given. His drawings are so sketchy that most of the characters mentioned by NOLTE are too delicate to be found. This applies e. g. to the pigmentation (which furthermore is able to vary rather much; cf. the larvae from the Isefjord!); the lenses of the eyes; the cilia of the tentacles and the tentacular cirri (cf. NOLTE p. X 240), which however, are not cilia, but sensory hairs! (observed very clearly on the Isefjord larvae, see figs. 11—12). Another objection is to be found in the fact that some of the stages of WILLEMOES-SUHM and LESCHKE cannot be compared owing to their different stage of development. The Metatrochophora of WILLEMOES-SUHM (his fig. 7, pl. XXXVI), e. g., is younger than the Metatrochophora of LESCHKE (fig. 4, pl. VII), a fact which may explain the different shape and development of the apical tuft.

Ignoring these small differences, which in my opinion are of slight value only, the main characters of the larvae are quite identical; a point of view also shared by THORSON (1946), and so it may be inferred that WILLEMOES-SUHM and LESCHKE have described the larvae of one and the same species only.

From the harbour of Copenhagen, SMIDT (1944) has described and figured a Metatrochophora of an *Eteone* species, which may belong to *E. longa*. However, the figure is rather rough and in some respects not correct; thus the first chaetigerous segment bears a distinct dorsal cirrus, a feature which according to FAUVEL (1923) is never found in any species of the genus *Eteone*.

In 1946 THORSON gave further information about the pelagic stages of an *Eteone* species from the Sound. The larvae were found in his so-called plankton main-series taken regularly

throughout several years in localities close to the island Ven and off Elsinore. The larvae were only collected in the spring and mostly in the months of March-April and in May. In June only two specimens have been taken. All the stages found belonged —according to THORSON—to one species only and were of the same type as described by WILLEMOES-SUHM and LESCHKE even if slight differences were present. Thus the tentacles of the larvae from the Sound developed at a much later stage than in the larvae of WILLEMOES-SUHM. On the basis of a. o. the discussion of NOLTE and of his own results THORSON concludes that the larvae of WILLEMOES-SUHM and LESCHKE are identical with the larvae from the Sound and that they belong to *Eteone longa*, this species being by far the commonest *Eteone*-species in the Sound.

THORSON gives figures of Metatrochophora II, the Nectochaeta, and the youngest metamorphosed bottom stages. However, the fig. 26 C and D cannot be correctly drawn. At the bottom stage figured in C, the left side of the body has only dorsal cirri (including the first chaetigerous segment, which—as emphasized above—in the *Eteone* species normally has no dorsal cirrus!) while the right side of the body of the same specimen has only the ventral cirri! Also in fig. 26 D both first chaetigerous segments erroneously have dorsal cirri.

In 1951 E. SMIDT again mentioned and pictured *E. longa* among his descriptions of annelid larvae from the wadden sea (p. 48). Only one newly settled bottom stage with seven chaetigerous segments and without any pelagic attributes at all has been found. The individual was taken in a micro bottom sample on 15-5-1942. Also SMIDT's drawing is incorrect owing to the presence of dorsal cirri on the first chaetigerous segments.

Up to now *Eteone longa* is the only species of the genus *Eteone* taken in the Isefjord area. It is among the commonest polychaete annelids and is found in all biotopes, especially characterizing the shallow sandy areas from about mean level to about 1—1.5 m below low water mark.

The adult individuals are generally found crawling in or on the bottom. However, from Vellerup Vig and off Frederikssund on the Roskildefjord several observations are available which show that adult individuals can be found—often in large numbers—swimming very actively near the surface of the water. In

Vellerup Vig, just outside the laboratory such swimming specimens were observed in March 1943, April 1944, and March 1949. At Frederikssund, in the very narrow passage under the bridge connecting the town with the peninsula Hornsherred, strong currents are very frequent. In this place drifting adult specimens were taken swimming near the surface in December 1943 and March 1945. Most of the animals from the spring were mature, while only a single female from December 1943 contained small eggs. Thus the usual time for finding swimming specimens seems to be the early spring, the time just before the beginning of the spawning, that is to say the last half of April, when the temperature of the low water rises to more than about 10° C.

As to the swarming of *E. longa* the following observation from another Danish locality, the low water area round the small island of Vorsø in the Horsensfjord (Eastern Jutland), in a rather instructive way supplements the Isefjord results.

On April the 13th 1949 I examined a small creek at the above-mentioned island. The creek was only about two metres broad and ten long and emerging directly in the large, sandy wadden area on the north side of Vorsø. The water was very shallow and the bottom contained a very thick layer of mud. The bright sunshine had raised the temperature in the small amount of water so that a considerable difference was present between the wadden sea outside (about 8° C.), and the creek. On my arrival just before noon I found a few swarming *Eteone longa*, but soon after, when the temperature passed 12° C., the whole creek was filled with large—up to 10—12 cm—swimming and wriggling *Eteone* individuals. I now made a series of temperature measurements from the warmest part of the creek and out to the wadden sea. Below 10° C. no individuals were seen in the water, but the bottom everywhere in these "cold" areas contained numbers of buried, large mature specimens. Also the swimming animals were mature; most of them being females. No real breeding nor already spawned eggs were observed. These observations together with the results of Meek and Storrow (1924) seem to confirm the swimming habits of *E. longa* as a component of the whole spawning process. Interesting is the swarming temperature mentioned by these authors, from 3.7 to 5.0° C., as against about 10° C. in Danish waters.

On April 1953, when working with the species in Vellerup Vig, I made an observation on a male and a female kept in aquarium which may indicate the existence of a sort of copulation before spawning. A fully mature male—about 20 mm in length—was seen winding itself round the body of a female—length 30 mm—but only with small eggs in the segments thus not being mature. The head and the foremost part of the body of the male moved in a trembling way while the male incessantly was putting its head into the spaces between the parapodia of the female. At small intervals the male projected its proboscis and adhered it to the body of the female although apparently without doing it any harm. During the action of the male the female kept quiet, only the "biting" of the male produced a slight starting in its body. After a while the male left the female and the pre-mating behaviour was not seen again, presumably owing to the immature condition of the female. The temperature during the observation was about 19° C.

The spawning time. It seems beyond doubt that *Eteone longa* everywhere where the species has been examined, spawns within a very short interval and always in the early spring. Both Willemoes-Suhm (1871) and Leschke (1903) mention the month of May as the time when the larvae occur in the plankton. Meek and Storrow (1924) found the swarming individuals in March and especially April. Thorson (1946) indicates also March-April and May as the time for pelagic larvae. The fact that the spawning of *E. longa* in the Sound, as in other areas, is much concentrated is rather interesting, because in a way it confirms the shallow-water distribution of the species. In that respect the Sound is a very good indicator owing to its two distinct fauna elements. Under the influence of the outflow of fresh water from the Baltic, the surface layer of the Sound, that is the shallow-watered areas along the shores, has a typical brackish water fauna with changing temperatures, while the deeper parts of the Sound receive water of permanently high salinity and rather low temperature from the Kattegat and the Skagerrak and for this reason contains a typical marine fauna. Consequently, the spawning time of *E. longa* coincides very closely with the rising of the surface temperature in the spring, 10° C. being the optimal temperature.

The young bottom stage described by SMIDT (1951) was also taken in May (15.5.1942).

In the Isefjord I have never observed the spawning under natural conditions. Judging from the appearance of the pelagic larvae in the plankton, it always seems to take place at the end of April or, as just emphasized, at water temperatures of about 10° C. In 1945, 1951, and 1953 I have observed the maximum of larvae very near the 1st of May. In 1951 when monthly plankton samples were regularly collected, the largest number, 104 specimens in one 8 metre vertical haul, was counted on May the 6th. The samples from 22.4 and 27.5 of the same year contained no *Eteone* larvae. In 1953 the last large larvae were collected in the plankton in Vellerup Vig on May the 10th.

In an aquarium I have observed the spawning once. On March 3rd, 1943, a female, which had been kept in a vessel since 2-2-1943, at a temperature of 10° C., spawned its eggs freely on the surface of the muddy bottom layer without forming any sort of surrounding mucus, an observation in full agreement with the results published by MEEK and STORROW (1924). Owing to lacking fertilization no development of the eggs took place.

The egg size. The new-laid egg, which had a distinct egg membrane, was quite globular in shape and had a light yellow colour. The diameter of the egg including the membrane was 0.1 mm.

The cleavage and the early development of the eggs could not be followed, since, as mentioned above, the eggs available were not fertilized.

The following description is based exclusively on larvae taken in the Isefjord, Vellerup Vig, with a small conical plankton net with gauze no. 12. The material was collected in the years 1945, 1951, and 1953.

Metatrochophora I (fig. 10) is the youngest stage found. It has a total length of about 270 μ; in comparison with figures 6 and 7 of WILLEMOES-SUHM the larva pictured here represents a middle stage between these two. The sizes of the stages of WILLEMOES-SUHM are remarkably small; 73—85 μ.

The larva from the Isefjord is rather transparent and of a yellowish hue. Grass-green chromatophores (g. ch.) are present only in the hindmost part of the body, beginning on the second

segment after the prostomium, while in the front part chromatophores are totally lacking. According to WILLEMOES-SUHM's figures the green pigment is regularly distributed over the whole larval body, while conversely LESCHKE's youngest larva (his fig. 3, Pl. VII) has only green pigment on the foremost part of the body. THORSON only mentions the presence of the green colour, his drawings of the pelagic stages having no distinct chromatophores.

The prostomium (p.), which is rounded, has in front a distinct group of apical cilia (ap. ci.), in which the tips of the first two tentacles—ventrally based—can be seen (t.). As pointed out by THORSON (p. 59), his pelagic larvae differ from the older descriptions in having the tentacles developed much later.

On each side behind the apical tuft a group of fine twisted tubes can be seen, undoubtedly a pair of mucus glands (m. g.).

There are four red eyes (ey.), the largest of which are the dorsal ones, which have distinct lenses. The smaller ventral eyes are darker red and have no visible lenses.

On the next segment, with the ciliated mouth ventrally, a long-ciliated prototroch is developed round the body (pr.), the cilia being shortest most dorsally.

In the next three segments, which in the older stages represent the chaetigerous segments, the chromatophores are arranged in thin transverse bands, a feature most distinct in the anal segment (b. g. ch.). Setae are not yet visible outside the segments; only a few thin capillary setae (c. se.) are present within the body. When only a little older—when four segments are developed—the parapodia with bristles will appear; this is the stage described by WILLEMOES-SUHM in his fig. 7, Pl. XXXI; fig. 4 Pl. VII in LESCHKE seems to be a little older.

The intestine is thick-walled (in.), especially in the hindmost part just before the anal opening (an.), which has a surrounding, glandular epithelium (g. ep.). The intestinal colour is light green and a few small oil globules are found in the wall. At this stage of the development no telotroch was seen.

The older Metatrochophora II (fig. 11) is from 310 to 320 μ long and has four distinct setigers. It corresponds rather closely with WILLEMOES-SUHM's Metatrochophora (fig. 7). However, the larva from the Isefjord has a more conical prostomium and is

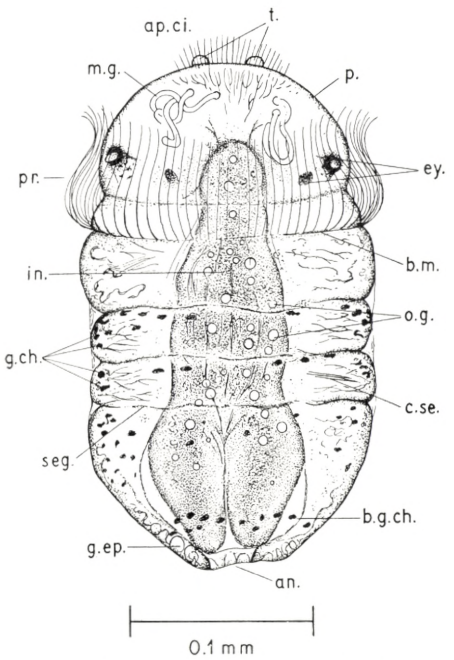

Fig. 10. *Eteone longa* (Fabricius).
Metatrochophora I, dorsal view; the Isefjord, Vellerup Vig, 1.5.45.
an., terminally placed anal opening; ap. ci., apical cilia; b. g. ch., transverse band of green chromatophores round the developing anal segment; b. m., basal margin of the prototroch, cf. the text on p. 40; c. se., capillary setae still inside the body wall; ey., eyes; the largest one (most dorsally) with a distinct lens; g. ch., grass-green chromatophores; g. ep., glandular epithelium (mucus?) round the anus; in., intestine; m. g., mucus glands?; o. g., small oil globules in the intestinal wall; p., prostomium; pr., prototroch; seg., beginning segmentation; t., first two tentacles.

much larger in comparison, 310—320 μ long as against 85 μ in the bay of Kiel larva. LESCHKE's fig. 4, Pl. VII, must be younger, among other things owing to the incomplete formation of the parapodia. LESCHKE gives no indication of the sizes of his pelagic larvae. THORSON's Metatrochophora II (fig. 26 A, p. 60), which is his youngest *E. longa* larva found in the Sound plankton, is remarkable in several respects. As previously mentioned, the tentacles, in spite of its advanced stage of development, are not yet visible and the tentacular cirri and the dorsal cirri of the parapodia have not yet appeared. Another very peculiar feature in his larva of fig. 26 A is the apparent presence of a further segment with a pigment band between the prostomium and the mouth segment, a detail absent in the Nectochaeta in fig. 26 B,

which, however, has the pigment band. Perhaps THORSON's drawing is to be explained in the way that the line dividing the mouth segment into two parts is only the basal margin of the prototroch, as also seen in the larva pictured in fig. 10 in this paper (b. m.). The total length of Metatrochophora II from the Sound is rather great, in comparison with stages from the Isefjord and especially with the *Eteone* larvae of WILLEMOES-SUHM, 460 μ. Five chaetigerous segments are seen in THORSON's Metatrochophora II.

On the Isefjord Metatrochophora the green chromatophores (g. ch.) are distributed over the whole body; on the two anal cirri (a. c.) their colour is darker green. Just before these lobes a transverse band of brownish chromatophores is placed round the body (b. pi.). The variations in the concentration of the green pigment of the larger *E. longa* larvae from the Isefjord were remarkable, and very often quite clear and yellowish larvae with few chromatophores were collected together with larvae which were of an intense green. The older Metatrochophora pictured here represents a rather light type.

The prostomium (p.), which has now a shape more like the head of the adult, has four well-developed tentacles (t.) with sensory hairs (s. h.) and placed in their permanent position. The four red eyes (ey.) are still situated in front of the prototroch on the prostomial segment, even if the smaller ones are on the border of the mouth segment. In the latter, with still well-developed prototroch, the mouth is distinctly seen as a transverse, ventrally situated opening (m.), behind which a thick-walled pharynx (ph.) has now been formed. The tentacular cirri (t. c.) are present, one pair on each side. The parapodia with their characteristic compound setae (se.) have all—except in the first setiger—dorsal cirri (d. c.).

The intestine (in.) with distinct and rather thick walls, is dark grass-green and is rather granular. In the above-mentioned brownish-coloured pigment band close to the anal lobes a telotroch is present (te.). As compared with the cilia of the prototroch its ciliary equipment is short and for this reason difficult to see even at high magnifications. In the literature the telotroch has only been observed once in connection with the pelagic larvae

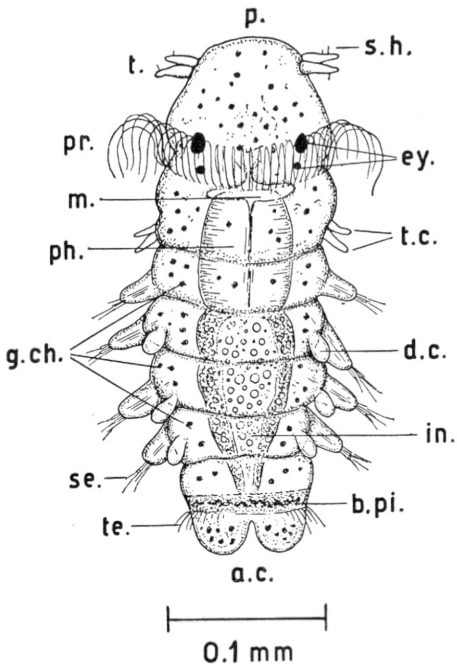

Fig. 11. *Eteone longa* (Fabricius).
Metatrochophora II, dorsal view; as to pigmentation the larva pictured represents a rather light type; the Isefjord, Vellerup Vig, 3.5.53.
a. c., anal cirri; b. pi., brownish pigmentation; d. c., right dorsal cirrus of the 2nd setiger; ey., eyes; g. ch., green chromatophores; in., the rather thick-walled, granular intestine; m., mouth aperture visible through the transparent larval body; p., prostomium; ph., the thick-walled, muscular pharynx; pr., prototroch; se., composed setae of a parapodium; s. h., fine sensory hairs on the tips of the tentacles; t., the tentacles; t. c., developing tentacular cirri; te., telotroch.

of *E. longa*; in the old Nectochaeta figured by Leschke (fig. 5, Pl. VII) a short telotroch can be seen.

The Nectochaeta (fig. 12) has a total length ranging from 480 to 500 μ. The corresponding stage from Willemoes-Suhm (fig. 8, Pl. XXXI) is only 123 μ; Thorson (fig. 26 B, p. 60) states 905 μ for a Nectochaeta consisting of 6 chaetigerous segments. Thus there is still a considerable variation in the size of the larvae described. In my samples from the Isefjord I also always found specimens varying greatly in size even if the same number of segments were present. Also when metamorphosing, the young individuals have different sizes.

Besides the green pigmentation, which on the central part of

the head very often forms a triangular figure (g. pi.), dense and dark brownish pigment is found close to the base of the tentacular cirri (t. c.) and on the centre of segment no. two (d. b.pi.).

The eyes (ey.) are now separated by the prototroch (pr.), as also described by LESCHKE, a feature which NOLTE (p. X. 239), however, denies. The colour of the eyes is still red when seen in falling light; when transmitted light is used in the microscope, the eye-colour will appear dense black.

The large, muscular pharynx (ph.) has a typically oblique position in the body; in the Isefjord larvae always turning on the right with the posterior end (cf. figure), as also figured by LESCHKE. It now reaches the posterior margin of setiger no. three. The grass-green intestine (in.) is still very opaque and even more granular than in the younger stages. It ends in a very short and thin-walled rectal part (re. in.).

The two ciliary bands, the prototroch and the telotroch, are still very well developed, especially the prototroch, which has long and very distinct cilia.

The pigmentation of the segments, as usual subject to great variation, form transverse bands, a character also mentioned by THORSON (cf. his fig. 26 E, p. 60, 1946). The ground colour of the larval body is faintly greenish.

During swimming, which in the larger larvae is brought about by the aid of the prototroch, the parapodia are laid along the body with their tips pointing backwards.

The Nectochaeta pictured here—very often with up to six chaetigerous segments—is the largest free-swimming larval stage of *E. longa* found in the plankton in Vellerup Vig.

Only slight differences are to be found in the newly settled bottom stage in comparison with the Nectochaeta. Of course all pelagic attributes, such as the prototroch and the telotroch, disappear. The changes are found e. g. in the development of the intestine. The anterior end of the pharynx is provided with papillae and the intestinal wall is more distinct in its contours and almost devoid of oil-globules so that the lumen of the intestine can be seen.

When summarizing the above, the results here published as compared with the descriptions hitherto given by WILLEMOES-SUHM, LESCHKE, NOLTE, and THORSON, are remarkable, especially in one respect. They show that even if the pelagic larvae of

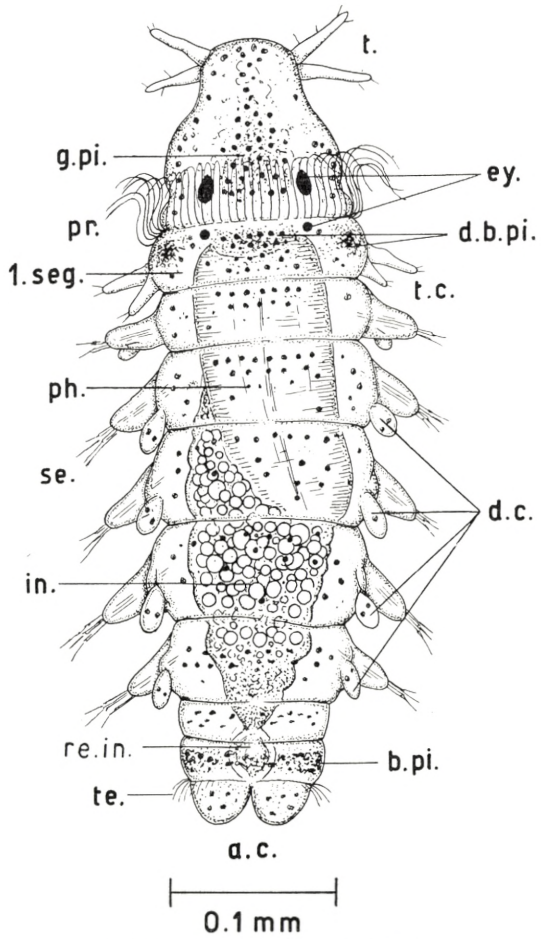

Fig. 12. *Eteone longa* (Fabricius).
Nectochaeta, dorsal view; the Isefjord, Vellerup Vig, 3.5.53.
a. c., anal cirrus; b. pi., transverse band of brownish pigment; d. b. pi., dark brownish pigmentation close to the base of the tentacular cirri and on the centre of the segment; d. c., dorsal cirri of the setigers nos. 2—5; ey., eyes, the posterior (more ventrally than the anterior ones) and smallest pair now behind the prototroch; g. pi., green pigmentation concentrated in a triangularly shaped figure both on the dorsal and the ventral part of the prostomium; in., intestine full of oil globules; ph., the large, muscular pharynx in its characteristic oblique position; pr., the prototroch now separating the eyes; re. in., rectal part of the intestine; se., the composed setae; 1. seg., first segment with the ventrally placed mouth aperture and the two pairs of tentacular cirri. Parapodia and setae not developed; t., tentacles with fine sensory hairs; t. c., tentacular cirri; te., reduced telotroch.

Eteone longa belong to a rather characteristic and rather easily recognizable type, *they differ very much not only from place to place, but even within one small water area.*

The variations mentioned above refer to:
(1) *The size of the larvae.* Thus Metatrochophora II is about 85 μ according to WILLEMOES-SUHM; about 460 μ in the Sound (THORSON) and from 310 to 320 μ in Vellerup Vig in the Isefjord. The Nectochaeta correspondingly, 123 μ (WILLEMOES-SUHM); 905 μ (THORSON), and the larva pictured from the Isefjord, about 500 μ. Even the young newly settled bottom animals vary rather much (e. g. many sizes in one sample from the Isefjord).
(2) *The pigmentation.* As emphasized above, larvae from the Isefjord could be found lacking almost any pigmentation, while others in the same sample were characterized by an intense and dense green pigmentation.
(3) *The time of the appearance of the tentacles.* While in WILLEMOES-SUHM's paper (his fig. 7) the late Trochophora (length about 85 μ) has distinct tentacles, the large Metatrochophora II of THORSON (fig. 26 A, p. 60), length 460 μ, has no traces of tentacles!
(4) *The larvae being monotrochous or exotrochous* (cf. NOLTE, p. X 66, 1938). LESCHKE's Nectochaeta (fig. 5, Pl. VII) according to NOLTE is exotrochous, i. e. it has both the prototroch and the telotroch developed. The corresponding stages of WILLEMOES-SUHM and THORSON have only the prototroch and are consequently of the monotrochous type. On this point the Isefjord larvae correspond closely to the exotrochous type.

In my opinion the correctness of this variation may or may not be right, since in my own experience the telotroch is so poorly developed that at a less careful examination of the larvae at low magnification it can very easily be overlooked.

4. *Magalia perarmata* Marion and Bobretzky.

The larval development of this species is mentioned by few authors.[1] Thus LANGERHANS (1880) states that the eggs are slightly purplish.

[1] After the finishing of my manuscript I have received two papers by L. CASANOVA describing and figuring the larval development of this species (L. CASANOVA, *Les annelides du plankton dans le Golfe de Marseille*, pp. 29—36, and *Note sur le développement de Magalia perarmata Mar. et Bobretzky (Polychaeta, Hesionidae)*, pp. 155—162, Pl. I—III, in: *Recueil des Travaux de la Station Marine d'Endoume*, fasc. 8, 1953, and fasc. 13, 1954). The early development is not mentioned and the

On June 24th, 1942, during dredging in Vellerup Vig, some specimens of this small delicate hesionid were taken as new to the Danish fauna. Later collecting has shown that the species is common in practically every biotope in the Isefjord area. Since the first finding in 1942 the species has been taken through the whole year. *M. perarmata* is a very fast moving species and very often it was observed swimming.

The first mature individuals will occur in the middle of May and from now on every single specimen examined will contain mature eggs or sperm. After the culmination of the breeding in July the number of mature individuals will decrease. The last mature specimens were collected in October (cf. fig. 1, p. 6). The percentage of males and females was examined in July 1944. About 70 % of the animals were females, as against 30 % males. The mature females seem mostly to be larger than the males; the largest adults found, about 8 mm in length, thus being females. The greatest number of chaetigerous segments noticed was 31.

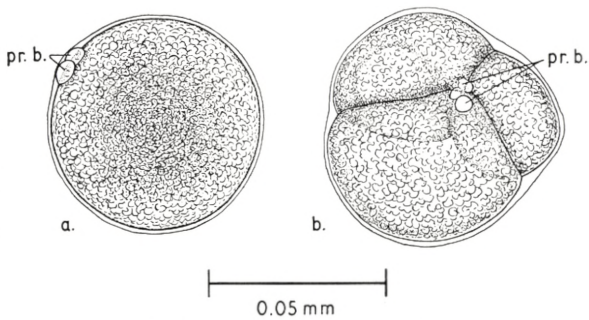

Fig. 13. *Magalia perarmata* Marion & Bobretzky. a. Undeveloped new-laid egg, spawned in aquaria; b. Egg in cleavage; pr. b., polar bodies. The Isefjord, Vellerup Vig, 15.6.44.

In the mature adults the sexual products extend to any part of the body segments. The females can be so distended with eggs that the usually very distinct pharynx is completely hidden.

first larval stage in the planktonic life, which is of short duration, is the Metatrochophora. This stage is 250 μ long and besides the apical ciliation only the prototroch is found. The number of segments, which consist of two groups, are five. On the ventral side of this stage a median longitudinal pigmentation of brown or dark green colour is found. The next stage, the last pelagic one, is a transitional stage between the Metatrochophora and the Nectochaeta with six or seven segments. Also the young bottom stage is described, length 400 μ. The author emphasizes that the development of *M. perarmata* is of short pelagic duration and that the stages show morphological resemblance with larvae of Phyllodocidae and Aphroditidae. Further the presence of provisional larval bristles as in the Spionidae is mentioned.

The paper from 1953 gives some information concerning the occurrence of the larvae in the plankton of the gulf of Marseille.

The eggs are shed freely in the water. The single new-laid egg is very small, about 58 μ across, and quite transparent (fig. 13). Its colour is slightly yellowish and the yolk is granular. The segmentation of the egg seems to be extraordinarily fast, and within a short time the early, ciliated gastrula is formed. At this stage, which has a girdle of fine purplish pigment cells round the body, the embryo leaves the egg-membrane and begins the pelagic life, which in relation to the small size of the egg and the poor content of deutoplasma in the yolk may be of rather long duration.

In spite of repeated experiments to rear larvae from the eggs, I did not succeed in getting the pelagic stages. Also plankton samples taken in the fjord during the spawning time gave no results, and no larvae, which with any possibility could be referred to this species were taken in the period 1942—1952. There may be some reason for this peculiar phenomenon, since the pelagic life may be so short that no larvae will form part of the plankton proper.

From a faunistic point of view *Magalia perarmata* and also *Scolecolepis fuliginosa* (Claparède), the larva of which is described later in this paper (p. 60), are interesting species, first, because they form part of the group of polychaete species of the Isefjord fauna whose main distribution is south of Danish seas. Out of the 45 species of polychaetes—up to now (September, 1954)—recorded from the Isefjord area, 12 species belong to this southern group, which inhabit places with rather high summer temperatures. Of these twelve species, eight—collected during the last few years—were not hitherto recorded from Danish waters and some of them even not from Scandinavian waters. These eight species are: *Eulalia* sp. (this species was quite recently found in Vellerup Vig and has not yet been determined with certainty as to species, however, it seems beyond doubt that it is new to our fauna); *Magalia perarmata* M. and B.; *Grubea limbata* Claparède; *Autolytus rubropunctatus* (Grube); *Autolytus edwardsi* St. Joseph; *Nereis* (*Neanthes*) *succinea* (Leuckart); *Scolecolepis fuliginosa* (Claparède) and *Polydora antennata* Claparède.

The presence of this southern element in the Isefjord constituting about 27 % of all polychaetes found here and especially

the new records within this group, seems to me to be of great interest.

One is tempted to connect these findings with the rise of the temperature and the higher salinity of our seas, which has been noticed during the last decades. As, however, since the thorough investigations of C. G. Johs. Petersen (1889), no systematic collections of Danish marine invertebrates have been performed, the possibility cannot be excluded that the species mentioned are in reality no newcomers in Danish waters, but only hitherto have been overlooked.

However, it is out of the scope of this paper to give further details and information about these peculiar findings, which I hope will be dealt with in a later paper.

5. *Microphthalmus sczelkowi* Mecznikow.

This small peculiar hesionid worm has only been found a few times since the first description was given by Mecznikow (1865) on specimens from the North Sea off Heligoland. From Scandinavian waters the species has only been recorded from the Sound (from two localities) by A. Eliason (1920). Nothing is known concerning the spawning and larval development of this species and on the whole its biology is totally unknown.

Mecznikow states that he found females only. Those with eggs had about 33 body segments and the eggs appeared from the 13th to the 24th chaetigerous segment. Those without sexual products had only 23 segments in all. Mecznikow gives no information about the spawning season.

In the Isefjord area *M. sczelkowi* (fig. 14) was found for the first time in June 1942. The species proved to be extremely common at depths from 3 to 10 metres and always in muddy bottom. So far the species has been collected in Vellerup Vig, in the Inner Broad and in the Roskildefjord off Frederikssund, but no doubt it is distributed in the whole fjord area where the bottom consists of mud. The number of individuals in the dredgings has been very varying, from several hundred specimens in even small samples of mud to only a single specimen in one sample. The species was collected in February, March, April,

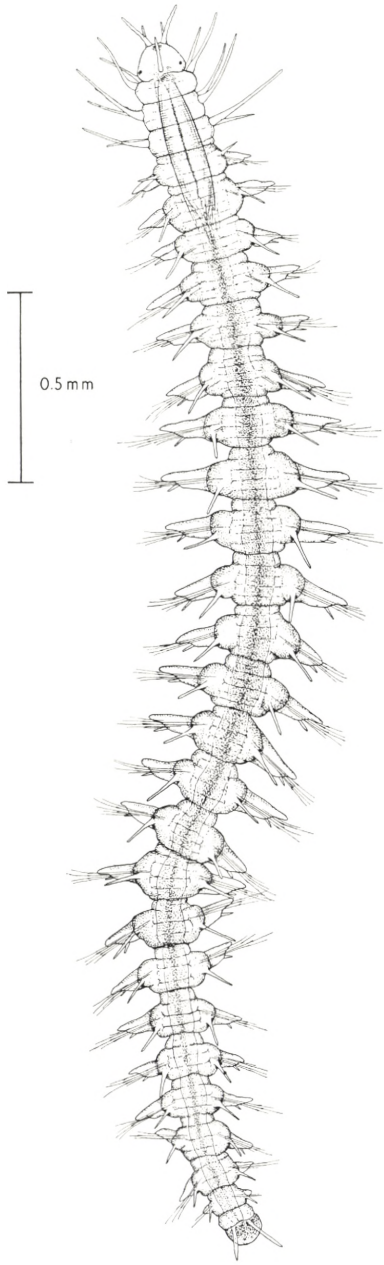

Fig. 14.

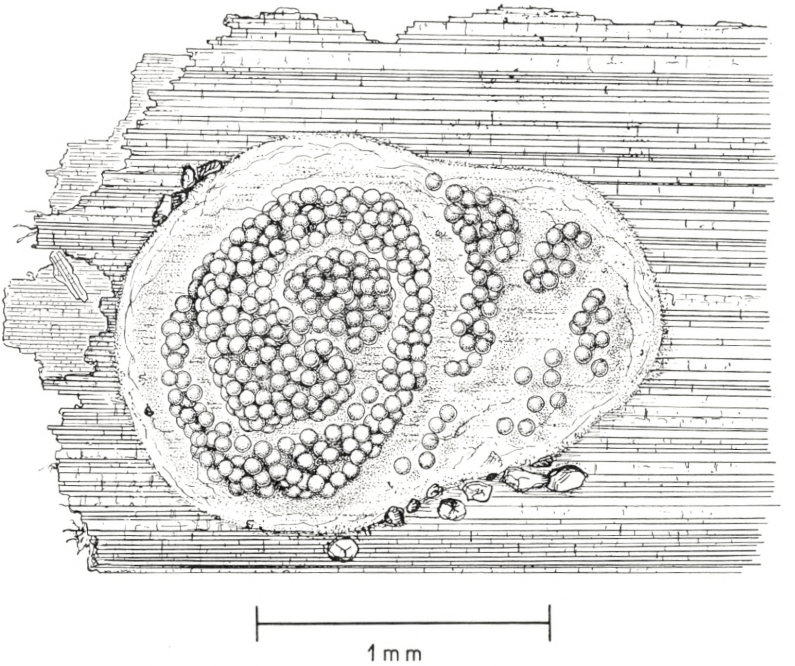

Fig. 15. *Microphthalmus sczelkowi* Mecznikow.
Egg-mass attached to a piece of a *Zostera* leaf.
From the Isefjord, Vellerup Vig, 3,5 m, muddy bottom; 13.3.43.

June, August, and October, thus in almost every season. After 1944 no specimens of this interesting polychaete worm could be found in spite of a thorough search for it. Animals with sexual products in their segments—both males and females—were collected in February, March, and June; all individuals from these collections were large specimens with 23 to 30 chaetigerous segments and from 3.7 to 4.0 mm in length. Most of the animals taken in the autumn were smaller, from 13 to 21 segments and without any traces of eggs or sperm. According to these observations the spawning time seems to occur early in the year. The colour of the mature adults, both males and females, is whitish, also the mature eggs are quite white. In the Danish individuals the eggs occur from the 13th to the 22nd chaetigerous segment.

Fig. 14. *Microphthalmus sczelkowi* Mecznikow.
Adult individual from Vellerup Vig, the Isefjord, 20.8.42.
The drawing was made after a living specimen.

A female with 26 chaetigers taken on February 8th 1942 off Frederikssund in the Roskildefjord was so full of large eggs that the intestine was seen only as a very thin line. When mature, both males and females have their usual shape and their normal supply of bristles.

In order to study the spawning and the larval development several attempts were made to "mate" ripe males and females in aquaria, but without results. In one case only a mature female, collected in Vellerup Vig 13.3.1943, discharged its eggs in the water. The eggs of this "spawning", however, did not seem to have been fertilized, since in a short time they all decayed. Most of the eggs were not laid freely in the water, but were shed surrounded by a clear and very sticky mucus of no definite shape. When just laid, the globular quite white eggs had a diameter of about 75 μ (fig. 16).

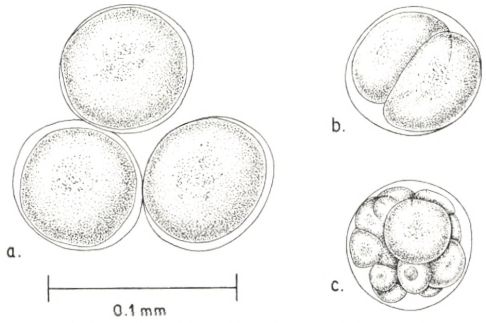

Fig. 16. *Microphthalmus sczelkowi* Mecznikow.
a. Undeveloped new-laid eggs.
b. Two-cell stage.
c. More advanced stage of cleavage. From Vellerup Vig, the Isefjord, 20.3.43.

In a sample from a depth of about 3.5 metres, together with many mature individuals used for the breeding experiments mentioned above, also an egg-mass was found, which no doubt had been spawned by a *M. sczelkowi* female (fig. 15). The shape was irregularly oval and it measured about 1.9 mm in length and about 1.4 mm in breadth. The surface was globularly vaulted. The mucus was quite clear and colourless and was very sticky so that small bottom particles adhered to its surface. The eggs, which in size and colour agreed with the eggs spawned by the above-mentioned female, were placed in an irregularly shaped coil. The number of eggs was about 3—400, but no exact counting was made. The egg-mass was found placed on a piece of a decayed *Zostera* leaf (fig. 15).

Shortly after the collection, the egg-mass began putrifying and no details concerning the development of the eggs were obtained. Thus the larval stages of this species are still unknown. The

small egg size, 75 μ, seems to suggest a development connected with a pelagic life.

No larvae, however, which with some reason could be referred to this species, have been found in the plankton of Vellerup Vig.

6. *Nereis pelagica* L.

Spawning has been observed by NORDGAARD (1912), who in August near Bergen in Norway found a pelagic *Heteronereis* specimen spawning its eggs; these were 190 μ across.

The larval development of this species is rather well-known. Thus HERPIN (1925) from the Channel described the spawning in December to January also by epitoquous individuals. The eggs are shed freely in the water, and the new-laid egg, still unsegmented, is 170 μ across. The larvae are hatched seven days after the fertilisation as quite non-pelagic animals only able to crawl on the bottom. The development of the young bottom stage is very slow; after 28 days only four chaetigerous segments are formed. In contrast to this description WILSON (1932) has followed up the whole development at Plymouth (in February) and has reared another type of larva. The egg, which is about 180 μ in diameter, is pelagic and also spawned by *Heteronereis* stages. The hatching takes place $3^1/_2$ days after the spawning. The larva has a typical pelagic stage lasting for about 18 days. This type of larva (WILSON, figs. 4—5) has three chaetigerous segments with ciliation, length about 400 to 450 μ. There are four brown eyes with patches of large brown pigment anterior and lateral to them.

From the plankton of the Sound THORSON (1946, fig. 29, pp. 64—65) during four years' investigations only obtained two larvae, which can be referred to *Nereis pelagica*. They are very similar to those described and figured by WILSON (*loc. cit.* fig. 6), but have no pelagic attributes at all. They were taken in June and November. THORSON concludes that *Nereis pelagica* in the Sound normally will develop in a non-pelagic way.

Mature epitoquous specimens of the same species were found by me in the Isefjord (Vellerup Vig) for the first time in 1953. During dredging on stony bottom on April the 2nd, seven—almost mature—individuals (3 males and 4 females) were collected in

their sandy tubes on stones and among the hapters of the seaweed: *Fucus serratus* and *Fucus vesiculosus*. Depth only one metre; temperature about 7° C.

The live males were from 5.0 to 6.0 cm in length and with a total number of chaetigerous segments ranging from 67 to 70. The body was divided into two distinct parts, the anterior and unchanged one consisting in all three males of 16 chaetigerous segments. The rest of the body, containing the sexual products, was shaped in the usual way found in the male epitoquous individuals: the parapodia with crenulated dorsal cirri and large lamella and with numerous fine swimming bristles. The primary colour of the living males was faint orange to yellowish-brown with an iridescent tinge and without distinct pigment cells. This characterization especially applies to the anterior part of the body. The epitoquous section had brighter colours, dorsally the foremost segments of this part had an almost dustlike dark-purplish pigmentation fading in intensity towards the pygidium, which had no special papillae except the usual two anal appendages. In the median line close above the dorsal blood vessel a very distinct dark-purplish pigment line was seen. The primary colour of the epitoquous section was more reddish than of the atoquous one and the whole surface had a light blue silky lustre. The parapodia were bright orange-red with the white sperm masses visible in the most thin-walled parts. The furrows between the parapodia were bright yellow. The ventral side of the epitoquous section had the same colour as the dorsal side, but was a little brighter. The eyes were large and of a bright purplish colour. The space between the hindmost pairs of eyes was formed as a white triangle. All three males were full of sperm.

The live females were 3.0, 6.5, 7.5, and 8.0 cm long and had 70, 68, 75, and 77 chaetigerous segments. The division of the body into two parts was only distinguishable in one individual (7.5 cm, 75 chaet. segments), which had an atoquous part consisting of 19 segments. Only this female had large eggs in the segments, the others having only small oocytes. The diameter of the eggs in this individual was 160 μ, thus smaller than the smallest egg-size described, Herpin (1925): 170 μ. The colour of the egg cell was light bluish-green and the yolk was very dense and opaque.

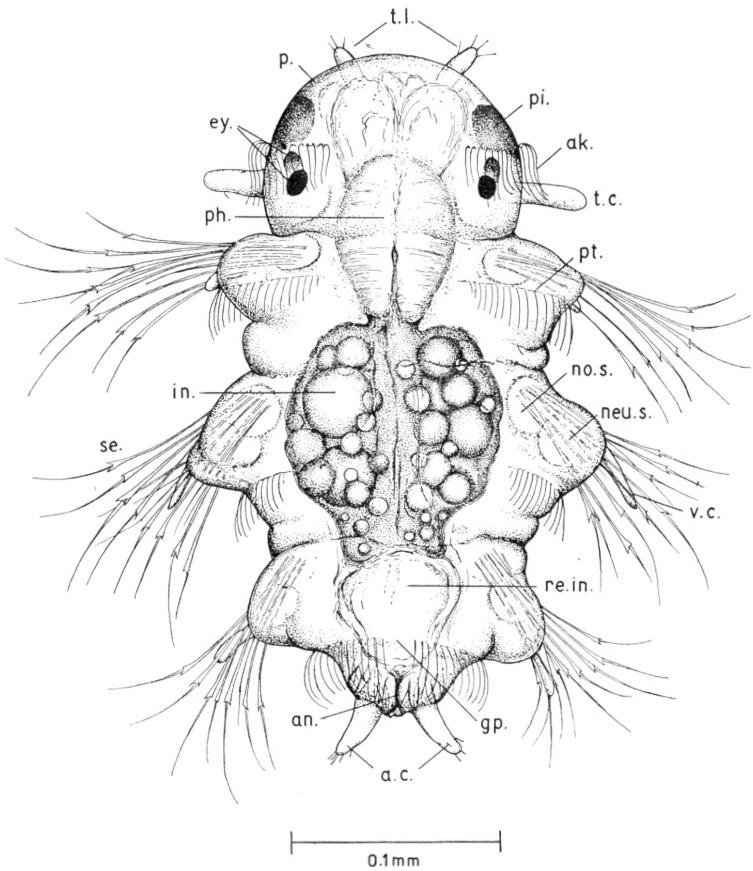

Fig. 17. *Nereis pelagica* L.
Nectochaeta, dorsal view; the Isefjord, Vellerup Vig, 13.5.44.
a. c., anal cirri with sensory hairs; ak., right akrotroch; an., anus; ey., rust-red eyes; gp., a slight gap separating the two hindmost paratrochs, no telotroch developed; in., intestine with a granular mass of different-sized oil globules; neu. s., neuropodial seta-sac; no. s., notopodial seta-sac; p., prostomium; ph., pharynx, still without visible paragnatha and jaws; pi., right rust-red pigment patch just before the eyes; pt., right paratroch of the first chaetigerous segment; re. in., rectal part of the intestine; se., bundle of the characteristic articulated setae; t. l., the developing first pair of tentacles with sensory hairs; t. c., the developing first pair of tentacular cirri; v. c., central cirrus of a chaetigerous segment (no. 2).

The primary colour of the above-mentioned female was much darker and more yellowish-brown than found in the males. The epitoquous section had a bluish-green hue owing to the content of eggs being visible through the skin. The parapodia in the posterior body segments were only a little changed and the cirri were not crenulated as in the male specimens.

The rather immature condition of the females collected, confirm that the *Heteronereis* of *N. pelagica* here described were not yet quite ready for spawning; an observation in agreement with the appearance in May of the pelagic larvae in the plankton (see text below).

The species is common in the area especially in sandy tubes on stony bottom with algae.

The results concerning the larval development of the species are based on plankton samples collected in 1944 and 1950 to 1951 and in 1953 in Vellerup Vig and in 1942 in the harbour of Copenhagen (the Sound). As to the samples 1950—1951 see p. 4.

The larvae from Vellerup Vig, all of the same type (fig. 17), were very similar to those described by WILSON. It was a Nectochaeta with three segments having noto- and neuropodial seta-sacs (no. s. and neu. s.). All setae were strongly built and of the characteristic articulated type well-known from *Nereis*-species. There was no prototroch, but distinct akrotrochs as mentioned by WILSON, forming "a row of cilia on each side" of the prostomium. The eyes and the pigment patches were rust-red and at the anterior end of the prostomium the first pair of tentacles with sensory hairs were visible. Also the first pair of tentacular cirri were developed. In the pharyngeal region no jaws were seen, the strongly built and muscular pharynx being the only detail marked out. The parapodia of all three body segments were not yet distinguishable, but had distinct ventral cirri and had paratrochs (pt.), the last one with a slight dorsal gap. There was no telotroch. The colour of the intestine was bright green, its contents being a granular mass with oil globules of different size. The anal cirri (a. c.) were well developed and had fine sensory hairs. The total length of the larva was about 350 μ. The larvae could swim as well as crawl. When swimming, the bristles, as also pointed out by WILSON (p. 207), are laid along the body with their tips directed backwards.

The larvae of *Nereis pelagica* were only taken in May. In 1944 larvae of this type were very common from 13.5 to about the beginning of June. In the eight month period 1950—51 with regular investigations only two larvae were collected on May the 6th. In 1953 no larvae were collected at all.

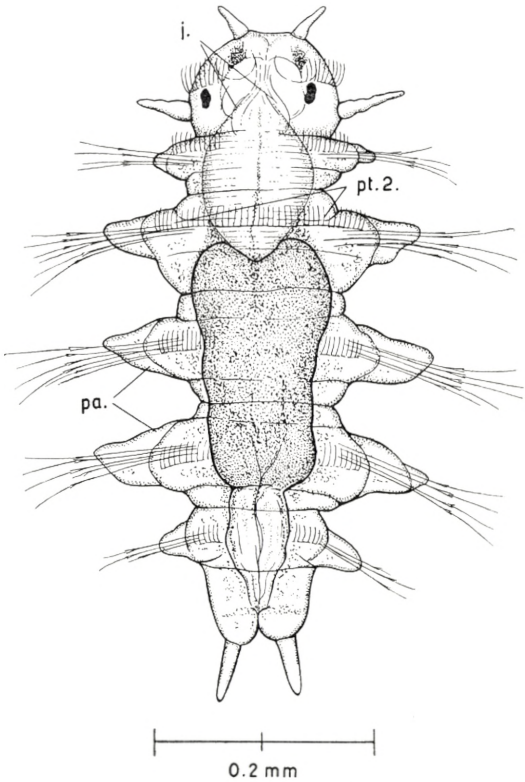

Fig. 18. *Nereis pelagica* L.
Large Nectochaeta in dorsal view, from the plankton of the South harbour of Copenhagen, the Sound, 13.10.42.
j., jaws; pa., well-developed parapodia still without any dorsal or ventral cirri; pt. 2, paratrochs of segment no. 2 united in one row of cilia.

On October 10th, 1942, a pelagic *Nereis*-larva, probably belonging to *N. pelagica*, was found in a horizontal plankton haul in the harbour of Copenhagen (southern part). The larva (fig. 18) was of about the same appearance as the larvae described and pictured by WILSON. However, it was interesting in being larger and still more pelagic than those hitherto known.

The total length was 650 μ and five chaetigerous segments were present. The prototroch was completely absent and only the akrotrochs were visible on the lateral parts of the prostomium. The four eyes and the pigment patches, which were small and pale, had the characteristic dark red colour. In front of the pharynx the first signs of the jaws could be seen (j.). The dorsal

parts of the body segments had distinct paratrochs, which, with the exception of the second (pt. 2.), had broad gaps. This larva, in comparison with the type from the Isefjord, is interesting in having well-marked parapodia, but without any dorsal and ventral cirri. No telotroch was visible. The middle portion of the intestine was very finely granular, but without any oilglobules at all, while the rectal part was quite clear and transparent. The intestinal colour was yellowish green.

Only one larva of this type was taken in the plankton sample. The presence of this individual in the south harbour of Copenhagen with its rather brackish water is peculiar, since the species has never been reported from this part of the Sound, neither as adult nor as larva. No doubt the larva has been transported to the place by currents from a more northerly locality in the area. The most interesting thing, however, is the fact that *N. pelagica* besides its probable non-pelagic mode of development in the Sound (see THORSON, 1946, p. 65) has a very distinct pelagic one of rather long duration.

Other observations from the Isefjord (1953) seem to confirm that the same species within the same small area may also have the direct non-pelagic development, as described by HERPIN.

On May the 3rd some samples of the uppermost fine mud-layer of the sandy bottom just outside the laboratory were taken. They were collected at depths of 30 to 100 cm. Normally the adult *N. pelagica* is never found there.

The samples contained large numbers of small *Nereis* individuals, which all apparently belonged to *N. pelagica*. The number of chaetigerous segments were from 5 to 7 and no animal had ciliated bands or other pelagic attributes. Anteriorly to the four eyes many of the small bottom stages had the characteristic brown-red pigment patches, which in other specimens had either totally disappeared or were present only on one side of the head. The intestine of all the individuals examined was bright green and the total colour was yellowish-red. Some of these bottom animals were reared in the laboratory and in May 1954 when having a length of 2 to 3 cm they could easily be determined as typical *N. pelagica*.

Plankton samples taken at the same place in April and May did not contain a single free-swimming *N. pelagica* larva. Thus the

only explanation of the occurrence of the bottom stages must be a non-pelagic development. No further observations of this mode of development were made.

Another very peculiar thing is the substratum of the small individuals. As emphasized above, the adults in the Isefjord are never found on open flats of pure sand, such as e. g. *Nereis diversicolor*, but always in sandy tubes attached to stones, shells or the hapters of seaweed and always in larger depths than the small worms.

Since the youngest stages of *N. pelagica* are found outside the natural biotope of the adults, it is a possible conclusion that the small individuals are unable to compete with the other *Nereis* species usually found there, especially a large and to *Nereis kerguelensis* McIntosh closely related species, (*N. southerni* Abdel-Moez and Humphries 1955). This species is now extremely common in the shallow sandy areas in the fjord and owing to its size (maximum length about 30 cm) and voracity it has, in the last few years displaced the normally very common *N. diversicolor* from this area, so that this species can only be found in the most brackish areas, where the low salinities prevent the large *Nereis*-species from being able to survive.

Another noteworthy thing as to the small *N. pelagica* is their free-living existence, an observation which was confirmed in aquaria. The specimens with 5—7 chaetigerous segments were very actively crawling through the fine sandy particles. The size and age of the small *N. pelagica*, when they will begin their tube-building, was not, however, observed.

Besides the above-mentioned species of *Nereis* two others are found in Vellerup Vig near the laboratory, *Platynereis dumerili* (Aud. and M. Edw.) and *Nereis* (*Neanthes*) *succinea* (Leuckart). The former is very common in tubes placed among different species of algae. The latter, which is fairly common among the mussels in shallow water, was found by me for the first time in 1953 and is hitherto unrecorded from Danish seas (see also the text above, p. 48). No results concerning the reproduction and larval development of these species are available from the Isefjord.

7. *Scolecolepis fuliginosa* (Claparède).

The reproduction and larval development of *Sc. fuliginosa* have been described by several authors.[1] Lo Bianco (1909) mentions the spawning season at Naples from October to April. Claparède and Mecznikow (1869) and McIntosh (1915) give short accounts of the egg-laying and early cleavage of the egg. The oval egg cells, which are about 120 μ in length, are laid in a common jelly of irregular shape and the yolk of each egg is surrounded by a reticulated and wrinkled egg-membrane. Crowding of males and females during the spawning has been observed by Claparède and Mecznikow. According to these authors the young larva has two red eyes and a long apical tuft of cilia. At eight days old the larva has six eyes and three chaetigerous segments with long provisional setae (cf. fig. 1 M, pl. XII Claparède and Mecznikow).

Day (1934), who describes the whole larval life history, gives the following details of the development: the larvae were reared from the egg (160 μ). When about 24 hours old the trochophores are 200 μ long and of a brown colour. The first bundles of larval setae, which project through the persistent egg-membrane, are developed two days after the fertilisation. At the age of eighteen days to three weeks the larvae, length 776 μ, have seven to nine chaetigerous segments and pairs of dense black pigment spots are present on the dorsal surfaces of the 3. to 6. chaetigerous segments. The larva with 14 segments, length 1000 μ, and from 34 to 36 days old, is the largest free-swimming stage of the species and normally metamorphosis will take place at this size and age. The larval description given by Day is supplemented with excellent drawings. Day and Wilson (1934) have studied the relations of the metamorphosing *Sc. fuliginosa* larvae to the substratum and have shown a distinct importance of the character of the bottom to the process of the metamorphosis. If the right type of bottom is absent, the larvae ready for settling are able to continue their pelagic life a week or more until the suitable

[1] In a paper received after the finishing of my manuscript L. Casanova (*Les annelides du plankton dans le golfe de Marseille*, pp. 29—36, *Recueil des travaux de la Station Marine d'Endoume*, fasc. 8, 1953) gives some details concerning the occurrence of the pelagic larvae of this species in the plankton of the gulf of Marseilles.

substratum is found. DAY (1937) mentions the fact that metamorphosis of the same species may take place at the swimming stage.

On August the 11th, 1941, during dredging in Vellerup Vig this spionid worm was taken by me for the first time in Danish seas. The species was found in mud at depths of about four metres only. Since then, it has proved to be extremely common in the innermost parts of the Isefjord area, especially in the shallow-watered sandy areas along the shores.

Mature specimens have only been collected in July and August; small and quite young larvae were also, however, taken in plankton samples from June, the species thus being a typically summer breeder (cf. fig. 1, p. 6). In spite of the frequency of the adults the pelagic larvae have only been collected in rather few individuals and only a few times. The present description is only based on these larvae; the spawning and the early development not being observed. Thus the material may be too insufficient and the morphological deviations found by me cannot give quite a correct picture of the actual conditions in connection with the spawning and habits of this species in the Isefjord area.

The early stages found, in June, with two to three chaetigerous segments have an appearance very close to the description given by DAY (cf. his fig. 5). The most characteristic feature noted by me was the presence round the larval body of the lobulated egg-membrane, easily seen, owing to the transparent space between it and the larval surface.

The larva with nine chaetigerous segments (fig. 19) is the oldest and largest stage hitherto found in my samples. It has a total length of about 500 μ. The body is slender and the prostomium is distinctly broader than the rest of the body and is "square in front" with a shoulder on each side bearing the swimming cilia of the prototroch as also pointed out by DAY. However, the front margin of the Danish larva has a greenish hue and on each side just before the shoulders there is a distinct spot of dark pigment. The number of eyes is not six placed on a transverse line across the head, but only five, of which the three, the most dorsal ones, are placed anteriorly to the two others. As in the English larvae the apical tuft of cilia consists of five short fine cilia. The cilia of the prototroch used for swimming are very long, especially in the lateral parts.

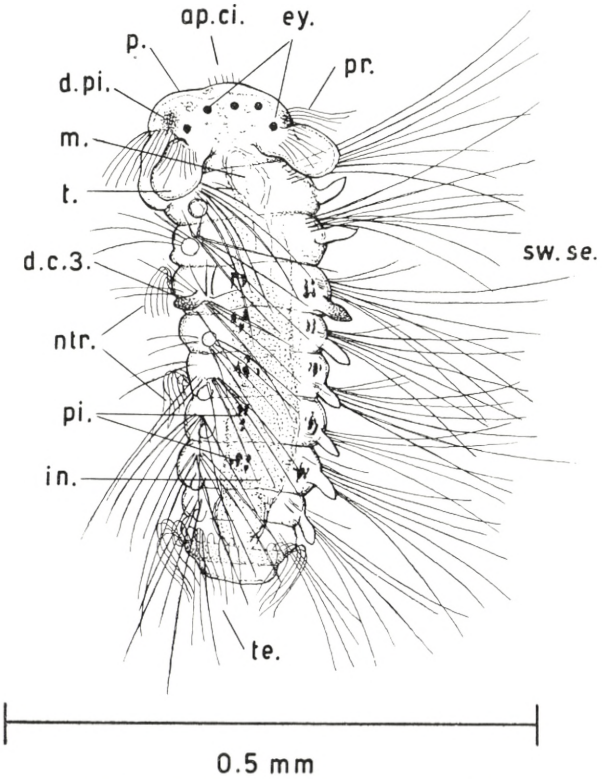

Fig. 19. *Scolecolepis fuliginosa* (Claparède).
Medium-sized larva, 9 chaetigerous segments, seen from its left side and partly from above; the Isefjord, from the plankton, Vellerup Vig, 15.8.41.
ap. ci., apical ciliation; d. c. 3, dorsal cirrus of chaetiger no. 3, with its characteristic purplish pigment; d. pi., dark pigment spots on both sides of the prostomium; ey., eyes; in., intestine; m., mouth visible through the rather transparent anterior part of the body; ntr., the neurotrochs, on chaetiger nos. 3 and 5; p., the broad and angular prostomium bearing "shoulders" on each side; pi., paired, black pigment spots on the dorsal surface of chaetiger nos. 3 to 7; pr., long cilia of the prototroch; t., lateral appendages of the prostomium, the developing tentacles; te., telotroch with a slight dorsal gap in the row of cilia; sw. se., long larval swimming setae.

The lateral appendages of the head, the palpi of Day, are in the Danish specimens peculiar in their placement on the head. They do not rise from the lateral parts of the prototroch ridges but from the dorsal section of the head behind the eyes, and may be considered as the developing tentacles (t.).

The segments of the body are furnished with the characteristic long provisional larval setae (sw. se.) from about 250 to 270 μ

in length, the longest in the first chaetigerous segments. Each segment is well marked off and the parapodia are rather distinct. However, only the dorsal rami of the parapodia are visible, the third one (d. c. 3) being very conspicuous owing to its purplish pigment, a character also emphasized by DAY (p. 643). In the English larvae the dorsal surfaces of the segments were furnished with pairs of dense black pigment spots from the third to the sixth chaetigerous segment. This pigmentation is also found on the larvae from the Isefjord, but with some differences. Each single spot consists of about four distinct black pigment dots and pigmentation is also found on the seventh segment. The dorsal bands of ciliation across the single segment were observed by me, but as their extent and placement could not be noticed with accuracy, I have chosen to omit this detail in my drawing. The neurotroch ciliation, however, was rather easily distinguished. Thus rows of cilia were seen on the ventral surfaces of the third and the fifth segment. The telotroch had very large cilia, about 100 μ in length and with a gap in the ciliation dorsally before the anus. The glandular cells with their papillae found by DAY on the pygidium were not visible on the Danish material. The mouth and the intestine can be seen dimly when the larva is viewed dorsally (cf. fig. 19).

In spite of several differences between the thoroughly described larvae of DAY and the type of larva from the Isefjord, I have nevertheless, although with some hesitation, referred my larva to *Scolecolepis fuliginosa*. The main deviations are: *smaller size, 500 μ as against 800 μ; dark pigment on the lateral parts of the head, number of eyes five, the placement of the palpi, the character of the pigmentation on the dorsal surfaces of the chaetigerous segments and the apparent absence of the glandular cells on the pygidium.*

The justification of my determination is to be searched in the following facts: the larvae from the Isefjord, apart from the deviations mentioned above, are in the main identical with larvae of DAY. Thus several structural details are common: *The pigmentation of the third parapodial ramus and the presence of the pigment on the dorsal part of the segments. Furthermore the large stages can be connected by transitional stages with the young Trochophora-stage with its attached egg-membrane very typical of Sc. fuliginosa.*

In the innermost part of the Isefjord proper, *Scolecolepis fuliginosa* seems to be the only species of this spionid genus, and other species of the same genus such as *Sc. ciliata* (Keferstein) are very rare on the whole in Danish seas.

The other spionid species in the Isefjord are: *Spio filicornis* (O. F. Müller), which in 1953 for the first time was found by me in the Vellerup Vig, where it is common now; *Pygospio elegans* Claparède, *Polydora ciliata* (Johnston), *Polydora quadrilobata* Jacobi, and *Polydora antennata* Claparède, this species also being hitherto unrecorded from Danish seas.

The reproduction and the development of *Spio filicornis* and *Pygospio elegans* are rather well-known, and the larvae of both species are of quite another type than the larva described above. They are partly developed on the basis of "nurse-eggs" (cf. Thorson, 1946, pp. 83—88).

The larvae of the *Polydora* species are so characteristic in comparison with the type described here, especially by their modified fifth chaetigerous segment, that they can also be excluded as possible parents of the larvae mentioned above.

8. *Heteromastus filiformis* (Claparède)?

According to Fauvel (1927) *H. filiformis* spawns from September to April; Linke (1939), however, has found spawning only early in the spring in Jadebusen. Smidt (1951, pp. 65—66) is the only one to describe and picture from the Danish waddensea planktonic larvae at various stages, which can be referred to this species. However, no direct observations of spawning and development of the eggs are available and his determination may still be a little doubtful. All his stages, which show great conformity with the larvae of another capitellid species, *Capitella capitata* (see p. 75), are dark and opaque and have ciliated bands on each segment. According to Smidt these larvae are lecithothrophic, e. g. they do not take any food from the plankton, but subsist on the yolk mass in their body. The largest one has about 9 segments and a total length of about 0.6 mm. The larvae were found most of the year in the plankton with maxima in the spring and the autumn.

In the innermost part of the Isefjord, where the species has been examined, adult individuals can be found commonly in practically all sorts of bottom material, from soft mud in the largest depths to sandy layers or more stony bottom in shallow water. It can also be collected in the *Mytilus* layer on the piers, but it seems to have its preference in the shallow areas consisting of pure fine sand.

As to the taxonomy of the collected mature specimens several very interesting deviations were found. According to FAUVEL (1927, p. 150) the genus *Heteromastus* among other things is characterized by the presence in the thoracal region of five segments bearing capillary setae. EISIG (1887, p. 839) and later on E. WESENBERG-LUND (1941, p. 38) state that juvenile specimens have four capillary segments only. In the Isefjord not only the new-settled individuals (see p. 72 below, fig. 23), but also any mature male or female—with a maximum length of five to six cm—have only four capillary segments!

A rather large number of adult animals of both sexes has been thoroughly examined and they all showed this character. In this connection it is noteworthy, however, that typical specimens of *H. filiformis*, in full agreement with the description given by FAUVEL, were found by me under the same ecological conditions in other Danish sea areas. Off the small island of Vorsø in the Horsensfjord (Eastern Jutland) I have found such individuals, which in every character were typical *H. filiformis*. Besides the absence of the last capillary segment other anatomical features were different in the Isefjord animals. Eyes should only be present on quite young bottom individuals and gills should be developed on the hindmost segments of the body of the adult. All mature animals from the Isefjord had distinct eyes, but very slight traces of gills, and their colour—owing to the red blood cells—was a brighter red than in the typical specimens from the Horsensfjord, which had a pronounced bluish-red colour.

Now, it is outside the scope of this paper to give a further description of the taxonomy of these two Danish populations of *Heteromastus filiformis*. The deviations described above are only mentioned here in order to give a sort of explanation of the two different types of pelagic larvae now described and pictured from the species in question (cf. SMIDT, 1951). From the facts

available it seems to me as if the diverging specimens from the Isefjord population and perhaps also the material from the Ringkøbingfjord (see E. Wesenberg-Lund, 1941), may belong to a special ecological race, or—as may be more probable—a species of its own. However, until a complete and detailed taxonomic description has been made, the results about the reproduction and larval development of the species in question will be published under the name of *H. filiformis*.

The breeding biology has been examined by me in 1944 and 1951 to 1953 in Vellerup Vig. In contrast with the observations of Thamdrup (1935) concerning *H. filiformis*, the Isefjord animals are not living freely in the bottom, but have permanent, vertical sandy tubes and form characteristic masses of excrement on the bottom surface just as *Arenicola marina*, an observation also made by Linke (1939).

The first mature males and females can be found long before the actual time of spawning, which occurs early in the spring dependent on the water temperature. Thus in January 1944 and 1953 many mature individuals were collected. In 1944 and 1951 the spawning itself, which as a rule seems to be finished within a short interval, took place in the very beginning of April at water temperatures of 6 to 7° C. (cf. fig. 1).

The eggs are laid on the surface of the bottom just above the aperture of the tube of the mother animal in globular jelly egg-masses, which are anchored in the bottom by a hollow thread of mucus (fig. 20). Through this appendage the mother animal will withdraw into its tube when spawning has stopped. The quite clear mucus of the egg-mass is stiff and elastic and very often its surface is covered with small bottom particles. The maximum diameter of the egg-mass is found to be about eight mm. In deeper water with a mud bottom, egg-masses of a more irregular shape are found. They often stick closely to the bottom particles.

In April 1953 I succeeded in following the spawning in detail during most of the breeding time. On April 2nd the first few egg-masses were observed in quite shallow water. The weather was then warm and the sea was calm and had a temperature of about 7° C. As the temperature in deeper water was rising and passing the level mentioned above, the rest of the population began

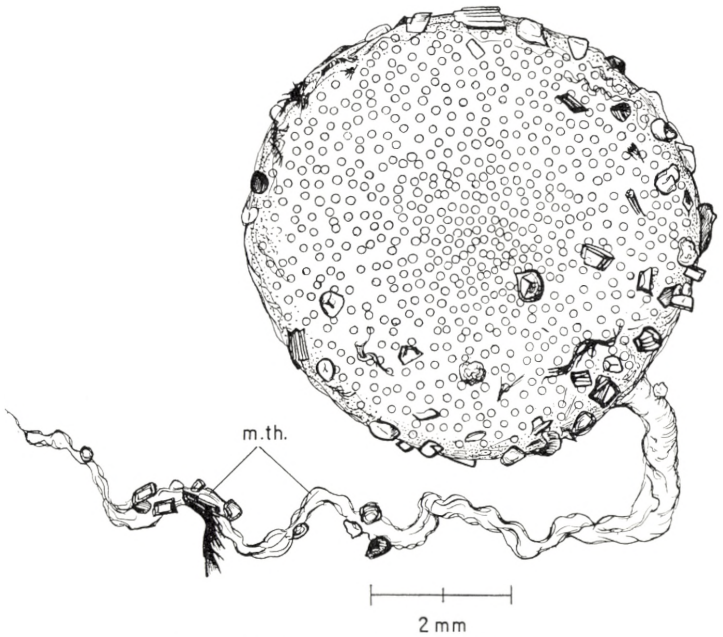

Fig. 20. *Heteromastus filiformis* (Claparède).
Egg-mass from the Isefjord, Vellerup Vig, 15.4.44.
The sticky surface with grains of sand and other bottom particles.
m. th., hollow thread of mucus, by which the egg-mass is anchored in the bottom by the spawning female.

spawning. When on April the 12th the spawning culminated, every type of bottom at any depth was covered with the characteristic yellowish egg-balloons. During the next few days the weather changed and became more windy. On this occasion it was very interesting to note how large a percentage of the egg-masses which was in danger of being destroyed by the sea. In the most exposed parts of the area all egg-masses attached were swept away by the waves and washed ashore. Without doubt the relatively short time from the spawning of the eggs to the hatching of the larvae is among the critical periods in the life of the species since the mature specimens of the area are spawning all at the same time. Thus a heavy gale just in this period may considerably reduce the number of eggs, i. e. the new generation of the year.

On the unsegmented new-laid egg the yolk is 110 μ across and is opaque with a faint yellowish-green colour (cf. LINKE, 1939, p. 316) and the egg membrane is not visible. Soon after

the spawning, however, the membrane and the polar bodies can be seen. The exact number of eggs per spawn was not counted, but a single egg-mass may contain several hundred eggs.

The segmentation and early development of this species have not been examined in detail. The young Trochophora seems to be ready for hatching two to three days after spawning (no measurements of the temperature). In aquaria the early pelagic larvae were reared from egg-masses laid here by adult specimens from the fjord and were found to be identical with larvae taken in the plankton from the fjord.

The young Trochophora (fig. 21) is from 140 to 155 μ long. It is very transparent and has a faint greenish colour. In the broad prototroch there are grass-green chromatophores. The cilia are long and placed at the uppermost edge of the band. The two distinct eyes with lenses are dark-red. From the mouth a narrow row of fine cilia runs along the ventral surface, the neurotroch. The apical tuft is well-developed with long and rather thick cilia. Most of the digestive system with the stomach and the oesophagus is visible owing to the transparency of the larva; the characteristic division of the intestine of the Metatrochophora can already be seen.

Metatrochophora II (fig. 22), about 300 μ in full length, has nine chaetigerous segments, most of them with separate notopodial and neuropodial sections (no. and neu.). In the dorsal and ventral part of the first three segments there are distinct capillary setae, while the rest of the setae are typical hooks. The single segments are mutually well demarcated and separated by the dissepiments, which in the figure can be seen especially between the intestine and the body wall (d.). This larval stage, which is very clear and transparent, is more yellowish than in the Trochophora. The grass-green chromatophores, now very conspicuous owing to their more dark-green colour, are present especially above the mouth and as irregular borders behind the prototroch and the telotroch.

The ciliation on the surface of the larval body forms four groups; the first and most anterior one is the apical tuft about 75 μ long, with a surrounding ring of short cilia (r. ci.). The next and most pronounced one is the muscular, thickened prototroch girdle with two rows of cilia. One anterior row consisting

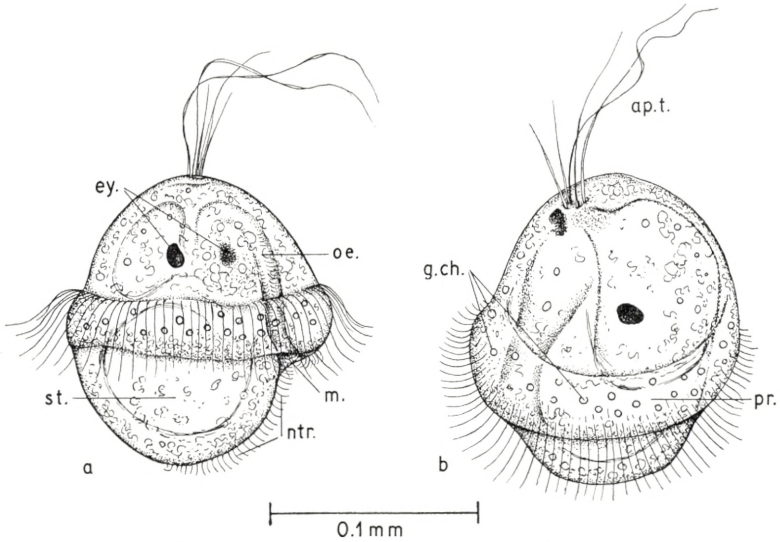

Fig. 21. *Heteromastus filiformis* (Claparède).
Two young trochophores a few hours after the hatching from the egg. Reared in aquaria from egg-masses taken in the Isefjord, Vellerup Vig, 22.4.44.
a. from the right side.
b. from the left side and partly from above.
ap. t., apical tuft of cilia; ey., eyes with lenses; g. ch., grass-green chromatophores in the prototroch girdle; m., mouth; ntr., ventral ciliary groove, the neurotroch; oe., oesophagus; pr., thickened prototroch girdle round the larval body with the long cilia fixed at its uppermost edge; st., larval stomach.

of long and strong cilia and another posterior row of short cilia stopping on each side of the mouth. The telotroch has only one unbroken line of long cilia. The fourth group comprises the cilia in the narrow central groove, the neurotroch, ending before the anus in a small tuft of longer cilia. During swimming the apical tuft is turned backwards along the body. There are no cilia on the segments as described by Smidt (1951).

Close behind each of the two round, dark-red eyes, which have lenses, there is a small group of oblong refracting cells, maybe mucus cells or a sort of glandular cells. Large refracting mucus cells are also observable on the anal protuberance close behind the telotroch.

The digestive system seems rather complicated in organization and is divided into distinct sections. The mouth (m.) is broad, about 40 μ, and densely ciliated and leads to a strongly built, ciliated oesophagus (oe.). On each side of the mouth aperture

there is an oblong ciliated sac, both of which might be the pharyngeal diverticles. The main part of the intestine comprises two large ciliated sections containing a few oil globules in their walls. The first one, behind the oesophagus, is the larval stomach, followed by a more opaque section, the intestine proper. There is a short thickwalled rectal part with the anus placed dorsally to a protuberance representing the anal cirrus of the adult (a. c.). The intestinal system has no coloration of its own, only the larger section is darker and more opaque.

Older Metatrochophora. The largest pelagic stage of the species collected in the plankton of Vellerup Vig has about eleven chaetigerous segments and a maximum length of about 400 μ. This stage was still very transparent, but the colour has changed to a more reddish hue. The green chromatophores have completely disappeared. The total shape is now more longish and not so conical as in the previous stage. The ciliation is just the same as before; however, the apical tuft looks shorter. The eyes are darker, almost black. The protuberance of the posterior end is longer and more separated from the anal segment. The movements of the larva are rather slow. A close examination of the plankton samples showed no older stages and the stage described above seems to be the last free-swimming stage before settling (cf. text below).

In the period 1950—51, when the quantitative plankton samples were taken in Vellerup Vig (see p. 4), larvae of all the types mentioned and pictured above were recorded in the fjord plankton on April 22nd, in two hauls, 13 specimens only

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Fig. 22. *Heteromastus filiformis* (Claparède).
Metatrochophora II, right-side view, the Isefjord, Vellerup Vig, 2.5.45.
an., dorsally situated anus; a. c., developing anal cirrus; ap. t., apical tuft of cilia; c. se., notopodial capillary setae of the first three chaetigerous segments; d., dissepiments between the segments; ey., eyes with lenses; g. ch., grass-green chromatophores; h., notopodial and neuropodial hooks of the hindmost chaetigerous segments; in., intestine; l. ci., tuft of long cilia at the posterior end of the neurotroch and just before the anus; m., mouth aperture; m. c., mucus cells; neu., neuropodial section of a chaetigerous segment; no., notopodial section of a chaet. segment; ntr., neurotroch; o. c., group of large, oblong cells close behind the eyes; oe., strong thick-walled and ciliated oesophagus, the posterior part with a narrowing; o. g., oil globules in the wall of the intestine; ph. d., ciliated pharyngeal diverticle (?); pr., muscular prototroch girdle with two types of cilia, the long anterior and the shorter posterior; r. ci., ring of short cilia round the apical tuft; re. in., short rectal part of the intestine; st., larval stomach w. ciliation st. ci. close to the opening between the oesophagus and the stomach; te., telotroch.

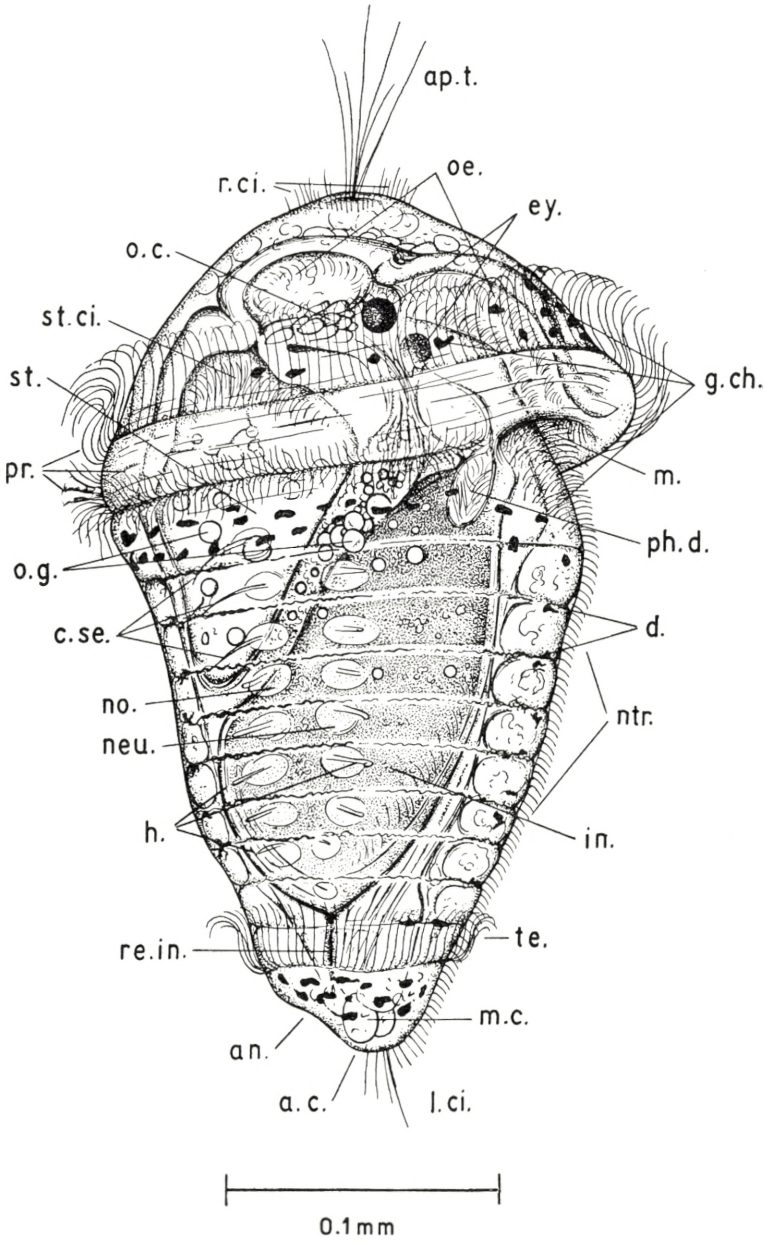
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Fig. 22.

trochophores and on May 6th, in one haul 23 old metatrochophores. Samples from May 27th did not contain a single larva, and the plankton occurrence in April—May seems to be the only and normal one of the year (see under the spawning p. 66). As to the duration of the free-swimming stages, about three weeks to one month will be the normal range, but the water temperature may again play an important rôle.

The young, newly metamorphosed bottom stage (fig. 23). In May 1953 a number of the largest pelagic larvae of the type described above were collected in the fjord plankton and were placed in vessels with fjord water and containing a thin layer of fine sand. Within a few days most of the larvae had metamorphosed.

The animal just settled has a total length ranging from 535 μ to 640 μ, of which the anal cirrus makes up the 85—90 μ. The full number of segments comprises thirteen. The opaque and granular prostomium, which at the foremost edge has sensory hairs (s. h.), is rounded conically and has two small palps on the ventral surface. These are post-larval organs and are not present on the larger bottom stages. The eyes, which persist in the adults, are now densely black and have no visible lenses. In the next segment, the achaetous mouth segment, the mouth aperture is situated at the foremost edge. When closed the mouth is about 40 μ broad: that is exactly the same as in Metatrochophora II.

The following ten segments the chaetigerous segments constitute the completely finished thoracal section of the adult individual. In comparison with the young bottom stages of other capitellid worms, cf. *C. capitata* (Eisig 1887 and 1899) and *Notomastus latericeus* (Wilson, 1933), this species is remarkable in having from the beginning the whole number of thoracal

Fig. 23. *Heteromastus filiformis* (Claparède).
Young metamorphosed bottom stage in right-side view and partly from below. Metamorphosed in aquaria, 17.5.53. Taken as Metatrochophora II in the plankton, Vellerup Vig, the Isefjord.
a. c., anal cirrus with blood vessel closed distally; a. v., anastomosing blood vessels; b. c., red blood corpuscles; d., dissepiments; d. c. se., bundles of dorsal capillary setae; d. h., dorsal hooks; d. v. l., dorsal longitudinal blood vessel; ey., eyes; m., mouth; m. s., mouth segment; o. g., oil globules in the foremost part of the intestine; p., prostomium; ph., muscular, protrusible pharynx; pl., palps; s. h., sensory hairs in front of the prostomium; v. se., bundles of ventral setae; v. l. v., ventral longitudinal blood vessel.

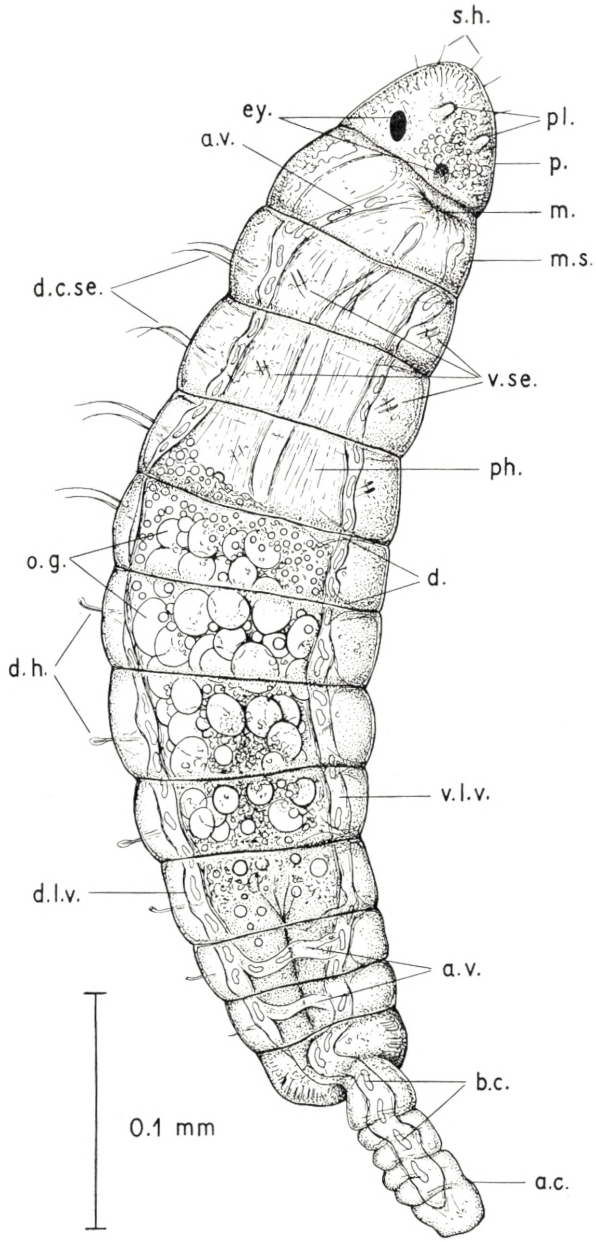

Fig. 23.

segments, eleven in all and not twelve as there should be in the genus *Heteromastus* according to FAUVEL (1927, p. 150). The first four chaetigerous segments have 1—2 long, curved capillary setae in their dorsal parts (d. c. se.); the ventral setae are a little shorter. The following six segments have only hooks and only one in each group (in the figure only the dorsal hooks are visible, d. h.). The last segment is the pygidium with the single, finely wrinkled anal cirrus, with the dorsally placed anus.

The vascular system is remarkably well-developed and longitudinal vessels, dorsal and ventral, are visible even at small magnifications. Round the anterior part of the pharynx and in the last few segments fine anastomoses connecting the dorsal and ventral vessels can be seen. In the anal cirrus there is a well-marked, distally closed vessel. Everywhere in the vascular system blood corpuscles (b. c.), faintly red, can be seen.

The intestinal duct has two main sections. The foremost part is the very thick-walled, protrusible pharynx, which is about 140 μ long and extends to the fifth chaetig. segment. The rest of the intestine is very opaque owing to its contents of often large oil globules. The intestinal colour is reddish brown.

The whole animal is quite transparent and of a faint reddish hue. Dissepiments between the segments are present.

The movements of the very contractile bottom stage are rather quick and very much similar to those of a common earthworm.

9. *Capitella capitata* (Fabr.).

The reproduction and larval development of this species is well known. The following authors: EISIG (1887 and 1899), CLAPARÈDE and MECZNIKOW (1869), LESCHKE (1903), HOFKER (1930), DAY (1937), THORSON (1946), and SMIDT (1951) give almost identical, illustrated accounts of the breeding habits, which, in contrast to what is found for this species in the Isefjord include a full pelagic stage in its development. The details known may be summarized as follows:

The new-laid eggs are found in the tube of the mother animal, which protects the brood until the larvae hatch 10 to 14 days later.

The young larva, which is provided with distinct ciliary bands, both prototroch and telotroch, has from 12 to 13 chaetigerous segments. The first three segments have capillary bristles, while the rest only carry hooks in their bundles of setae. There are two distinct red eyes in older larvae. The mouth and the intestine are well developed. The colour of the intestine, which according to DAY contains no food until after the metamorphosis, is most varying, from greyish to olive-green or navy-blue.

After a free-swimming stage of seven days, the pelagic attributes disappear and the larva goes to the bottom metamorphosed as a fully equipped bottom stage, having the same number of chaetigerous segments as the plankton larva. The size of the plankton stage is about 400 to 500 μ.

In the Isefjord area *Capitella capitata* is a wide-spread, but not very common species. Regularly only a few specimens can be found, even if thoroughly searched for, and only very seldom, e. g. in the Roskildefjord near Frederikssund, it occurs in considerable numbers.

The species has been taken in all months of the year. Mature specimens with eggs or larvae in their sandy tubes were only recorded in two periods: from April to May and from October to November, but there seems to be some evidence of a spawning also in the intermediate summer months. Individuals examined from January to March contained no eggs or larvae in the tubes. Since my observations of the extension of the breeding time of *C. capitata* are so fragmentary, I have found it natural to omit the species in fig. 1.

As other marine invertebrates in the Isefjord fauna, this species is remarkable by having two modes of larval development and even at the same time of the year. As described in the literature (see above) it has free-swimming larvae with a short pelagic stage and another and—as far as I can see, not previously known —nonpelagic, direct development.

The pelagic larvae, which were taken in the plankton in Vellerup Vig, correspond very closely to the description given by several authors.

They were collected in May 1944 and 1945, very few in number in horizontal hauls. They had a length of 500 to 600 μ and had always 13 chaetigerous segments in all, the first three

of which carried capillary setae. The rest of the segments were only supplied with hooks. There were two dark red eyes on the conical head and a distinct prototroch and telotroch. The colour of the larval body was always faintly yellowish and almost transparent. The intestine was dark with greenish contents and the foregut in the foremost three segments was coiled. The larvae were very fast swimming and could assume any shape owing to the great elasticity of the body. One larva taken in the plankton on May the 14th, 1944, metamorphosed 60 hours after being collected. The temperature in this period was constantly 9° C.

Adult female individuals with eggs or embryos in their tubes were collected by me in October—November 1949 and in April—May 1954. The material from the autumn gave no evidence of a deviation in development from that hitherto known, since the embryos were too young. They were placed in a mucus string built as a transparent tube in the walls of which the embryos were lying close together. Thus the sandy tube of the female was as a case round the egg-string.

The material of *C. capitata* tubes from May 1954 gave the proof of a completely non-pelagic development with individuals going directly from the mother tube as fully equipped animals to the bottom life. One quite intact egg-string from 16.5.1954 contained individuals with a length ranging from 1100 to 1200 μ. Most of the specimens dissected out of this egg-string were of the same stage of development while very few represented an earlier stage. Most of the young animals had 13 chaetigerous segments and only a single one comprised 14 segments with setae.

Any individual removed from the mucus was a typical bottom stage without any pelagic attributes at all and crawled away from the tube. Moreover, they all agreed with the type of the young bottom stage hitherto described; thus the first three segments besides hooks in their dorsal parts also had capillary setae and the following segments contained hooks only. The main colour of the body was more whitish than yellowish as recorded about the pelagic larvae from the same locality (May 1944 and 1945). There was one very conspicuous feature which furthermore characterized the young bottom stage. The intestine in all the specimens was opaque and filled with a dense yolk

mass, which began in the fifth chaetigerous segment and stretched through the whole rest of the body to the anus.

The presence of this yolk mass seems to me to explain the non-pelagic development of *Capitella capitata*. Probably the egg-size and with that the yolk volume have been larger than usually found. However, a development connected with nurse-eggs cannot be excluded as a possibility. In this connection it is worth while calling attention to the extraordinarily large size of the bottom stage when leaving the egg-string, maximally more than 1200 μ or exactly twice the size of the bottom stage developed from pelagic larvae.

As mentioned above, a double mode of larval development is also known from other marine invertebrates of the Isefjord area. Within the polychaetes, up to now 14 species out of the 45 species known from the innermost part of the Isefjord have been examined by me as to breeding biology. Three of these species have both a development by pelagic larvae and a direct one without a free-swimming stage at all. The three species are: *Nereis pelagica* L. (described in this paper p. 53–59), *Pygospio elegans* Claparède, the non-pelagic mode of which is based on nurse-eggs; both modes occur simultaneously within the same egg-string (the investigations of this species have not yet been finished), and finally *Capitella capitata* Fabr. just described.

Within the Gastropoda nine species have so far been examined out of the 41 species recorded from the Isefjord (See E. Rasmussen, 1944 and 1951). Of the nine three in all have the pelagic as well as the non-pelagic development, viz: the two prosobranchs, *Rissoa membranacea* Adams (not published) and *Brachystomia rissoides* (Hanl.) and the nudibranch, *Embletonia pallida* A. and H. (cf. Rasmussen, 1944 and 1951). This rather high percentage of invertebrate species, which in the same area have two quite different modes of development is extremely interesting.

10. *Metatrochophora* sp.

On May the 13th, 1944, several young metatrochophores were collected in plankton samples from the Vellerup Vig.

The species represented by these free-swimming and typic-

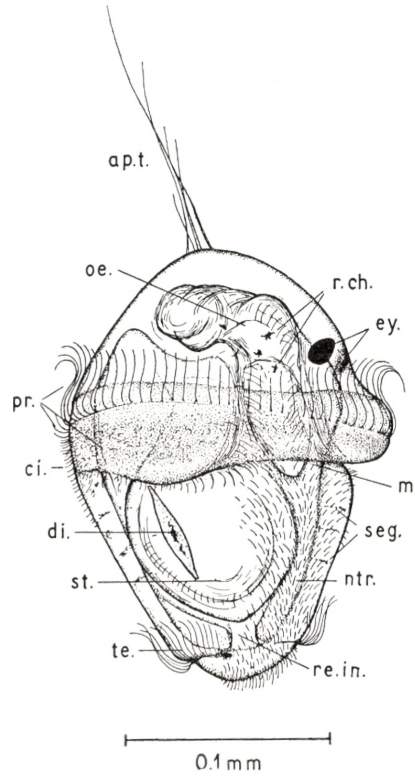

Fig. 24. *Metatrochophora* I sp.
The Isefjord, Vellerup Vig, 13.5.44.
ap. t., apical tuft of long cilia; ci., a dense cover of fine cilia on the prototroch girdle, only visible at the contours; di., diatom in the stomach; ey., dark red eyes, no lenses visible; m., mouth aperture beneath the prototroch girdle; ntr., neurotroch; oe., oesophagus; pr., broad prototroch girdle, rather opaque and with one row of long anterior cilia and short posterior cilia; r. ch., red chromatophores; re. in., rectal part of the intestine; seg., slight traces of a developing segmentation; st., larval stomach; te., telotroch with a ventral gap between the cilia.

ally pelagic larvae, is either rare in the area or the larvae may have been transported by currents from elsewhere, as the larvae in question have only been taken on this single occasion.

The total length of the larva (fig. 24), without the apical tuft of cilia, was about 200 μ. The colour was slightly yellow with a few small red chromatophores behind the large dark-red eyes. The prototroch formed a complete ring of long cilia arranged in a single row anteriorly in the broad, densely ciliated girdle round the body. The ciliation on the larval surface was limited to a

longitudinal groove, the neurotroch running from the mouth aperture to the anus on the hindmost tip of the animal. The telotroch was not a complete ring, for a slight ventral gap was present where the longitudinal ciliation was passing through. The segmentation of the body was very incomplete and no setae were visible. The apical tuft of cilia was very long, about 140 μ, consisting of a few stout cilia. The oval mouth had a largest diameter of about 60 μ and was followed by a ciliated oesophagus leading into the larval stomach. The whole intestine was rather thick-walled and had a dense ciliation. The rectal part of it was very short.

The identification to species of this larval type when no other stages are available is of course very difficult, but there might be some reason to refer it to the capitellidae, owing to various typical details in its structure. For instance the ciliation, the apical tuft and the neurotroch, cf. the description given above of the larval stages of *Heteromastus filiformis*; cf. also WILSON (1933), who describes the development and larval stages of another capitellid sp., *Notomastus latericeus* Sars.

Summary.

An account is given of the occurrence, reproduction and larval development of 10 polychaete annelids from the Isefjord (Zealand, Danmark).

The species are: *Harmothoë imbricata* (L.), *Harmothoë impar* Johnston, *Eteone longa* (Fabr.), *Magalia perarmata* Marion and Bobretsky, *Microphthalmus sczelkowi* Mecznikow, *Nereis pelagica* L., *Scolecolepis fuliginosa* (Claparède), *Heteromastus filiformis*? (Claparède), *Capitella capitata* (Fabr.), and Metatrochophora I of an unidentifiable species.

The temperature is shown to be the main factor initiating spawning, (cf. fig. 1).

H. imbricata. The larval stages, which are truly pelagic, including Trochophora, metatrochophores I and II (figs. 3—5) and the young newly metamorphosed bottom stage, are described (fig. 6). It is shown that the pelagic larvae dominate the zooplankton in January. Metamorphosis normally occurs in midwater and younger adults live a semi-pelagic life.

H. impar. The pelagic larvae—Metatrochophora II is described —and the young bottom stage (figs. 7—9) rather much resemble those of *H. imbricata.* It is pointed out that the pigmentation is subject to so much variation within the single species that its value as a systematic character is doubtful.

E. longa. Metatrochophores I and II, the Nectochaeta and the young bottom stage are described and pictured (figs. 10—12). Variations in the types of larvae in comparison with older descriptions are also given.

M. perarmata. A species new to the Danish fauna. The results are very scanty and the larval development is not described (fig. 13). In connection with the description of the breeding habits a brief discussion of the zoogeographic composition of the polychaete fauna of the Isefjord is found. Out of 45 recorded species 12 constitute a faunal element, the main distribution of which is south of the Scandinavian seas. Out of these 12, 8 are hitherto unrecorded in Danish seas.

The possible relation of these findings to climatic changes in our seas is briefly touched on.

M. sczelkowi. The spawning, the egg-mass and the egg-size are described (figs. 14—16). No observations on the larval stages are available.

N. pelagica. Epitoquous males and females were found. A description of these stages is given, based on live specimens. Planktonic larvae (Nectochaeta, fig. 17) were collected in the Isefjord and in the harbour of Copenhagen (the Sound); the latter represents a type indicating a pelagic stage of far longer duration than hitherto known (fig. 18). Evidence is brought forward to show that also a totally non-pelagic mode of development of *N. pelagica* is found within the same small area in the Isefjord. A short notice concerning the *Nereis* species of Vellerup Vig (vicinity of the laboratory) is given (p. 59).

Sc. fuliginosa. Pelagic larvae from the plankton in Vellerup Vig are described and pictured (fig. 19), and some deviations from former descriptions are pointed out. On p. 64 the other spionid species hitherto recorded in the Isefjord are mentioned and their larval development briefly compared with that of *Sc. fuliginosa.*

H. filiformis? The results obtained include the whole devel-

opment with descriptions of egg-laying, egg-mass, pelagic stages comprising Trochophora and Metatrochophora II, and the newly settled bottom stage (figs. 20—23). However, the determination as to species of the adults is doubtful owing to some important differences, which may indicate a new and undescribed capitellid species.

C. capitata. In the Isefjord the species has two modes of larval development even in the same period of the year. One —as known from the literature—with free-swimming larvae, and another, non-pelagic, direct development. The presence of this double mode of reproduction is discussed (p. 77) and a comparison is made with another invertebrate group, the Gastropoda, where similar conditions have been found in the Isefjord.

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BY

JOHS. BOYE PETERSEN and J. BENTH HANSEN

København 1956
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Synopsis.

The scales of a number of Synura species were examined by means of electron microscopy and thoroughly described and figured. Two main groups of species could be distinguished. One group includes S. Petersenii and S. glabra, the other S. uvella, S. echinulata, and S. spinosa. The last-named species occurred in different forms, which may represent a corresponding number of species, viz. f. spinosa, f. mollispina, f. longispina and f. Nygaardii, all of them new forms.

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In 1918 Boye Petersen called attention to the fact that the covering of the Synura cell is built up of scales which in a screw position make up a tight armour. Boye Petersen gave the name of *Synura uvella* to the species he had examined, the only species which up to then had been fairly well defined. Several authors (Lemmermann 1903, Pascher 1914) had attempted to establish new species of the genus on the basis of the length of the spines, as well as from a certain mesh pattern on the surface, and from the shape of the cell. No doubt the first two criteria may indicate differences of species, but the shape of the cell is of no value whatever as a criterion, as already pointed out by Boye Petersen in 1918, owing to the great variability of the cell shape. It will probably for ever remain an unsolved problem with what species the authors have been confronted, as there is no possibility of finding the original material from which electron microscopy of the scales can be made. On the other hand, *S. Adamsii* G. M. Smith (1924) and *S. australiensis* Playfair (1915) seem to be so adequately characterized that they are easily recognized. Other species have been established by Kisselew (1931); these, however, are not provided with any information about the scales and, therefore, can hardly be recognized without careful examination of the original material.

Korshikov (1929) made a thorough examination of the scales of a number of Synura specimens, which led him to the discovery that the scales have so many different patterns that he was able to establish a number of new species from the appearance of the scales. Above all he made it clear that Boye Petersen's Synura could hardly be identical with *Synura uvella* Ehrb. The latter species, especially in Stein's well-known picture (1878) is remarkable for strongly developed spines on the anterior portion

of the cell. Besides, however, he found a Synura species the scales of which were in complete accordance with those of Boye Petersen's species, for which reason he termed it *S. Petersenii*. Apart from that he found several other species with very characteristic scales. BIORET (1933) worked on the same lines without knowing about Korshikov's work. Like the latter, he found several types of scales; however, he did not establish any new species, assuming that the scales of *S. uvella* were just highly variable. Quite obviously he has not seen *S. Petersenii*. Finally, CONRAD, in a posthumous paper (1946), tried to carry on Korshikov's work; but he did not arrive at a satisfactory grouping of the species before his death. HUBER-PESTALOZZI (1941) collected all essential facts known about Synura species and their scales. He carefully gathered biological information about *S. uvella*; as this information, however, is based on the statements of the different authors, and as, moreover, these authors beyond doubt used the term *S. uvella* about all the species they observed, it is fair to believe that the biological data may apply equally to *S. uvella* and to other species. Consequently, along with investigations into the biological relations of the species, it will be necessary to start afresh for a correct definition of the existing species. It should be noted that KORSHIKOV (1941) described a most characteristic species, *S. splendida*. SCHILLER (1929) mentions two Synura-like organisms by the genus name of *Synuropsis*. As for these two species, there are no indications to show whether they have a covering built up of scales. About one of the species, *S. globosa*, it is stated that there are short bristles on the surface of the cell, whereas the other species (*S. danubiensis*) is mentioned as being completely smooth. Especially in the case of *S. globosa* it will be natural to ascertain whether the bristles in question are not in fact fixed on a scale armour. KISSELEW (1931) mentions some Synura species, one of which is identified as *Synura uvella*, the other as *S. reticulata* Lemm. As for the latter, it seems likely to believe that the species is *S. spinosa* Korsh.; and the former, which is figured and mentioned as having a completely smooth cell surface, can at least not be *S. uvella*. These species, accordingly, should be more closely examined.

It may be useful to realize fully which of the characters

hitherto used to distinguish the different species are in fact reliable as specific characters. As a main criterion, KORSHIKOV (1929, p. 287) among other things mentions the arrangement of scales, according as they are lying longitudinally or obliquely. To this may be objected that these relations are very difficult to determine, even in the exsiccated material, because the scales are, as a rule, more or less displaced in the exsiccation process. Besides, it is a fact that even if the scales are placed in a screw position, as may be seen in spruce cones, it is still possible to distinguish longitudinal rows; and at least it will be possible to observe oblique rows, even if the scales are arranged in circles. This last arrangement never seems to occur. Wherever we have been able to distinguish the arrangement of the scales, oblique lines have been discernible in all species. The cellular shape is used by several authors as a systematic criterion; as it has already been emphasized, this is highly objectionable because all Synura species are characterized by the great variability of the cellular shape. Within the same species, there are few-celled colonies with nearly spherical cells and large, many-celled colonies with long-stalked cells (see also KORSHIKOV 1929, p. 285; I. MANTON 1955, p. 310, and CONRAD 1946, p. 2). What makes all these variations possible, is among other things the very fact that the covering is built up of imbricated scales of a certain mutual movability. This, in the end, leads to the conclusion that the best specific characters are obtained from the structure of the scales. For this reason we have, by electron microscopy, examined the scales of the different Synura forms available. We have adopted the simple method of drying up the Synura cells direct on the formvar film. By this the covering of most cells will decompose more or less into its single scales, which, however, often retain part of their original arrangement. If the material is preserved in formalin, it pays to dry up a drop on a cover slip, and after that to heat it on a platinum or iron sheet. In this process the formalin evaporates completely, and superfluous organic parts are eliminated. After this the material may be transferred in the usual way with collodion to the formvar film. However, it seems as if this treatment makes the scales so brittle that they are easily crushed. As a rule the preparations have been shadowcast with palladium and then electron micrographed at different magni-

fications. These micrographs show a very different and highly characteristic structure in the single species. By shadowcasting the preparations a clear impression is obtained of the spatial relations in connection with the structure of the scales. For further confirmation of the structures observed we have prepared stereoscopic pictures with the electron microscope, and the structural relations thus observed have proved to be quite in agreement with those previously assumed.

Most of the species examined were identified, with comparative certainty, with Korshikov's species. Some species, however, presented difficulties. On the other hand, as original material will hardly ever be available, it is fair to regard our determinations and descriptions as emendatory descriptions of these species, and in future it will be necessary to make use of e. m. micrographs of the scales in order to determine the species with adequate correctness.

The genus Synura may be divided, according to the structure of the scales, into two sections:

I. Petersenianae, the scales of which have a hollow ridge in the middle, and no spine or, if any, a very short one.

II. Spinosae, without a hollow ridge in the middle, and the scales of which have a more or less strongly developed, hollow spine attached near the tip of the scale.

Section I Petersenianae.

Synura Petersenii Korsh.

Korshikov 1929, p. 283, pl. 11, figs. 54—58.
Synura uvella Boye Petersen 1918, p. 345, pl. V, figs. 1—9.
Synura caroliniana Whitford 1943, p. 159, figs. 6, 7.
Plate I, figs. a—c. Text, figs. 1, 2, 3.

The species was first mentioned by Boye Petersen (1918) by the name of *S. uvella*, which was natural as it was the only one fairly well-defined at the time. Later on, Korshikov (l. c.) succeeded in ascertaining that it could not be identified with *S. uvella*, as he found a different species far more in accordance with Stein's description and figure of this species. Whitford

(1943) has described *S. caroliniana*, an American species characterized above all by very long-stalked cells, and by very short thorns on the apical part of the cell. The scales of this species were presented by MANTON (1955) in e. m. photos from specimens selected from a culture made by WHITFORD. These scales are in perfect accordance with the scales of *S. Petersenii*; and as the other details of the description reveal a corresponding identity with the latter species, we do not hesitate to merge the two species into one. In consequence, the species must bear the older name, that of *S. Petersenii*. The scales of *S. Petersenii* are imbricated, and lie in a screw position like the scales of a spruce cone. The dimensions of the scales may vary a little. By measurings with the light microscope, KORSHIKOV (1929) found length ca. 4 μ, breadth ca. 2μ. In *S. caroliniana* — in e. m. photos by MANTON (1955, pl. V) — was found l. 3.2—3.8 μ, br. 1.9 μ. In our own pictures, in apical scales, we have found l. 4.7 μ, br. 2.3 μ. On the whole, the different values observed agree fairly well; and as the variation within the same individual is far from being negligible, and it cannot be decided with absolute certainty to what part of the covering the measured scales belong, the degree of accordance must seem satisfactory.

Fig. 1. *Synura Petersenii* Korsh. Border of cell showing the serrate outline. ×14.400.

In a live cell of *S. Petersenii*, along the edge, is seen a fairly distinct serrulation (fig. 1). In his paper of 1918, BOYE PETERSEN

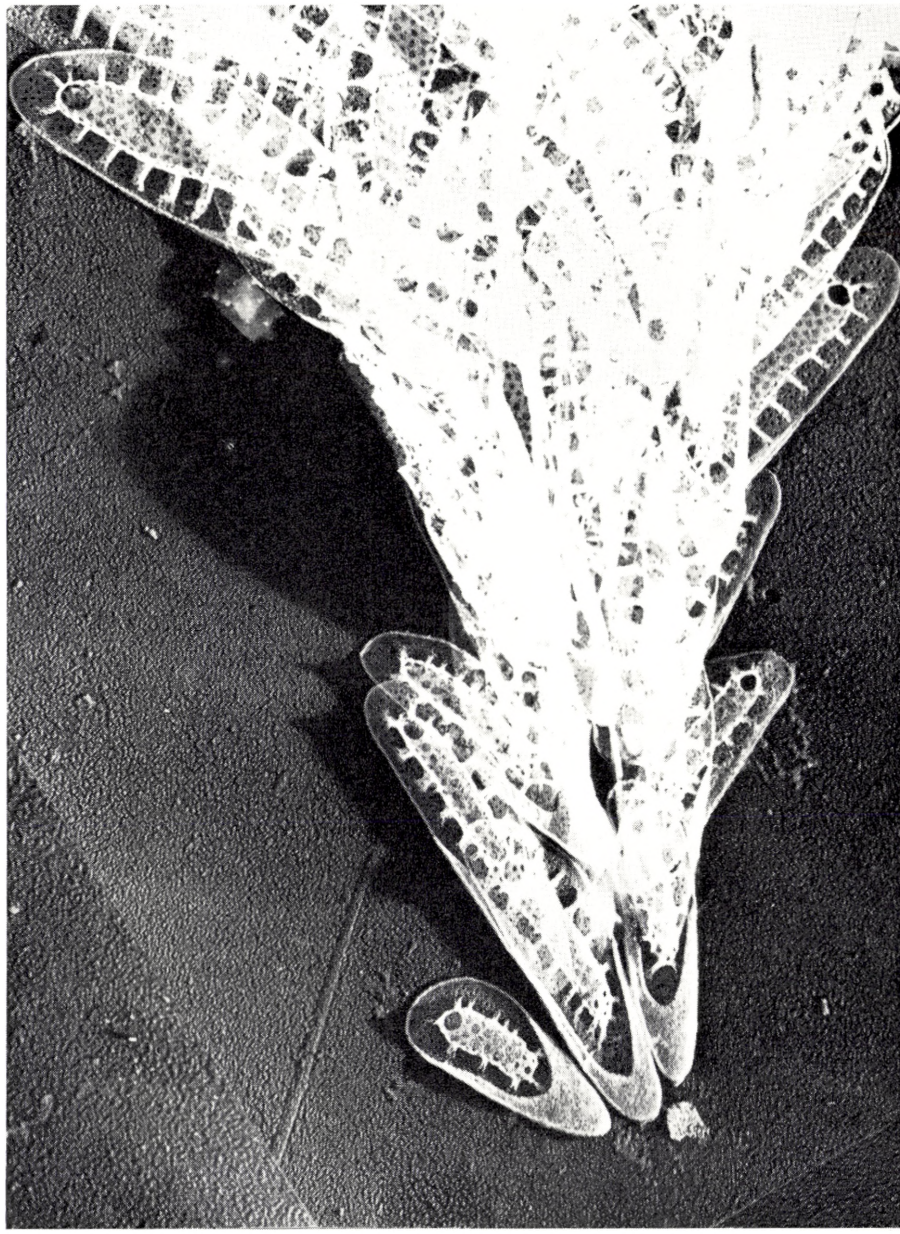

Fig. 2. *Synura Petersenii* Korsh. The stalk. ×14.400.

assumed these points to be due to the edges of the slightly projecting scales. We know now that they are caused by the short thorns to which the apical scales are tapered off. On the lower portion of the covering (the stipe), the scales have no spines; consequently the covering appears smooth (fig. 2). The description of the scales of this species in KORSHIKOV (1929) is very much to the point; equally, his figures are so carefully made that identification of the species with the light microscope is feasible.

In the neighbourhood of Copenhagen, *S. Petersenii* is the commonest species. It has been found in the pond of the Botanical Gardens; in the pond Bondedammen of Teglstrup Hegn; in the lake Bøgholm Sø, and in several minor ponds in northern Zealand. From the e. m. micrographs it is possible to give the following description of the scales:

Apical scales elliptical or oval, l. 3.6—4.7 μ, br. 2.2—2.5 μ. Along the lower two-thirds of the scale is an upturned edge (0.3—0.4 μ broad). The upturned portion is transparent enough for ribs and holes to be seen through it, and, accordingly, it is very thin. In the middle of the surface of the scale is a hollow, longish region, which at the front end tapers into a short, pointed thorn. The cavity is 2.7—3.3 μ long, 0.6—0.9 μ broad in front, narrowing gently towards the rear end. The thorn is ca. 1 μ long, conical. Inside the spine base is an opening inwards towards the cell (0.2—0.35 μ in diameter). The ceiling as well as the floor of the cavity is provided with little holes (ca. 0.1 μ in diameter). Evidently the outside wall is very thin, which implies that in many of the e. m. pictures it is impossible to discern its structure above the hole on the inside, and often it has been crushed during the preparation. The spine itself, on the contrary, has a rather thick wall. The surface of the scale round the hollow rim has numerous little holes (0.05 μ in diameter, ca. 12 in 1 μ), and from the cavity a number of strong ribs extend towards the edge. Most often they are not forked, occasionally they are bifurcate, and sometimes they are interconnected by transverse ribs (fig. 3). These ribs are of rather considerable height, as may be seen from the shadowcast e. m. pictures and, still more conspicuously, in the stereoscopic pictures. In some pictures they are seen in a somewhat oblique position, in which cases the vertical planes

are seen to contain small holes, which leaves the impression that they are folds originating from the inner surface of the scales, projecting from the middle cavity as a sort of buttresses.

Down the lower part of the cell the scales grow narrower and smaller. At the same time the spine disappears; the cavity,

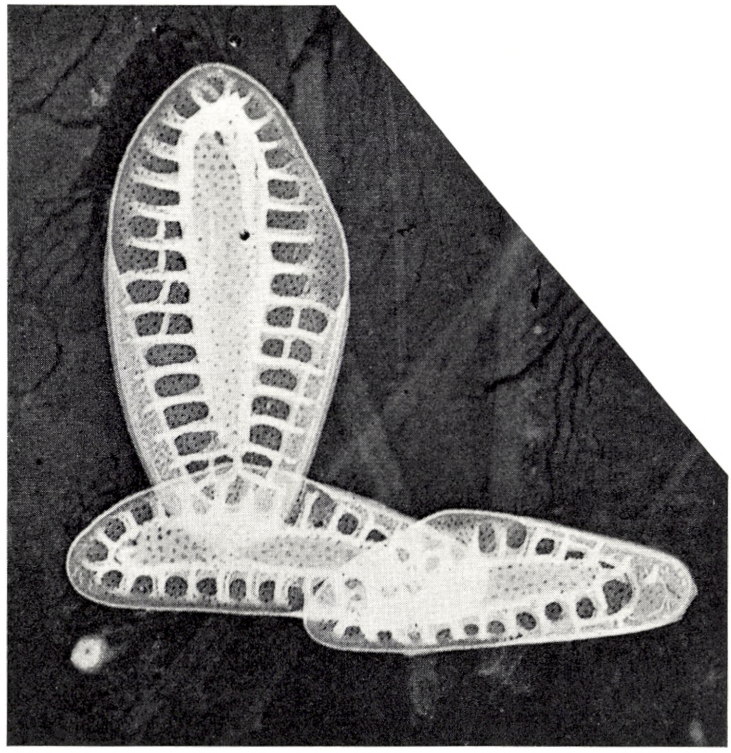

Fig. 3. *Synura Petersenii* Korsh. Three median scales. ×14.400.

however, remains right down to the very lowest scales. This is inconsistent with Korshikov's observations, because he was not able to see any "median ridge" on the basal scales (l. c., p. 284, table 11, figs. 57, 58). However, the objects in question are so extremely small that it is no wonder he was not able to see all the details revealed in the e. m. pictures.

The basal scales are shaped like slippers, elongate obovoid, the smallest and very lowest l. 2 μ, br. 0.6 μ. The lower point is covered by the upturned edge, the upper part has a narrow cavity and extremely small holes (ca. 0.01 μ). The upturned edge is

broad at the bottom (0.8 μ), narrowing upwards and reaching nearly halfway up the scale. Ribs are short, do not reach the edge. Cavity of the central part (l. 1.9 μ, br. 0.4 μ) narrow and short.

Synura glabra Korsh.

Korshikov 1929, p. 285, pl. 11, figs. 59—65.
Plate I, figs. d, e. Text, figs. 4, 5.

The species was established by Korshikov after some hesitation, because he suspected that it might be regarded as a variety of *S. Petersenii*. However, it has proved possible to distinguish the two species, even by means of the light microscope, and the e. m. pictures show such a marked difference, that the species must be considered without doubt different from *S. Petersenii*. Its main characteristic is a less silicified scale, the shape of which is more oval (at times nearly circular and slightly oblique in

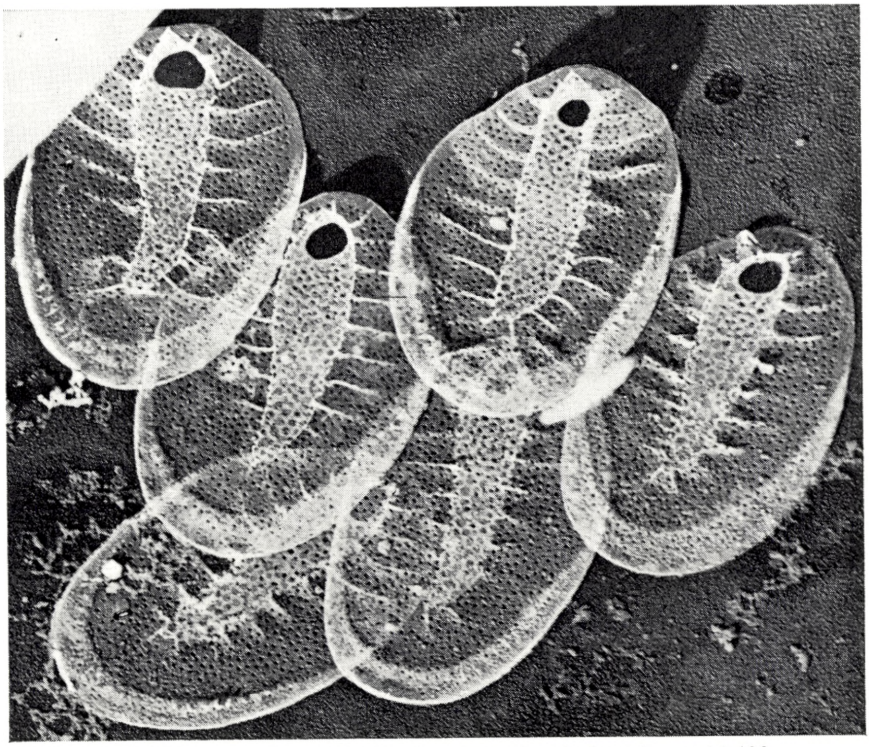

Fig. 4. *Synura glabra* Korsh. A group of apical scales. ×14.400.

front), a less developed middle cavity, which in front is only provided with a very short point, hardly discernible in the light microscope.

As already emphasized by KORSHIKOV (1929, p. 286), *S. Petersenii* and *S. glabra* make up a group within the genus which is

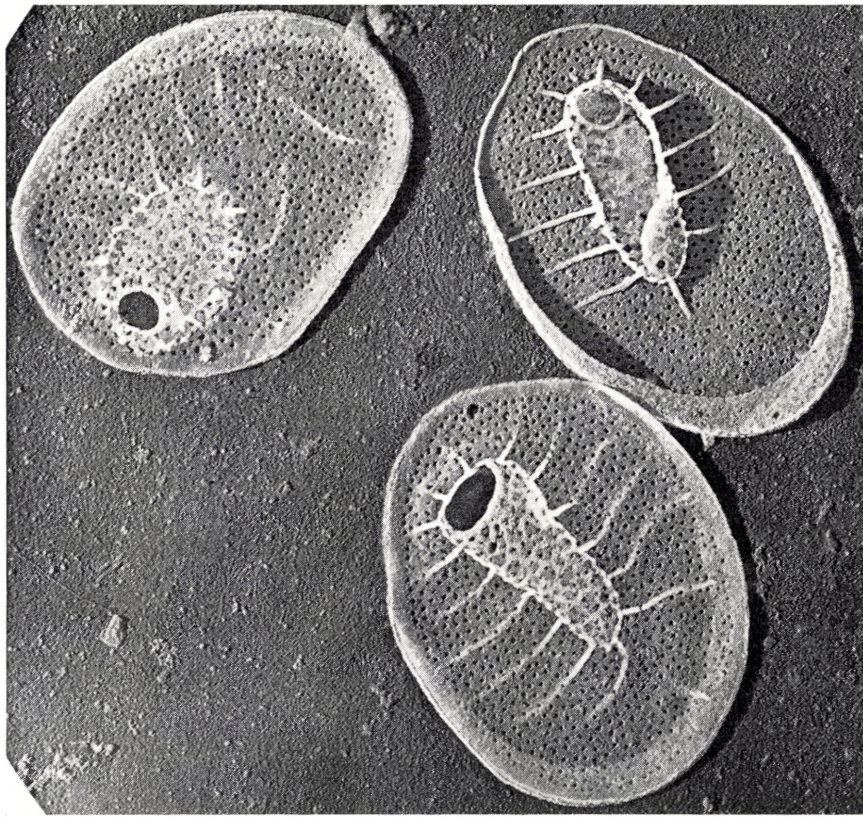

Fig. 5. *Synura glabra* Korsh. Three median scales. ×20.000.

quite different from the other species through the structure of the scales. KORSHIKOV has even considered the possibility of referring the two species to a special genus; he gave up this thought, however. We have joined him on this point, dividing the genus into two sections.

Apical scales oval, often cut off obliquely at the top, l. 3.4—3.6 μ, br. 2.3—2.6 μ. On the basal part of the scale an upturned thin edge 0.5 μ broad, reaching upwards ca. two-thirds of the

scale. The scale surface is very broad, with weak radial, hardly bifurcate ribs which do not reach the edge of the scale. Between, and on, the ribs are seen small holes (0.025 μ in diameter, ca. 14 in 1 μ). As in *S. Petersenii*, the central part is covered by a hollow ridge, tapering off, in front, in a very short thorn. The cavity is 2.6 μ long, 0.6—0.7 μ broad in front, gently narrowed at the back. At the very back it is often turned a little sidewards. The inside as well as the outside wall are provided with holes at small intervals (ca. 0.05 μ in diameter). Through a pore (diam. 0.4 μ) the cavity is connected with the surroundings.

Downwards on the narrow part of the cell, the scales grow smaller and more elongated. The smallest scales: l. 2.1 μ, br. 1.1 μ still contain the middle cavity with a hole on the inside, but the ribs are very weak, or there are none at all. The shape of the scale is rather oblong, with parallel sides, not elliptical as in Korshikov's drawings (1929, table 11, figs. 63, 65).

Found in Bøgholm Sø.

Section II. Uvellae.

Synura uvella Stein

Stein 1878, p. 15, table 13, fig. 24.
Korshikov 1929, p. 279, pl. 11, figs. 31—37.
Plate II, figs. a, b. Text, fig. 6.

Korshikov realized that what previous authors understand by *S. uvella*, evidently represents several species. Consequently he decided to accept the specimen depicted by Stein (l. c.) as the type species. This form is characterized by scales provided with strong spines which are discernible even through imperfect optical instruments. By this it is clearly distinguished from all the other species described. Korshikov (l. c.), gave excellent figures of the scales of this species, having made surprisingly good observations of the structure of these scales with the light microscope. The e. m. pictures testify in nearly all details to the correctness of his observations. It should be noted, too, that this particular species has larger scales, and these a coarser structure, than all the other species. Bioret (1933) in fig. 5 a—e depicted a number of scales evidently belonging to this species; this

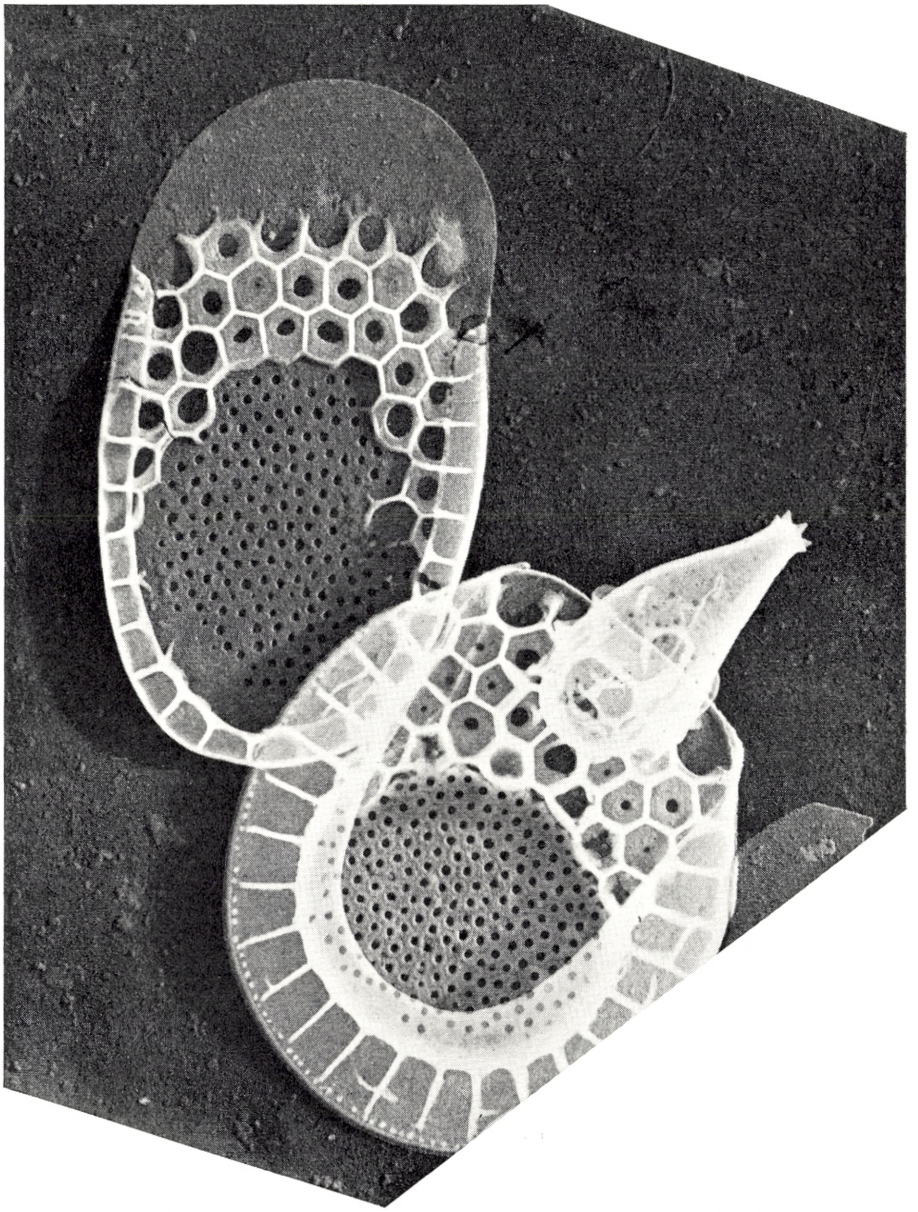

Fig. 6. *Synura uvella* Stein. An apical and a basal scale. ×15.400.

presumably also applies to fig. 7 f—i. As for Bioret's other figures of Synura scales, it will no doubt for some time to come be impossible to decide to what species these scales belong. CONRAD (1946) tried to base the establishment of new species on some of Bioret's drawings; however, it seems difficult to classify any more of his figures among Korshikov's species. No doubt some of his original figures belong to *S. uvella*, e. g. figs. 37—39.

The scales of *Synura uvella* may be described as follows:

The apical scales are almost circular, cut off rather abruptly in front; l. 4.9 μ—5.0 μ, br. 4.3 μ—4.5 μ. An upturned edge (1.2 μ broad) reaches upwards nearly as far as to the cut-off portion of the edge. Here the edge of the scale is very thin, supported by strong ribs. Below the upturned edge, too, are seen strong ribs at right angles to the edge (ca. 25 in 10 μ), and along the edge itself are a number of very small grains (0.05 μ in diameter, ca. 6 between every two ribs). Inside this portion of the edge, the surface of the scale falls into two sections of completely different structures. The front part to which the spine is attached has strong ribs connected as a mesh pattern forming hexagonal meshes, each of which is covered at the top by a thin membrane with a hole in it. The meshes are 0.5 μ in diameter, the holes 0.1—0.3 μ in diameter. At times, however, the membrane seems to be quite missing, or perhaps to have burst during the preparation. The posterior section is a continuous plane with round, evenly distributed holes (0.1 μ in diameter) which often appear surrounded by a fine thickened border. The spine issues from the front part of the scale, slightly inside the edge. It is thick, conical, and hollow, and ends in 3—5 teeth; l. 2.7 μ—3.0 μ, th. 1.2 μ—1.3 μ. At the bottom the inside of the spine is connected with the cell through a hole (ca. 0.2 μ in diameter). The spine appears to be rather thick-walled; however, the thickness of the wall is not easily measured in the pictures.

The basal scales are oblong oval, without thorns (l. 5.8 μ—6.0 μ, br. 3.2 μ—4.5 μ). The upturned edge along the basal part of the scale is narrow (0.3 μ) and only stretches ca. three-fourths upwards towards the upper end. The surface of the scale falls into three sections:

1) the upper part of the surface is thin, devoid of structure;
2) after this follows a section with strong ribs connected as a

mesh pattern, and forming hexagonal meshes (0.4 μ in diameter). In these, a thin membrane is sometimes stretched out, with a hole in the centre (0.05 μ in diameter); on the basal scales, however, this membrane is often missing;

3) from this section stretches a zone of radial ribs surrounding the rear part of the scale as a continuation of the middle section. It should be noted that the floor of this third section has a smooth surface with holes in it (0.1 μ in diameter).

Found in Hulsø (leg. J. Kristiansen), and other places.

Synura echinulata Korsh.

Korshikov 1929, p. 282, figs. 42—53.
Table I, figs. f, g, h. Text, figs. 7, 8.

In Korshikov (l. c.) this species is characterized particularly by the blunt-edged shape of the apical scales, the short stiff spine forming an angle to the surface of the scale, and by the small size of the scale. In reality the scale is best desribed as elliptical or ovoid, and the reason why Korshikov describes it as being blunt-edged in front, is no doubt that he was not able to discern the thin edge of the front part.

Erroneously he considered the front plane as a thickened portion of the edge. The scales are characterized particularly by the short stiff spine ending in one point, and by the front plane provided with linear thickenings making an area of broken lines. Apart from that, the scales are essentially built like those of *S. uvella*. The shape of the middle scales is more elongated, the spine completely or almost missing. On the basal scales the front plane with the broken lines is also completely missing.

The scales may be thus described:

Apical scales ovoid, l. 3.0—3.4 μ, br. 2.4—2.5 μ.

At the base is an upturned edge, which stretches upwards to slightly above the middle of the scale. Under it no structure is discernible. Its breadth is ca. 0.35—0.5 μ. It extends in front into a narrow thin edge with ribs at right angles to the edge (5 in 1 μ). Between each of the ribs is seen a small hole (ca. 0.03 μ). The front part of the scale surface, to which the spine is attached, is highly silicified and provided with small linear, sinuate thickenings (ca. 0.03 μ thick, 0.15—0.5 μ long). From here issues the

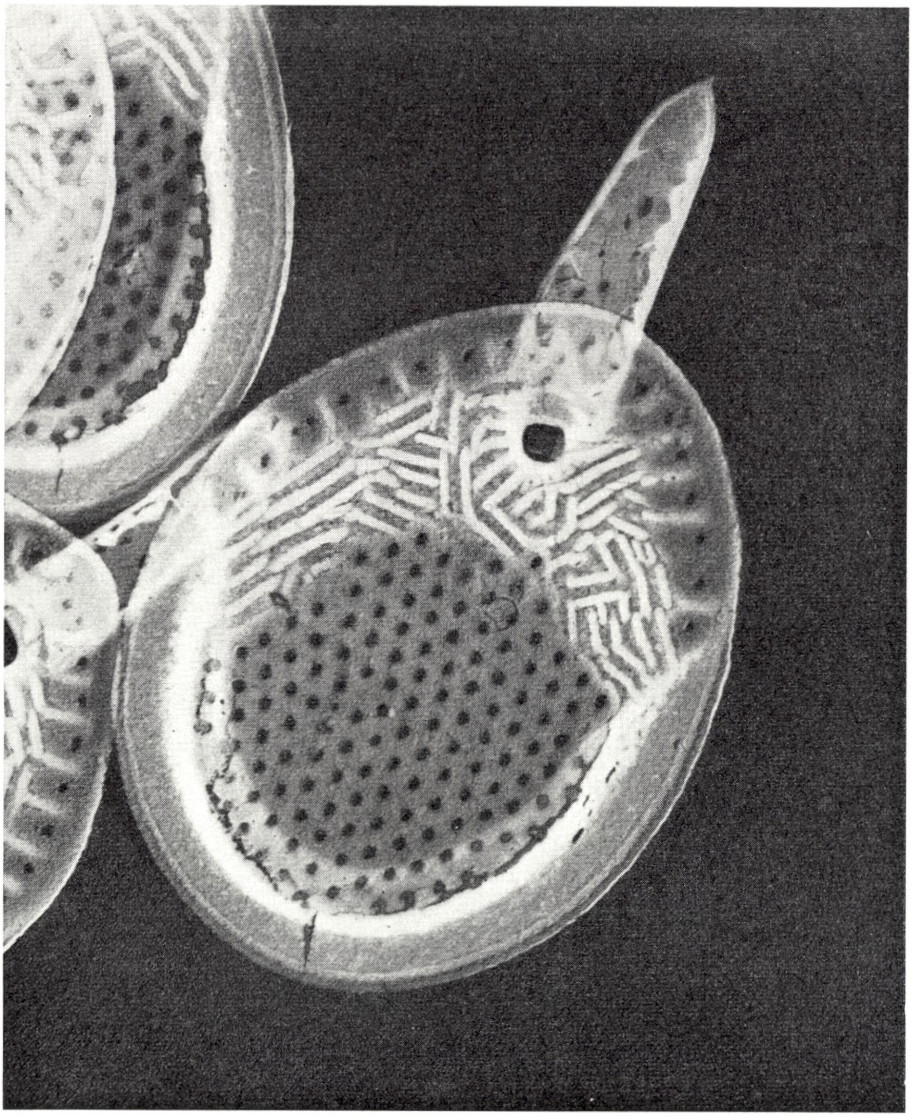

Fig. 7. *Synura echinulata* Korsh. Apical scale, seen from the inner side. ×32.400.

spine, forming an angle to the surface of the scale (l. 1.4—2.0 μ, thickness at the base 0.5 μ), narrowing gently upwards, and ending in one point. The spine wall seems to be provided with occasional holes, and it rests above a hole in the surface of the scale (0.2 μ in diameter). The posterior part of the scale surface

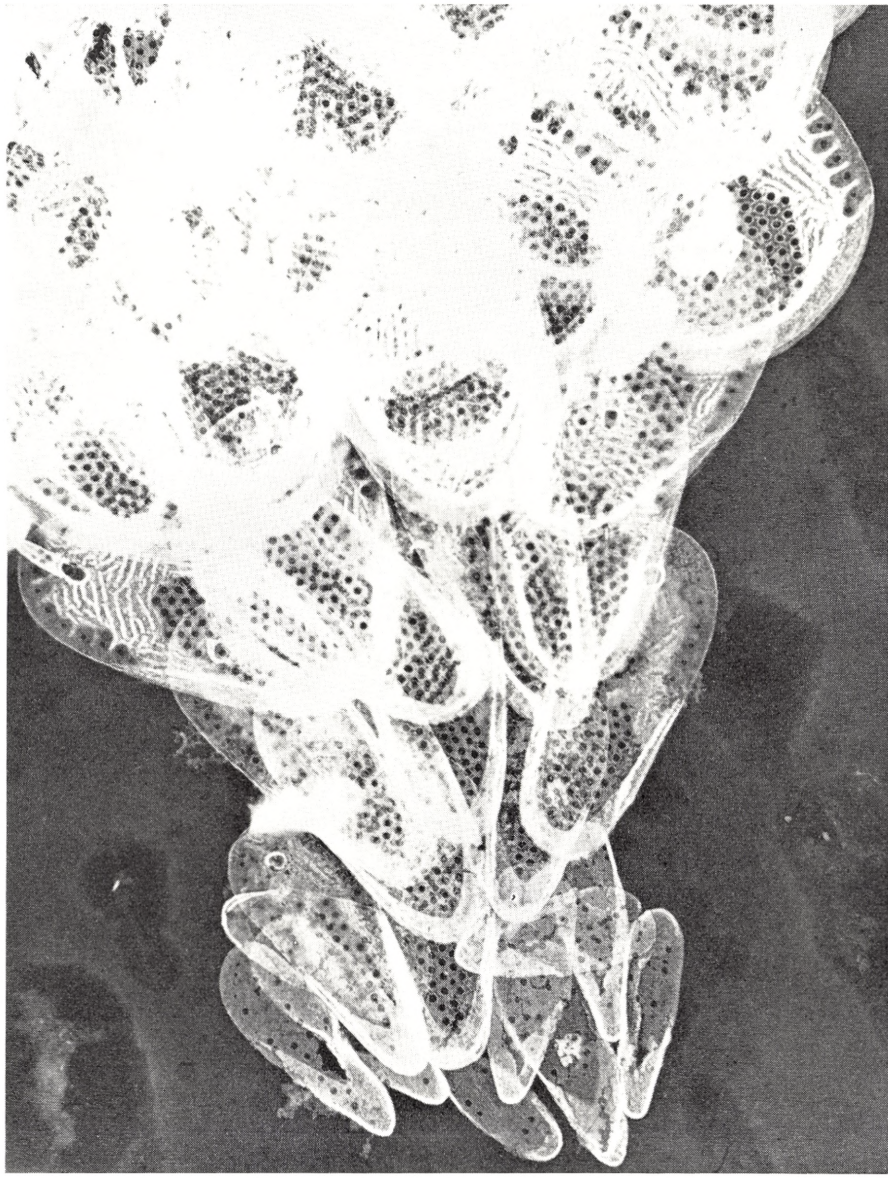

Fig. 8. *Synura echinulata* Korsh. The stalk. ×14.400.

is smooth, with holes (0.05 μ in diameter; ca. 8 in 1 μ), regularly arranged in rows following three directions.

In a downward direction on the cells the scales adopt a more oblong obovoid shape, and the spine becomes shorter. A scale of this kind (corresponding to KORSHIKOV 1929, table 11, fig. 49) showed the following dimensions: l. 3.5 μ, br. 1.7 μ. The basal upturned edge stretched as far as two-thirds of the length. The front edge had no ribs, but a row of small holes (ca. 0.05 μ in diameter), ca. 5 in 1 μ. The front part of the scale surface had linear thickenings arranged in slightly sinuate lines. The spine was quite short, pointed (ca. 0.5 μ long). The rear part of the scale surface had round holes (0.06 μ in diameter) arranged in rows following three directions (ca. 6 in 1 μ). The basal scales were still more simplified, shaped like slippers, broadest upwards (l. 2.3 μ, br. 0.7 μ); at the base they had an upturned edge reaching upwards ca. half the length. The surface had small holes (ca. 0.04 μ in diameter) lying irregularly.

Found in the Fuglsangsø (leg. J. Kristiansen), and in other places.

Synura spinosa Korsh.

KORSHIKOV 1929, p. 281, pl. 11, figs. 38—41.
BIORET 1931, p. 5, 6, figs. 2, 4a—e.
CONRAD 1946, p. 4, 8, fig. 20.

This species seems to be rather variable, or it may in fact include a group of closely related species. We have selected, as the type species, the form found in the pond Bondedammen and the lake Bøgholm Sø, the apical scales of which have spines of medium length, with two or three teeth at the points. Other forms have very long spines, which are blunt-edged at the points; besides these, there are short-spined forms the spines of which are truncated abruptly at the points, and are provided with up to seven teeth. Besides, the structure of the scales seems to be essentially identical within the different forms.

In the opinion of KORSHIKOV and later authors the spines of the apical scales of this species are attached to the edges of the scales, and are projecting at the same level as the surface of the scales. This is not so. The spine is always attached slightly within the uppermost edge of the scale and, in life, always forms an angle to the scale surface. Probably this angle is always acute.

In dried preparations the spine is sometimes lying on the glass or the formvar film, owing to the fact that the degree of silicification is not very high.

In this species KORSHIKOV has only observed the apical scales, whereas he has not been able to find the basal scales, on account of the sparsity of the material at his disposal. He describes the apical scales with great correctness, and gives these measurements: l. 3—5 μ, br. 2.5—3 μ; spine 2—3 μ long. These measurements agree fairly well with ours, as will appear from the figures given below.

Survey of the forms found within the species in question:

1. The whole scale covered by ribs forming a hexagonal mesh, spine slender and soft ..... f. *mollispina*
   Only front part of scale covered by ribs in mesh pattern, spine coarser and rigid 2
2. Spine ending in blunt point, 3.5—4.3 μ long.. f. *longispina*
   Spine ending in teeth, shorter 3
3. Spine 2.8—3.5 μ long, with 2—3 teeth ..... f. *spinosa*
   Spine shorter 4
4. Spine 2.7 μ long, having up to 7 teeth ..... f. *Nygaardii*
   Spine 1.9—2.4 μ long, having 3—4 teeth ..... f. *curtispina*

S. spinosa f. *mollispina* n. f. Plate IV, fig. a.

Apical scales ovate, l. 4.9 μ, br. 3.3 μ, upturned edge at base 0.5 μ broad reaching upwards about halfway. The whole of the plane covered with mesh-pattern ribs, mesh hexagonal (0.3 μ in diameter); in the middle of each a little hole (0.05 μ in diameter). Spine hollow, 2.8 μ long, 0.3 μ thick, only a little silicified, ending in two teeth. Spine attached slightly within the upper edge of the scale.

Found September 14, 1954 (leg. Berit Asmund) in the pond Bondedammen. Few apical scales observed, and none basal.

S. spinosa f. *spinosa* n. f. Plate II, figs. c, d, Text, fig. 9.

Apical scales with oval or slightly elliptical outlines, l. 4.2—4.8 μ, br. 2.9—3.0 μ. Upturned edge at base 0.5 μ broad, reaching upwards two-thirds of scale. Front plane provided with mesh-

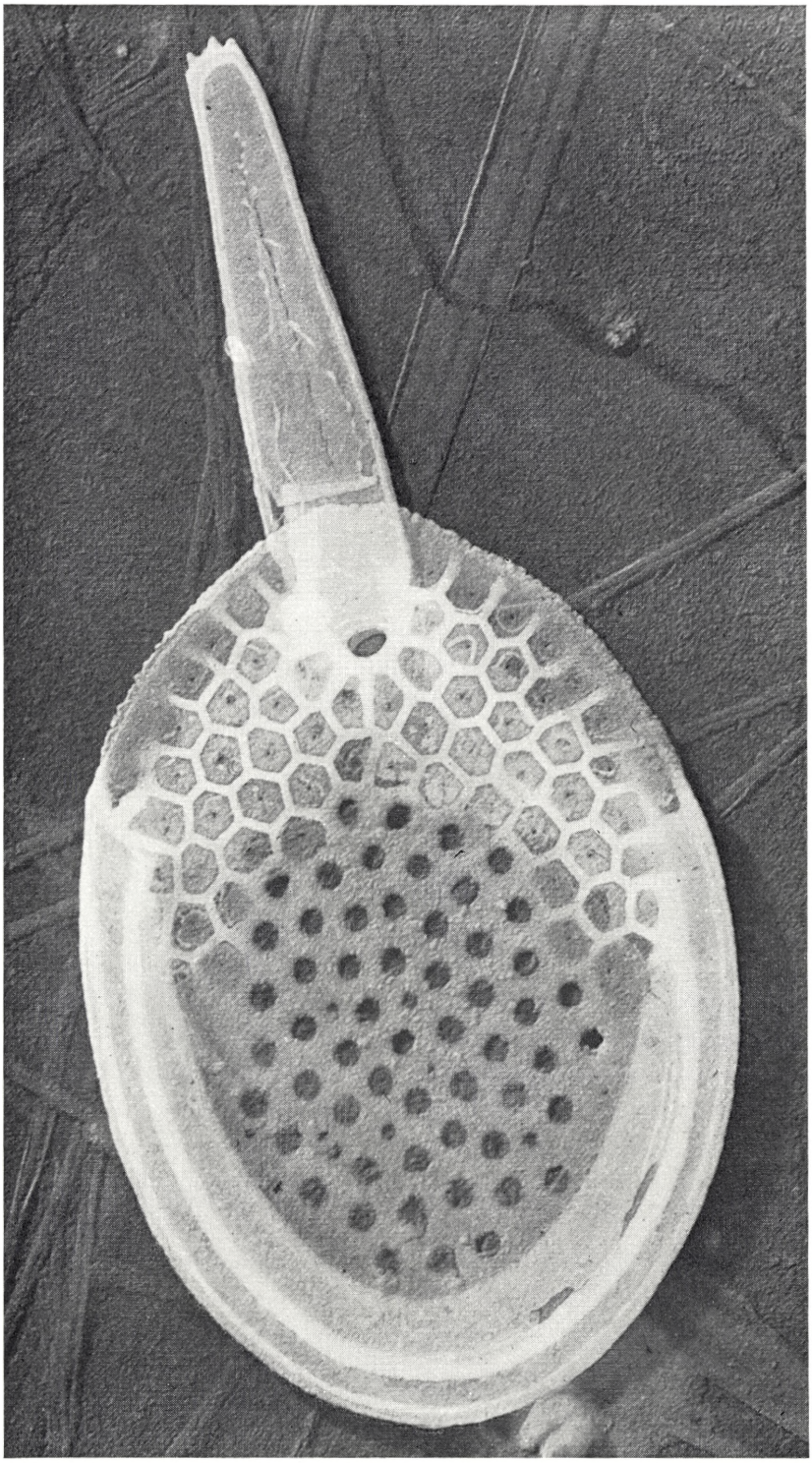

Fig. 9. *Synura spinosa* Korsh. f. *spinosa* n. f. Apical scale. ×32.400.

pattern ribs, meshes hexagonal (0.2 μ in diam.); each mesh covered with thin membrane containing small hole (0.01 μ).

Rear plane smooth, with round holes (ca. 0.1 μ in diam.), ca. 5 in 1 μ. The spine is 2.8—3.5 μ long, attached slightly within the upper edge of the scale, hollow, 2—3 teeth in front. Thickness at base, 0.5—0.7 μ, at top 0.2 μ. Thickness of wall 0.03 μ. At the bottom a hole, ca. 0.5 μ in diam.

Basal scales ovoid, tip turned upwards, l. 3.4—4.8 μ, br. 1.5—2.2 μ. Upturned edge all the way round (0.3 μ broad). Whole of surface smooth, with round, sparse holes (0.1 μ in diam.), especially in lower, broader part.

Observed in the Bondedammen and Bøgholm Sø.

S. spinosa f. *longispina* n. f. Plate III, figs. c, d, Text, fig. 10.

Apical scales, l. 3.9—5.0 μ, br. 2.5—3.7 μ. Spines 3.5—4.3 μ long, blunt-edged, no teeth at tip; 0.5 μ broad, seems rather thin-walled, apical portion apparently being attached to formvar film, collapsed and rather flat. Basal scales same size and equipment, as in f. *spinosa* (l. 5.3 μ, br. 1.9 μ).

Observed in the Sortesø on Aug. 27, 1948.

S. spinosa f. *curtispina* n. f. Plate III, figs. a, b.

Apical scales, l. 3.9—4.3 μ, br. 2.9—3.1 μ. Spine, l. 1.9—2.4 μ, thickness 0.4—0.7 μ, at tip with 3—4 teeth. Except at the top of the cells the scales are without spines, but still with mesh-pattern ribs on the front part of the surface, and small round holes at the rear of the surface. The basal scales on the stalk are shaped like slippers (l. 2.7 μ, br. 0.8 μ), without holes on the surface. These scales are very slightly silicified.

Observed in the Kathale bog on Nov. 12, 1955.

S. spinosa f. *Nygaardii*. Plate III, fig. e.

S. Adamsii Nygaard 1949, p. 139, fig. 72 bis.

The material representing this form was collected by Dr. E. Teiling in the Ryven lake in Sweden.

Nygaard mentioned and described it (l. c.) by the name of *S. Adamsii*. Evidently this description is based essentially on the

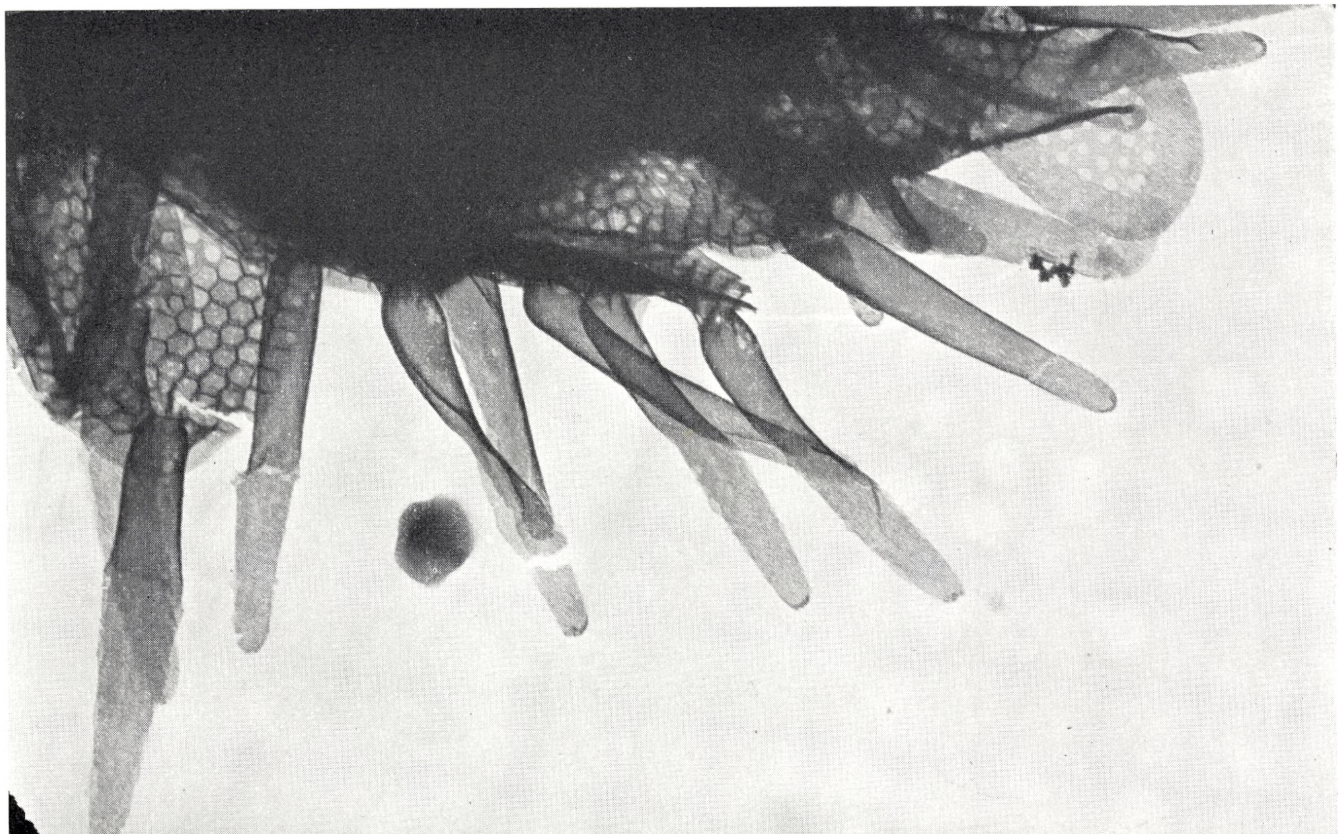

Fig. 10. *Synura spinosa* Korsh. f. *longispina* n. f. Border of a cell, showing the insertions of the spines. Positive, non-shaded print. ×14.400.

shape of of the cells, which have great similarity with *S. Adamsii* G. M. Smith. The cells are broadest in front, narrowing very gently towards the rear, without any clearly drawn stalks. Differing completely from the usual pictures is the fact that all the scales are provided with very distinct, projecting spines. It will, therefore, be fair to assume that the form in question has no connection with *S. Adamsii*. The subsequent e. m. examination revealed that it is very closely related to *S. spinosa*, as regards the structure of the scales. Owing to the sparsity of the material at our disposal, we have not been able to discern with complete certainty any particular basal scales without spines, as in other forms of *S. spinosa*. Provided Nygaard's drawing is correct (l. c. fig. 72 bis), such scales are not to be expected, either. It seems as if all the scales are more or less alike and have spines.

Most of the scales in our preparations appeared more or less crushed. An intact scale (table III, fig. e) showed the following measurements:

L. 5.2 μ, br. 3.4 μ. Upturned edge 0.7 μ broad, reaching upwards two-thirds from base. Front surface provided with mesh-pattern ribs forming hexagonal meshes (0.25 μ in diam.); each mesh covered by a membrane with a round hole (0.05 μ in diam.).

Rear part smooth with round holes 0.15 μ in diam., surrounded with a thickened border. Holes arranged in somewhat irregular rows, ca. 4 in 1 μ.

Spine 2.7 μ long, 0.7 μ broad at base, 0.35 μ broad at top. Point truncated abruptly with up to 7 teeth. Spine appears rather thick-walled and stiff.

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Diagnoses formarum novarum.

Synura spinosa Korsh. f. *mollispina* n. f.

A f. spinosa differt tota facie plana reticulatim costata spina molli, sicca collabente, denticulis binis terminata.

Synura spinosa Korsh. f. *longispina* n. f.

A f. spinosa differt spina longiore, obtusa, edentula, minus rigida.

Synura spinosa Korsh. f. *spinosa*.

Spina longa, rigida, denticulis ternis terminata, facie plana in parte anteriore modo reticulatim costata, ceterum foraminibus minutis perforata insignis.

Synura spinosa Korsh. f. *Nygaardii* n. f.

A f. spinosa differt spina breviore, apice truncata, denticulis usque ad 7 terminata.

Synura spinosa Korsh. f. *curtispina* n. f.

A f. spinosa differt spina breviore, crassiore, apice rotundata, denticulis ternis terminata.

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Plates.

All figures are from shadowcast preparations, except plate IV a. Enlargement: 14.400×, except Plate IV b and Plate V and VI.

Plate I.

Synura Petersenii Korsh. a. apical scale, b. apical scale, c. basal scale.
Synura glabra Korsh. d. apical scale, e. basal scale.
Synura echinulata Korsh. f. apical scale, g. middle scale, h. basal scale.

Plate II.

Synura uvella Stein. a. apical scale, b. basal scale.
Synura spinosa Korsh. f. *spinosa* n. f. c. apical scale, d. basal scale.

Plate III.

Synura spinosa Korsh. f. *curtispina* n. f. a. apical scale. b. basal scale.
Synura spinosa Korsh. f. *longispina* n. f. c. apical scale, d. basal scale.
Synura spinosa Korsh. f. *Nygaardii* n. f. e. apical scale.

Plate IV.

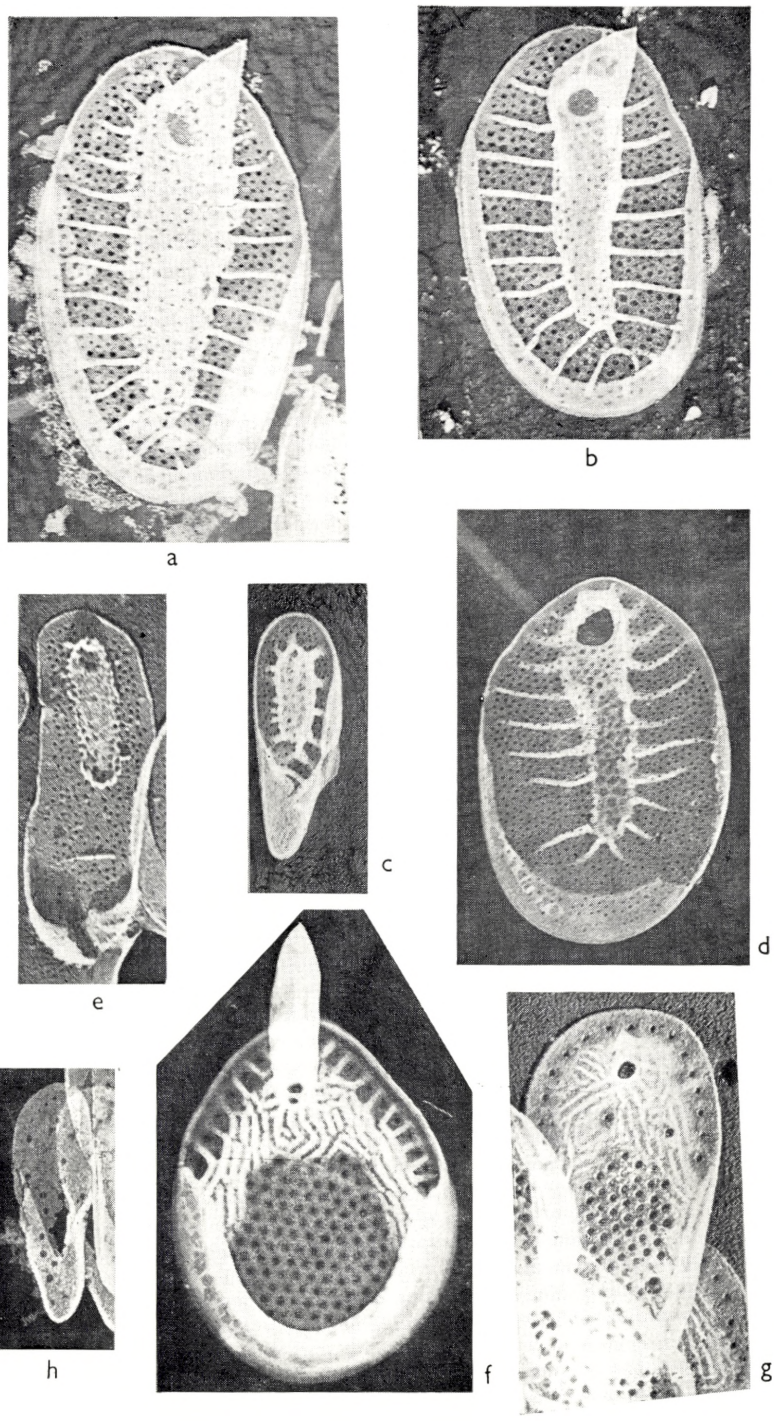
Synura spinosa Korsh. f. *mollispina* n. f. a. apical scale.
Synura spinosa Korsh. f. *curtispina* n. f. b. part of colony, Kathale bog Nov. 12, 1955. Mordanting and staining after Vilh. Jensen (cfr. Boye Petersen & Hansen 1954, p. 285). Light microscope 1650×. Note the two kinds of flagella: the long flimmer-flagella, and the shorter, smooth whip-lash flagella, and scales. Phot. E. Tellerup.

Plate V.

Stereoscopical pictures. Upper: Scales of *Synura Petersenii* Korsh. Lower: Scales of *Synura spinosa* Korsh. f. *spinosa* n. f. The scales are partly broken.

Plate VI.

Stereoscopical pictures. Upper: Scales of *Synura uvella* Stein, partly broken. Lower: Single intact apical scale of *Synura spinosa* Korsh. f. *spinosa* n. f.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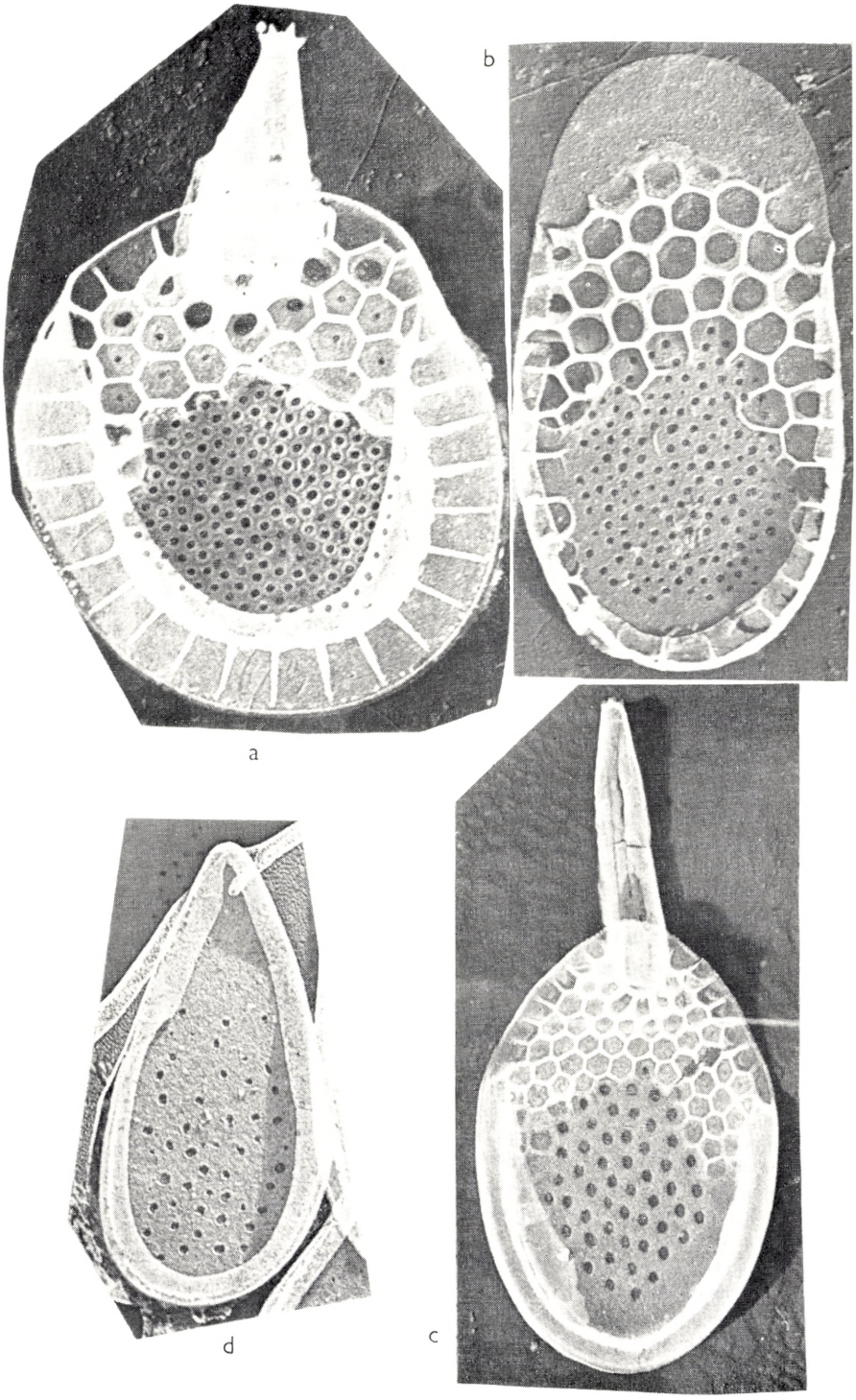

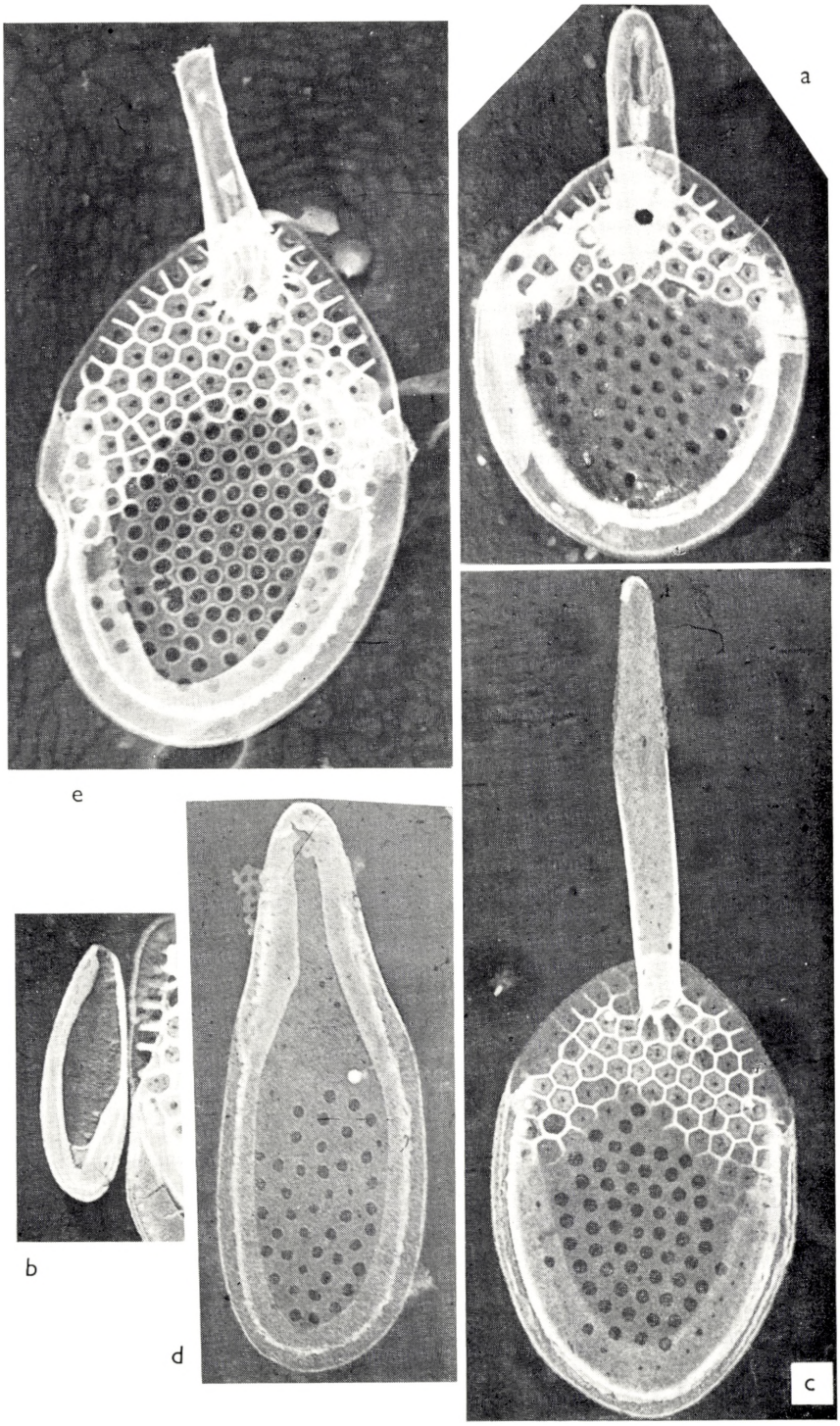
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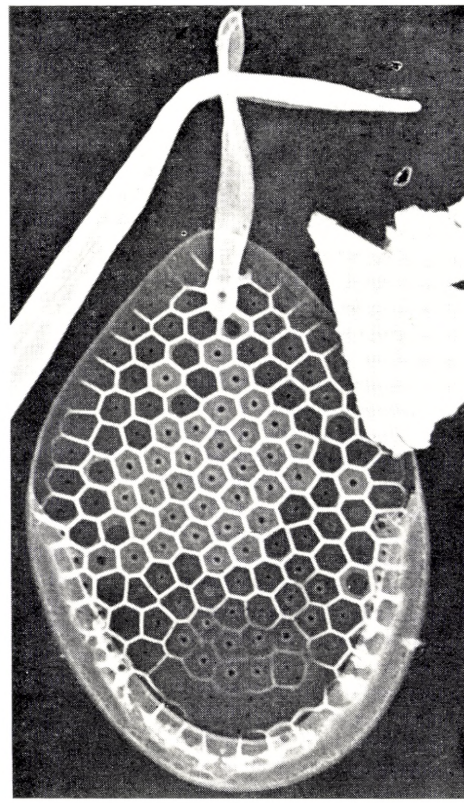
PLATE IV.



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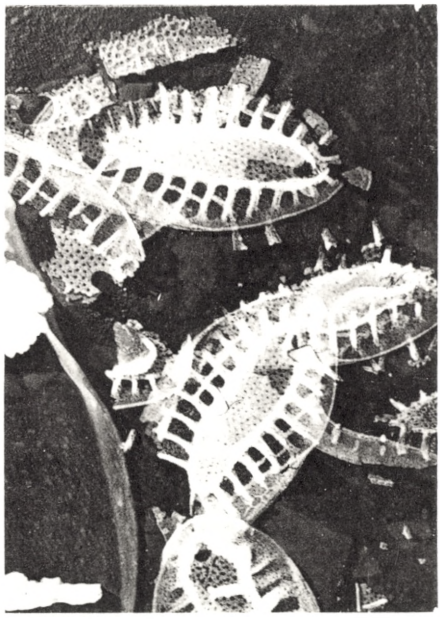

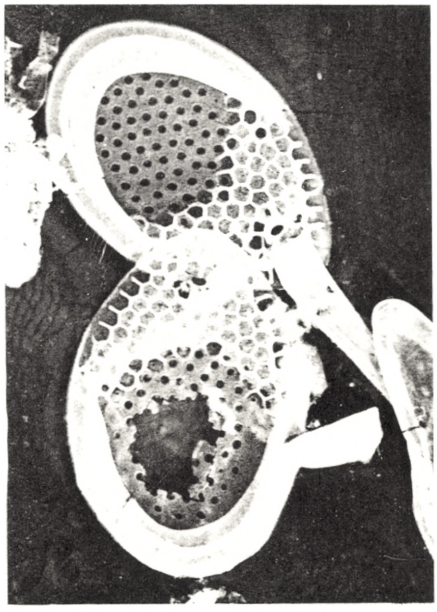

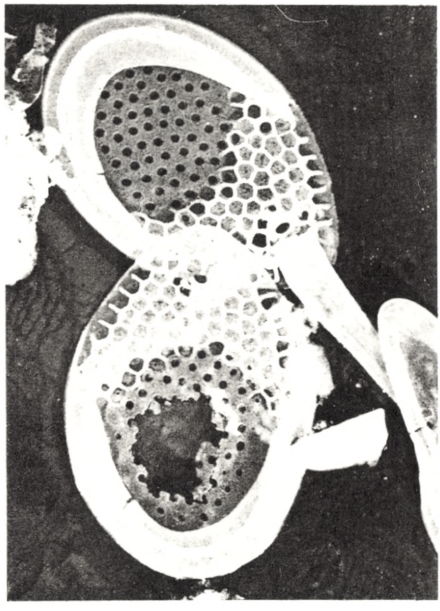

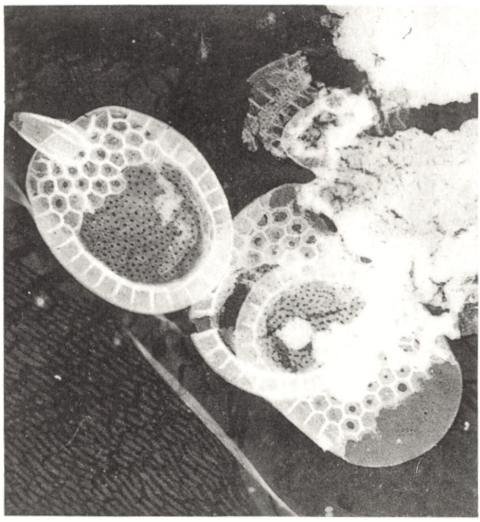

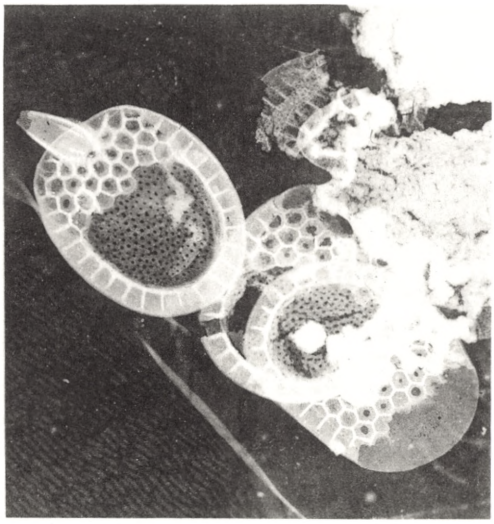

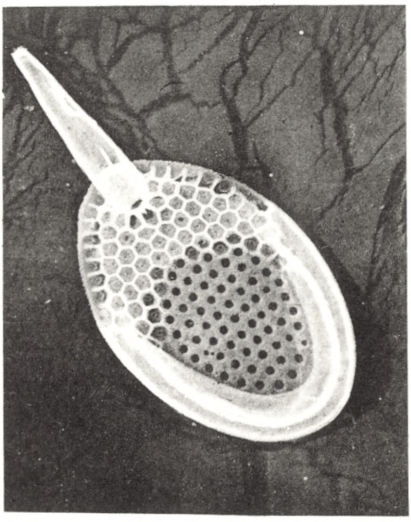

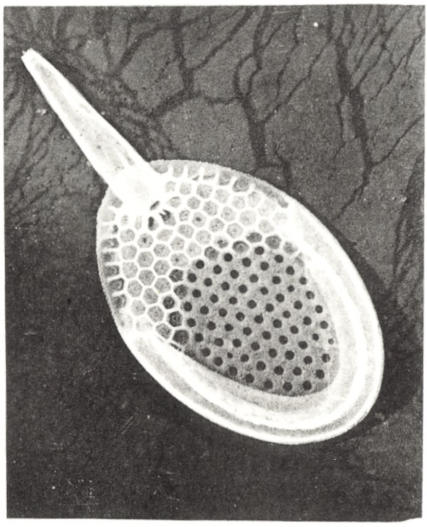

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WITH A DISCUSSION OF ITS MORPHOGENETIC SIGNIFICANCE

BY

H. V. BRØNDSTED

København 1956
i kommission hos Ejnar Munksgaard

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CONTENTS

Synopsis.

1. Transplantation experiments have shown that the time-graded head regeneration is a physiological property locally fixed to different parts of the planarian body. (I).
2. The bearing hereof on the problem of bilateral symmetry has been elucidated by means of median splitting and retransplantation at various levels. (II).
3. The significance of the existence of a time-graded field has been emphasized by the performance of twin parabioses. (III).
4. The spreading of inhibitory forces emanating from the highpoint has been clarified. (IV).
5. The starting rate of head regeneration is dependent on the place in the time-graded field; later quantitative growth of head structures is dependent on the number of neoblasts present. (V.)
6. The restoration of the time-graded field is conform with the morphological remoulding of the animal during morphallaxis. (VI).
7. A hypothesis is suggested covering common principles in embryogenesis and regeneration. (VII).

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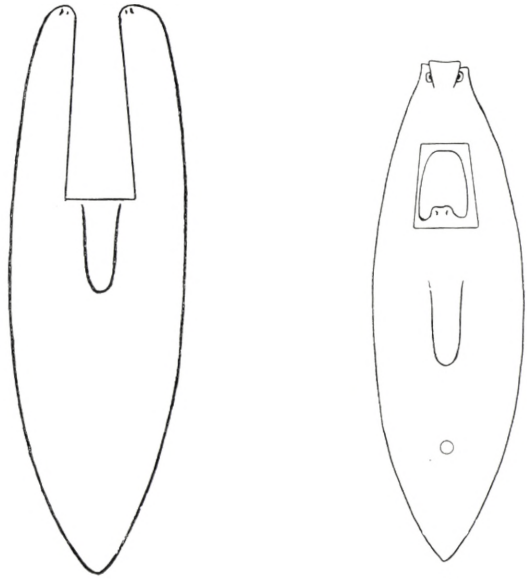
Introduction.

The existence of a time-graded regeneration field in planarians has long been established through extensive studies. Its structure and dynamics are, however, far from being explained satisfactorily. With the aim to elucidate some of the problems, certain experiments have been carried out and will be discussed in this paper.

Earlier experiments on *Bdellocephala* and *Dendrocoelum* (Figs. 1 *a*, *b*) have shown that every part of the body anterior to the pharynx has the power, time-graded in a certain way, to regenerate a head, if an anterior surface is exposed by a cut. The head regeneration rate confirms with a field as depicted in



a *b*

Fig. 1. *a. Bdellocephala punctata* regenerating heads on each of two "arms" after decapitation and excising of a median part of the body. *b. Bdellocephala punctata* regenerating a head in a "window" cut out from the anterior part of the body. (Brøndsted 1942, 1946.)

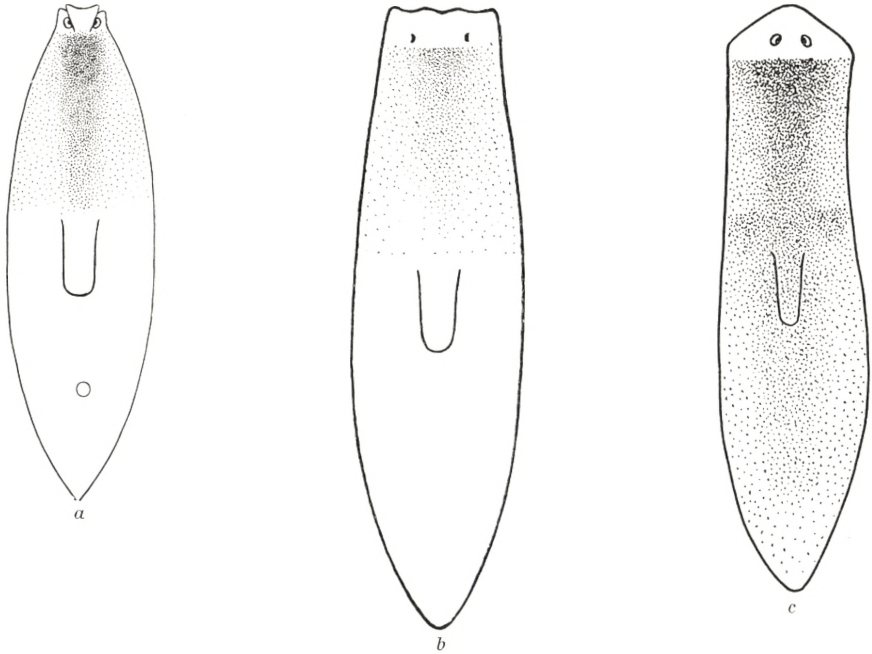


Fig. 2. The time-graded head regeneration fields of *a*, *Bdellocephala punctata*, *b*, *Dendrocoelum lacteum*, and *c*, *Euplanaria lugubris*. (BRØNDSTED, 1942, 1952.)

Figs. 2 *a*, *b* (BRØNDSTED 1942, 1946). Experiments on *Euplanaria lugubris* (A. and H. V. BRØNDSTED, 1952) indicate that the time-graded regeneration field extends over the whole body (Fig. 2 *c*). The field manifests itself when a wound surface is exposed and regeneration starts. I have denoted it a static and potential field.

The time-graded regeneration field secures that only one head is formed on a transverse cut, inspite of the fact that every part of the wound has the ability of regenerating a head. This may be explained by the assumption that an inhibitory influence is exerted by that part of the wound which is regenerating at the most rapid and vigorous rate. In view of its high regeneration rate this part will be called the *high-point*.

Harmonious regeneration seems to rely on two main factors, one of them organizing the totipotent, embryonic neoblasts which form the blastema, the other one inhibiting identical regeneration processes in other neoblasts in the lateral parts of the blastema. Thus, both factors compete in the blastema for determination and differentiation of the neoblasts.

I. Stability of the Time-Graded Field.

This problem was studied by means of a transplantation technique (BRØNDSTED 1939); a preliminary report of the results has been given elsewhere (BRØNDSTED 1954).

Euplanaria lugubris. Several animals were operated upon as shown in Fig. 3. With the aim to find out whether the time-graded field is dependent on humoral or structural factors in the body segments, the median segment, *a*, which in its proper place has a higher rate of regeneration (cf. Fig. 2 *c*), was interchanged with a lateral segment, *b*.

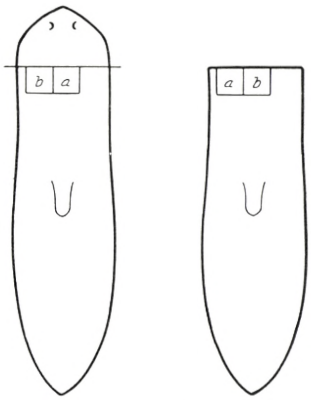


Fig. 3. *Euplanaria lugubris.* The animals were decapitated and the median a-piece was exchanged with the b-piece.

In Figs. 4 *a*—*c*, it is seen from the unpigmented demarcation lines that the transplanted median segments have retained their bilateral symmetry and have regenerated eyes at the same rate as they would have done prior to transplantation, i. e. 100 hours after the operation distinct eyes had been newly formed. On the other hand, the lateral segments, although implanted in the medial line of the body, have not reached the high regeneration rate characteristic of median segments. The three specimens exhibit a somewhat different behaviour. In *a*, after about ten days, an eye appeared in front of the right side of the body, making it probable that it had regenerated from the right part of the body and not from the transplanted lateral segment with which it has formed a common blastema. Here, the power to regenerate eyes seems to be inhibited (cf. Section IV). In *b*, the transplanted

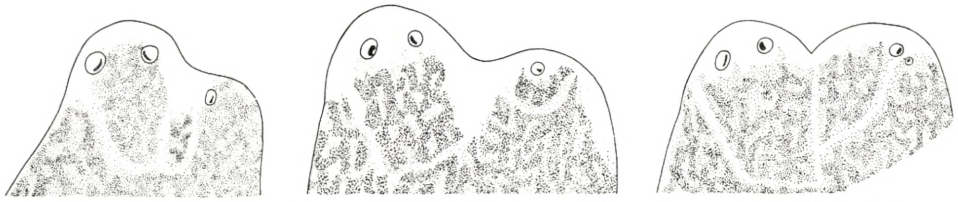


Fig. 4. *Euplanaria lugubris. a*—*c*. Three instances of the exchange experiments the principle of which is seen in Fig. 3. For details, see the text.

lateral segment has formed its own eye after seven days; in *c*, the lateral segment has regenerated an eye after eight days and, at the same time, the right body segment has formed an eye in the course of ten days.

The experiments seem to indicate that the time-graded field is dependent on some structural factor residing in the body parts. They also show that *bilateral symmetry is not attained by the*

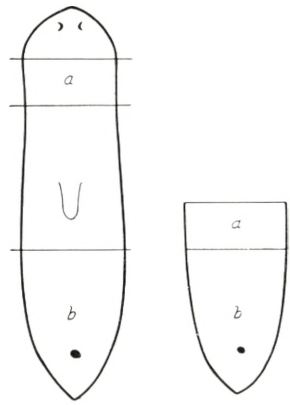


Fig. 5.

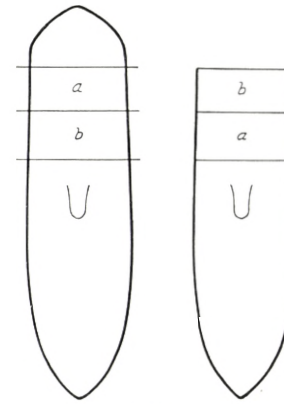


Fig. 6.

Fig. 5. *Euplanaria lugubris*. After decapitation, section *a* is transplanted to *b*; it retains its rate of regeneration.

Fig. 6. *Euplanaria lugubris*. After decapitation, section *a* is exchanged with section *b*, which regenerates a head at its inherent rate.

lateral segments when transplanted into the medial line of the body (cf. Section III).

Fig. 5 indicates the way in which *Euplanaria lugubris* was operated upon in order to elucidate whether the field is fixed also after being removed from its original cranial position to a caudal one, where the rate of regeneration is much lower (cf. Fig. 2 *c*). From 20 experimental animals six survived. They regenerated eyes after about 100 hours, which is the time required for eye formation at the original level in the normal animal.

From Fig. 2 *c* it is seen that the rate of regeneration from cuts made in the time-graded field almost in the middle between the eyes and the pharynx is somewhat lower than from cuts made just behind the eyes. 20 animals were operated, as indicated in Fig. 6, in order to find out whether the regeneration rate of more posterior segments of the body can be accelerated by

transferring them to anterior parts of the body. In fact, they regenerate eyes at the same rate as they do in segments left at their normal place. 120 hours after the operation, only four out of seven living animals had regenerated small eye spots, while all seven had regenerated eyes after 140 hours. (The mean rate of regeneration at this level has earlier been determined to 130 hours).

In *Bdellocephala punctata*, only the part of the body anterior

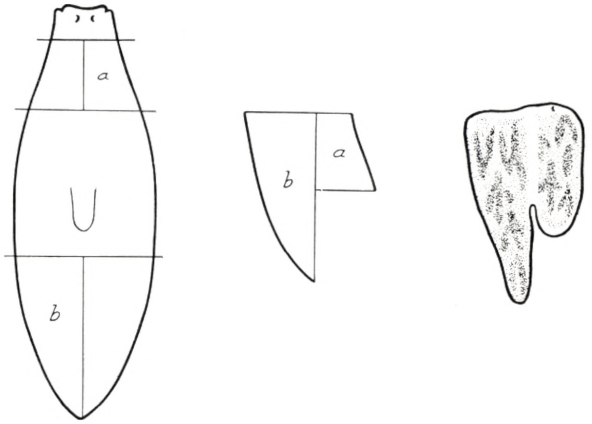


Fig. 7. *a. Bdellocephala punctata.* After decapitation, section *a* was transplanted to section *b*, where it regenerated a right eye; no eyes were regenerated from *b* in the common blastema, the tissue in *b* having no eye-forming competence.

to the pharynx has the ability to regenerate a head (cf. Fig. 2 *a*). In order to get information about whether also in this species the field is inherent in a given segment, irrespective of its situation in the body, two types of experiments were performed. In the first series (Fig. 7 *a*) 20 animals were operated upon as indicated; only one chimera survived, showing that the two pieces had confluent anterior and posterior blastemata. After 90 hours, one eye had been regenerated in the blastema of the piece *a* before the chimera cytolized, thus requiring the normal time for eye regeneration at the respective level in non-transplanted segments.

The second experiment was carried out in order to learn whether polarity in both the segment and the field is retained even if they are exposed to the action of a segment with opposite polarity. 40 animals were operated upon as indicated in Fig. 7 *b*.

On the first day, cuts I—III separated two segments of the body from the head and the tail and from one another; the following day, when the wounds had healed, new cuts bisected the segments, and pieces *a* and *b* were combined with reversed polarity. Two days after the operation, only one chimera survived, pieces *a* and *b* cohered only at about half of the medial line; again two days later the free parts of the chimera were removed. After six

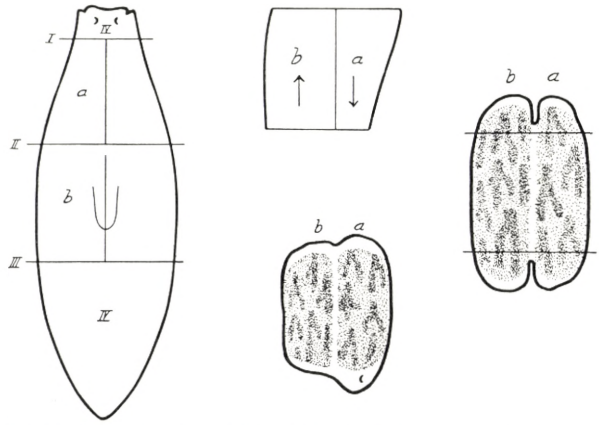


Fig. 7. *b. Bdellocephala punctata.* After decapitation, section *a* was transplanted to section *b* with reversed polarity; after some time, two clean transverse cuts were made as indicated. Hereafter, *a* regenerated a left eye, whereas *b* did not regenerate eyes in spite of common blastema with *a*.

days, piece *a* had formed a left eye in the left side of its blastema, which however is confluent with the blastema of piece *b*; the latter regenerated a tail; a tail was also developed in the caudal part of piece *a*, from a blastema confluent with the anterior blastema of *b* which does not regenerate eyes.

Thus, the two parts retain their polarity and field, irrespective of the close contact with one another. Actually, they behave as if they were belonging to two different organisms.

II. The Bilaterality of the Field.

In 1946, it was shown (Brøndsted 1946) that the regeneration of lateral segments starts at the same time as the regeneration of the organs belonging to the respective lateral part. A left piece starts, e. g., with the formation of a left eye, only later does a

right eye develop (Fig. 8). If a decapitated animal is cut into halves by a median cut, and separated, each half regenerates first its "own" eye; the symmetrical eye appears later and the other half of the body is regenerated subsequently. Thus, in fact, the bilateral animal is made out of two onesided animals acting together and controlling each other's ability to form a new symmetrical half. This example of inhibition may conceal a good deal of the morphogenetic riddle.

In view of the time-graded and bilateral antero-caudal property of the field, interesting results may be obtained by separating the field medially into two longitudinal halves and transplanting them together with the two half-fields transposed along the median axis.

In a series of 60 *Euplanaria lugubris*, the animals were separated by a median longitudinal cut, and the two halves were transplanted together in varying positions. After separation, the pharynges had to be removed, since they were protruding and prevented a proper coherence of the two halves; rather few animals survived.

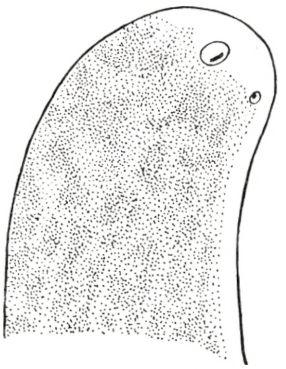


Fig. 8. *Euplanaria lugubris*. After decapitation and median splitting, the left half of the specimen first regenerated a left eye, only later the symmetrical right eye.

The procedure involves certain difficulties, one of which may be mentioned here. Due to muscular contraction, the halves tend to curve so that the longitudinal wound becomes concave; therefore, transplantation requires repeated readjustments. Nevertheless, several specimens may contract so strongly as to overlap, with the result that they cohere at the wound surface only slightly or not at all.

A separated half will regenerate a new head after decapitation (Fig. 8). A left half will first regenerate a left eye and after a couple of days a right one which, of course, is smaller than the left one in the beginning. This experiment may serve as a control of the transplanted half, showing the regeneration pattern of an isolated half which has no possibility of cooperating with the symmetrical field.

Example a. One of two animals was separated and the two

halves were joined (Fig. 9 *a*), transposed about $^1/_4$ of the length of the animal. After three days, the chimera was decapitated by a cut (as indicated in Fig. 9 *a*), which met the right time-graded field at a level of fast head regeneration, whereas the left field was met at a level with a lower regeneration rate. Seven days after decapitation, the right half had regenerated one large right

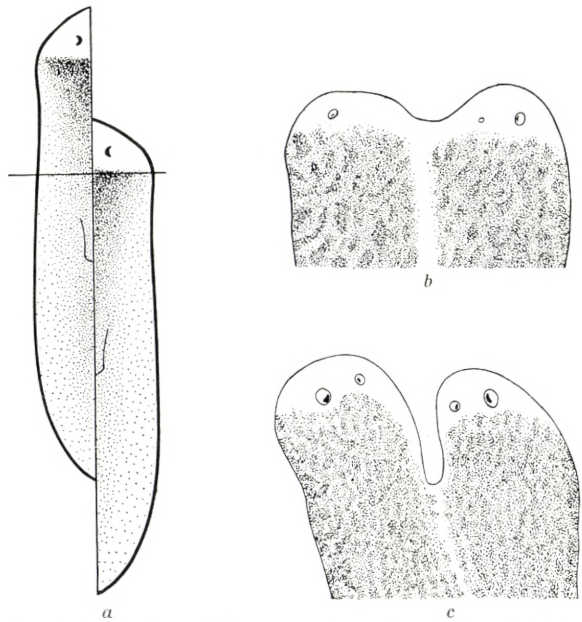


Fig. 9. *Euplanaria lugubris*. *a*. The two halves of a split animal are transplanted together with a grade of shifting of about $^1/_4$ of the body length. The stipling indicates the distribution of the time-graded field. After healing, the chimera was transsected as indicated. *b*. The situation after 7 days. *c*. The situation after 15 days.

eye and one small left eye. At the same time, the left half had regenerated a small left eye spot (Fig. 9 *b*). Four days later, both halves had regenerated distinct heads with two normal eyes each. This experiment shows that the two half-fields act like two separate entities which, when placed in a position impeding them from cooperation, regenerate the missing symmetrical half. Fig. 9 *c* demonstrates the situation after 15 days. In the beginning, the blastema formed at the transverse cut I is continuous from right to left.

The other animal (Fig. 10 *a*) which had regenerated two

heads at cut I was operated, as indicated in Fig. 10 *a* II, 8 days later. Here, the field was met at a level about $^1/_4$ caudad in the right half and about $^1/_2$ caudad in the left half.

After ten days a symmetrical head had regenerated. Obviously, the two levels met by cut II are not so divergent in their rate of

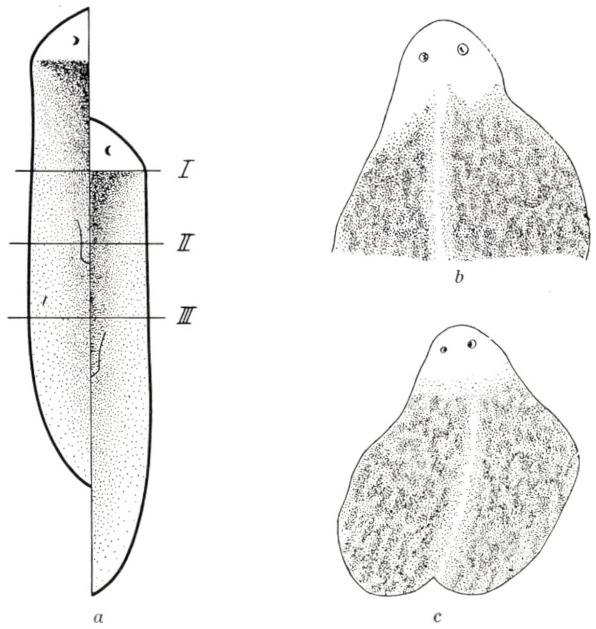


Fig. 10. *Euplanaria lugubris*. *a* indicates the experimental procedure. *b*, the regenerate after 10 days from the transverse cut II in *a*. *c*, the regenerate after 18 days from the transverse cut III in *a*.

regeneration as to prevent their cooperation. Fig. 10 *b* shows the result; the slightly larger right eye is especially noteworthy.

18 days after decapitation, the remaining hindpart was again cut transversely (Fig. 10 *a* III), the cut meeting the field in the right piece about half way down, in the left one about $^3/_4$ caudad. Fig. 10 *c* indicates that the two fields have again been able to cooperate.

Example b. The two halves were joined as shown in Fig. 11 a. The forepart of the left half cytolized (Fig. 11 *b*). Decapitation was performed after 72 hours. The cut met the field about $^1/_4$ down in the right half and $^3/_4$ down in the left half. Seven days later, the right half had developed one larger right eye and a

smaller left one, whereas the left half had not regenerated any eye at all. Fig. 11 *c* shows the situation after 15 days.

Example c. The two halves were joined (Fig. 12 *a*) and their foreparts separated a little bit (Fig. 12 *b*). 72 hours after transplantation a transverse cut was inserted (Fig. 12 *a*). After seven

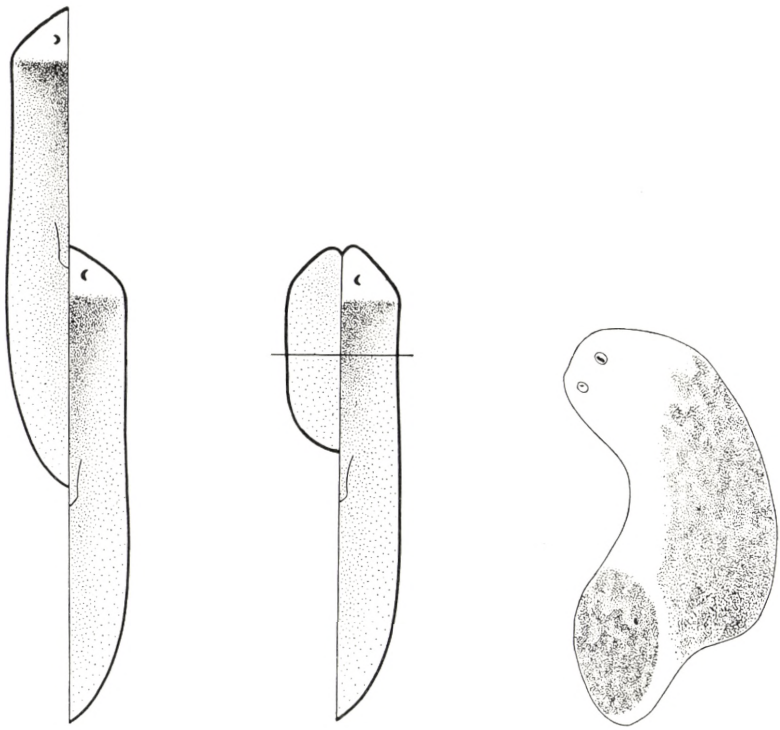


Fig. 11. *Euplanaria lugubris*. *a* shows that the lengthwise transplantation has been made with a shifting, covering about half the length of the animal. *b* shows the transsection after the forepart of the left half-animal had cytolized. *c* gives the result after 15 days of regeneration, only the right half has regenerated eyes.

days, the right piece with its high regeneration rate developed one larger right eye and a smaller left one. 11 days later, the situation was unchanged, no regeneration was observable in the left piece (Fig. 12 *b*).

Example d. The two halves were transplanted as in Fig. 13 *a* (i. e. almost in the same way as specimen a in Fig. 9). The free anterior part of the left half cytolized and the foreparts of the two halves did not coalesce (Fig. 13 *b*). The first transverse cut

was made about $^1/_4$ down in the right half and about half way down in the left one. Seven days after decapitation, the right half had regenerated two eyes, the right one being the larger. The left half had not regenerated any eye. Fig. 13 *c* indicates the situation after 16 days. Subsequently, the chimera was cut again (Fig. 13 *b*). The right half was met about midway, the left

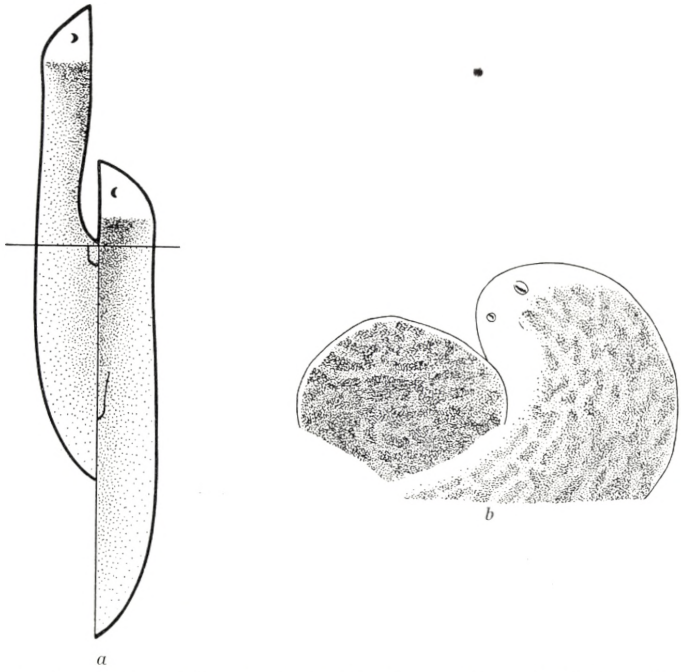


Fig. 12. *Euplanaria lugubris*. *a*, the experiment; *b*, the result which is the same as in fig. 11 in spite of the fact that the left half animal retains its integrity throughout the healing period.

one about $^3/_4$ down in the field. No cooperation had taken place; only the right half has regenerated two eyes, the right one being slightly larger than the left one (Fig. 13 *d*).

Example e. The two halves of four animals were transplanted; the fields were slightly shifted, about $^1/_6$ of the length of the specimen (Fig. 14 *a*). 72 hours after transplantation the foreparts were removed as indicated by the line. Ten days later, an almost symmetrical head had regenerated, the right eye being only slightly larger than the left one, indicating the slightly higher regeneration rate of the right field where the cut had hit (Fig. 14 *b*).

Example f. The two halves of three animals were transplanted with a very slight transposition, only about $^1/_{10}$ of the length of the animal (Fig. 15 *a*). 100 hours after transplantation a cut was made, as indicated by the line. Four days later, the common blastema had developed two symmetrical eyes, the right one being slightly larger than the left one (Fig. 15 *b*). Another trans-

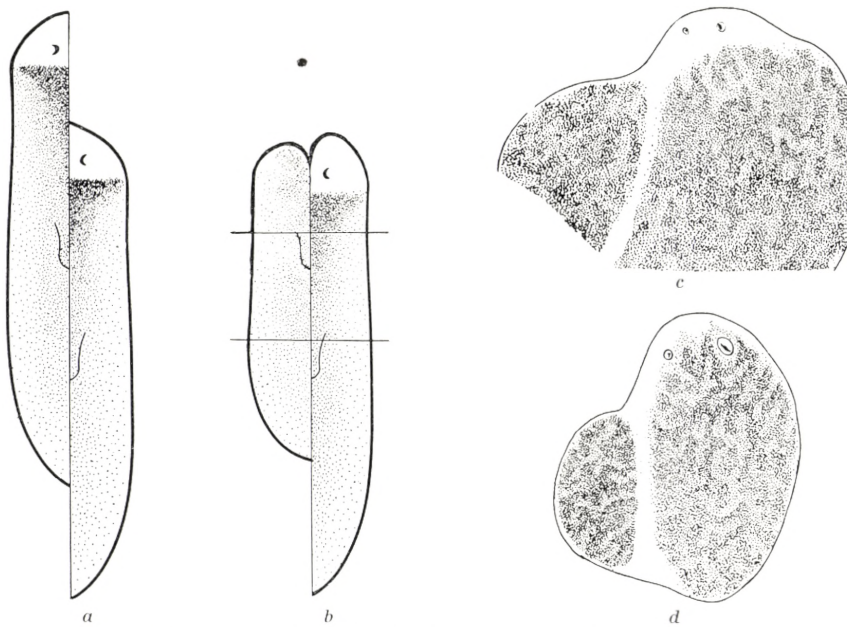


Fig. 13. *Euplanaria lugubris*. *a*, lengthwise transplantation as in Fig. 9 a; *b*, the anterior transverse cut was made somewhat more posteriorly than in Fig. 9; *c*, the result of this cut; *d*, the result of the more posterior cut seen in *b*; in spite of common blastemata, both *c* and *d* show eye formation only by the right half.

plantation was made at the same level and showed the same result, although no difference in the size of the eyes was detectable.

Example g. The transplantation was made in the same way as in specimen f; the two halves coalesced only along a small part of the hindbody (Fig. 16 *a*). 100 hours after transplantation the cut was made. Five days later, a common blastema had developed (Fig. 16 *b*); ten days later, a trace of a right eye could be observed, and four days later, two symmetrical eyes had been regenerated, the right being a trifle larger than the left one (Fig. 16 *c*).

Example h. The same transplantation as in *f*; also here the halves coalesced only slightly. A cut was made in about the same place as in *g*, but the wound contracted so much that no wound surface was exposed and, hence, no blastema formation took place. Nine days later, a new cut was made a little behind

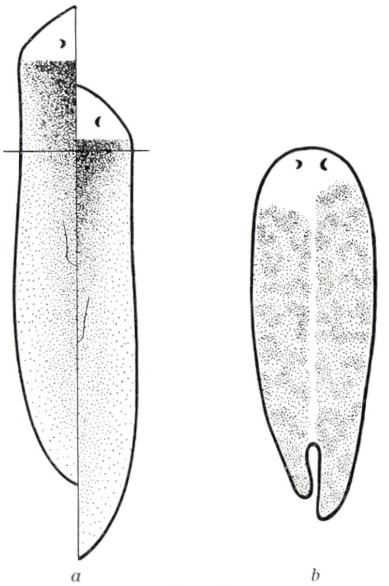


Fig. 14.

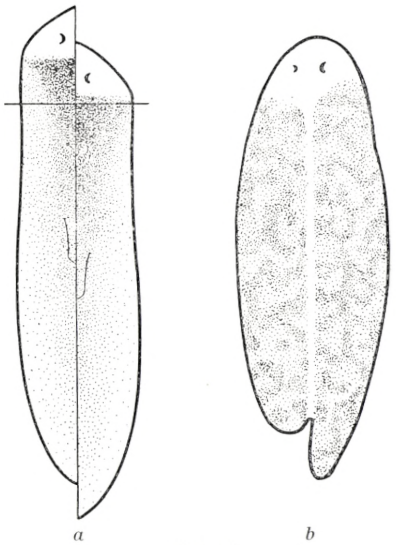


Fig. 15.

Fig. 14. *Euplanaria lugubris*. *a*, the shifting covers about $^1/_6$ of the animal's length; *b*, a bilateral symmetrical head is regenerated, the right eye, however, being slightly larger than the left one, due to the fact that the right half has been met at a level with higher regeneration rate than the left one.

Fig. 15. *Euplanaria lugubris*. *a*, shifting only about $^1/_{10}$ of the animal's length; *b*, the result as in Fig. 14.

the first one in order to obtain an exposed surface (Fig. 17 *a*); again the wound healed up and no blastema formation took place. 17 days later, another attempt was made by inserting two cuts (Fig. 17 *b*), but this was not successful either.

Similar transplantations were made on *Bdellocephala punctata*; only one of the operations was successful. The two halves were shifted about $^1/_5$ of the length of the animal. Six days after decapitation, a common blastema had formed one large right and one small left eye.

III. Parabioses and the Time-Graded Field.

In a previous paper (BRØNDSTED 1942), it has been shown that two longitudinal halves from different specimens, when joined and decapitated, form a common blastema with two

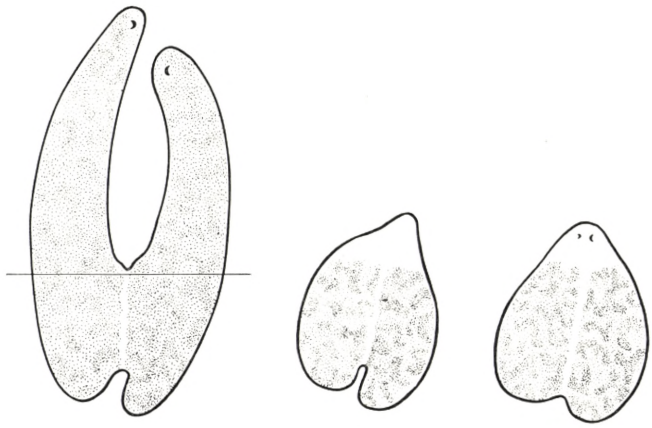


Fig. 16. *Euplanaria lugubris*. *a*, the shifting covered only about $^{1}/_{10}$ of that in Fig. 15; only the posterior third of the halves coalesced; the transverse cut was made as indicated by the line; *b*, the situation after 5 days; *c*, after 10 days; the right eye was a trifle larger than the left one; thus, also at this posterior level, the field displays itself.

symmetrical eyes. We were interested in learning the reaction of two whole animals to transplantation and subsequent decapitation.

The operation was carried out in the following way. In nicotine anesthesia, a longitudinal lateral brim was cut away on either the right or the left side of two animals, and the two pieces were united immediately.

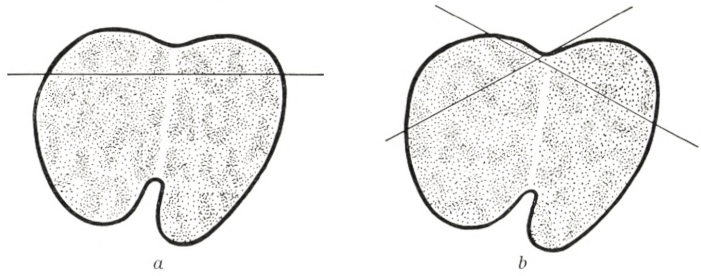


Fig. 17. *Euplanaria lugubris*. *a*, hind parts of an animal treated as in Fig. 16. A transverse cut resulted in closing up of the wound. *b*, two cuts made as indicated had the same effect, therefore no regeneration followed.

In a first series, 16 specimens of *Planaria torva* were treated as shown in Figs. 18 *a*—*b*. The fields of the two individuals were at the same level. Only one out of eight pairs survived; it was decapitated (Fig. 18 *b*) five days after transplantation. Four days later, the right "twin" had regenerated two eyes, the left one only one small eye, but four days later again, both animals had developed a head, each having two symmetrical eyes (Fig. 18 *c*).

In a second series, 6 specimens of the hardier *Euplanaria lugu-*

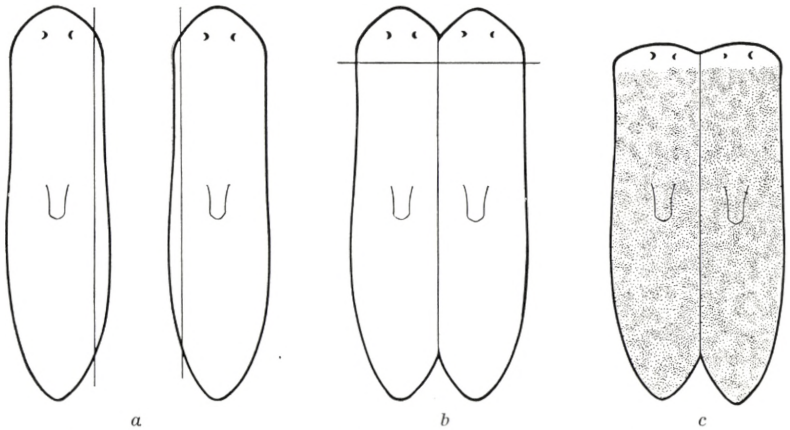
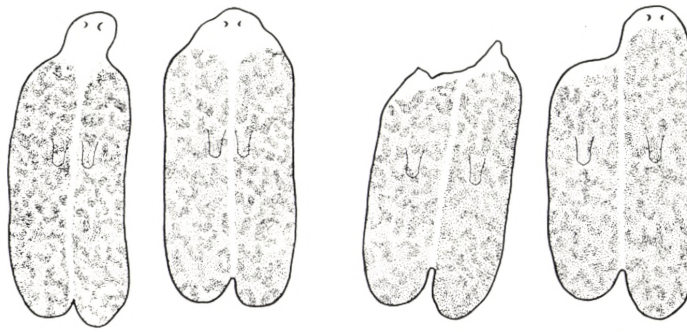


Fig. 18. *Planaria torva*. Parabioses. Narrow lateral brims were cut away from two animals, *a*; the specimens transplanted and decapitated, *b*; each animal formed its own blastema, in each of which two symmetrical eyes were regenerated, *c*.

bris were treated in the same way; they formed three pairs. The first pair was decapitated on the fourth day after transplantation. Three days later, a small blastema had developed, and six days after decapitation two symmetrical eyes in the common blastema had been regenerated, the right one being slightly larger (Fig. 19 *a*). The second pair developed in just the same way, however the two eyes did not differ (Fig. 19 *b*). On the fourth day after decapitation, each of the "twins" in the third pair had formed a blastema, although they were confluent; the right one was larger than the left (Fig. 19 *c*). In this pair, the pharynges were further apart than those in the first and second pair, indicating that narrower lateral strips had been cut away before transplantation. On the sixth day, the right animal had regenerated two eyes of equal size, whereas in the left animal no eye could be seen, and its blastema had not become larger. This situation

remained unchanged during one month, during which time the right animal had developed normally (Fig. 19 *d*), while the left animal still had no head.

The first and second pairs exhibited a continuous process of coalescence of the two animals, which appeared from the pharynges that approached the bisector more and more and almost met after a month, when they were fixed for histological examina-



a *b* *c* *d*

Fig. 19. *Euplanaria lugubris*. Rather broad lateral brims were cut away from four animals, which were then transplanted pairwise parabiotically. In both pairs a common blastema developed after decapitation, and in both only one head was regenerated; in *a*, the right eye was slightly larger than the left one. In *c*, more lateral brims were cut away than in Fig. 19 *a*. Hence each of the animals formed blastemata, but they were confluent. Only in the right animal a head was regenerated, *d*, presumably due to an inhibiting influence from the right to the left part of the common blastema.

tion. We are here confronted with a most fascinating phenomenon: two adult individuals merging into one by morphallactic processes. It would certainly be desirable to investigate this phenomenon in greater detail.

IV. Spreading of an Inhibitory Influence from the Median "High-Point".

The notion of a time-graded regeneration field consisting of separated points with different rates of regeneration presupposes that the high-point, exposed by the cut, acts in two ways. In the first place, it "organizes" the neoblasts into head and eye formation and, in the second place, it inhibits the formation of similar structures in other parts of the blastema, preventing them from developing their potencies for head formation.

The investigation of the subtly balanced biochemical processes involved in this phenomenon would be premature. However, suitable experiments might elucidate some details of the propagation regarding the postulated inhibitory influence from the high-point, especially with regard to time.

110 specimens of *Dendrocoelum lacteum* were cut in the way

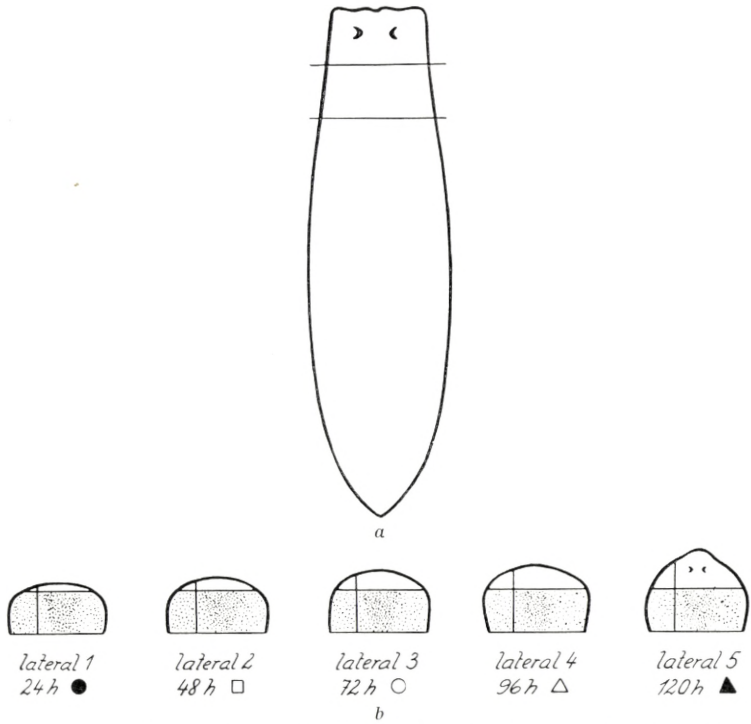


Fig. 20. *Dendrocoelum lacteum*. Representation of cuts mentioned in the text. *a*, after decapitation a transverse segment is cut out. *b*, segments with growing blastemata; the stippling indicates the time-graded field. Every 24 hours new groups of 15 individuals had their left third cut away.

indicated in Fig. 20 *a*. 10 segments served as controls, the remaining 100 segments were divided into five lots of 20 pieces each.

If an inhibitory influence from the median high-point prevented lateral parts from regenerating eyes, it should be possible, by separating the lateral parts from the median ones at different intervals, to obtain some information about the time required for the inhibitory force to travel laterally through the blastema.

The cut shown in Fig. 20 *b* separated, as sharply as pos-

sible, one lateral third of the segments from the remaining two thirds. It had to be made outside the area of normal eye-regeneration so that no tissue of the median high-point was included in the lateral third piece. On the other hand, the cut must not be made too far laterally in order to avoid too high mortality of the small pieces. Variations in the rate of regeneration cannot be completely excluded, since it is of course impossible exactly to hit the desired level of the regeneration field in all pieces; lots of 20 specimens should, however, provide a reliable mean value.

Lot 1 (Fig. 20 *b*) was cut 24 hours after decapitation, the other lots at intervals of 24 hours. The death rate of the lateral pieces was high, about 25—30 %. Several pieces bent so much towards the cut side that the wound closed completely and regeneration was inhibited.

The animals were placed in a dark room at a constant temperature of 20 ± 0.5° C. Regeneration was regarded as established when eyespots could be seen at a magnification of 25 in standard illumination. It should be remembered that the part of the segments which includes the median high-point regenerated two eyes, since it comprises both the left and the right parts of the bilateral field; on the other hand, the lateral parts regenerated first one eye only, e. g. that belonging to the corresponding part of the lateral field; only later is the symmetrical field built up as indicated by the regeneration of the second eyespot. In the lateral pieces, regeneration was of course considered complete when the first eyespot could be discerned (Fig. 21).

The median parts (72 survivors of 110 specimens, including 10 controls) regenerated two eyes after 170 hours, most of them (96 %) already after 140 hours. The lateral parts of lot 1 (9 survivors), lot 2 (8 survivors), and lot 3 (10 survivors) regenerated eyes at almost the same rate. Lot 4 (9 survivors) and lot 5 (10 survivors) exhibited a greatly retarded regeneration rate.

The inhibitory influence has reached the lateral piece before visible eyespots have been regenerated in the median piece. The inhibition is increased only slightly in the interval of 96—120 hours, which is about the time required for production of visible eyes in the median high-point. We shall see below that, when eyespots are regenerated, the time-graded field is restored, i. e. regeneration starts in every part of the new field at a normal rate.

Although the available material of *Planaria torva* was insufficient, an experiment was attempted on 50 animals. It would not be safe to base a hypothesis concerning the propagation of inhibitory forces on such a poor experiment, but in connection with the experiment on *Dendrocoelum* it might be permissible to regard it as a support of the hypothesis. The 50 animals available were divided into five lots and treated in exactly the same way as described above. In *Planaria torva* the median pieces regenerated two eyes at a somewhat lower rate than in *Dendrocoelum*.

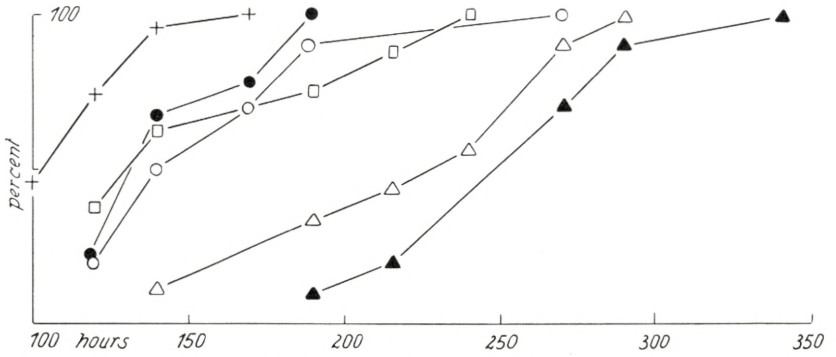


Fig. 21. Comparison with the symbols in Fig. 20 shows how the regeneration is retarded in the lateral pieces from those groups which were separated first after 96 and 120 hours.

Mortality and distortion of the lateral pieces were very great. Lot 1 (2 survivors), lot 2 (2 survivors), and lot 3 (5 survivors) also here regenerated eyes at a normal rate, whereas lots 4 and 5 (2 survivors each) were retarded.

A third experiment was carried out on *Bdellocephala punctata* in order to find out whether the result was changed if the lateral pieces were not removed from the main body during regeneration. 95 animals were decapitated at an anterior level. A lot (no. 0) of 10 specimens was cut as shown in Fig. 1 and employed as controls for the regeneration rate of "arms". This operation removes the median high-point with its inhibitory influence. 75 specimens were divided into 5 lots of 15 each (Fig. 22); they were operated upon in the same way, at 24 hours intervals after decapitation. 10 animals, which were only decapitated to serve as controls for normal median regeneration rate, had regenerated two eyes after 110 hours. It may be mentioned here that *Bdel-*

locephala, when decapitated at an anterior level, regenerates eyes at a higher rate than *Dendrocoelum*, a fact which is important for the evaluation of the results in Fig. 23.

Experiments of this type involve some difficulties. The lateral "arms", resulting from the removal of the median part, frequently detach themselves from the main body, curl up, distintegrate or close their wounds so that no regeneration occurs. In other cases, the two arms stick together and must often be separated repeatedly, a procedure which has to be performed very carefully so

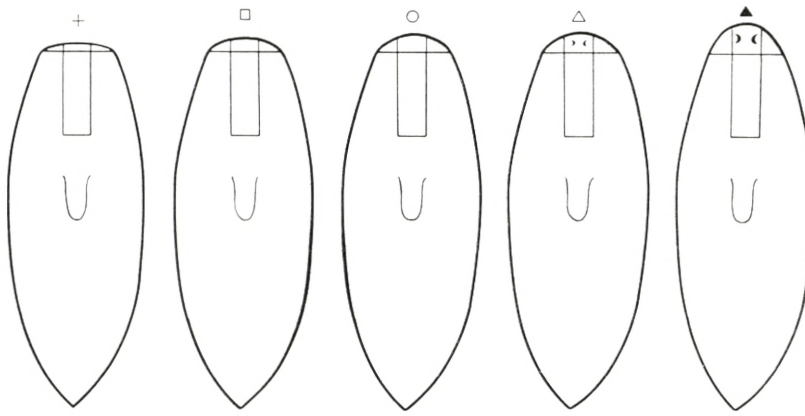


Fig. 22. *Bdellocephala punctata*. The median part was cut away after 24 hours +, 48 h. □, 72 h. ○, 96 h. △ and 120 h. ▲.

that no newly formed blastemata are removed. A fine glass needle with a little bead instead of a point proved to be a very well suited tool. Although 15 animals were operated in each lot, thus providing 30 "arms", only 48 out of 150 were useful. The percentages given in Fig. 23 refer to the number of arms. Lots 0—2 regenerated at about the same rate; lot 3 showed a somewhat retarded regeneration, while the regeneration of lot 4 was highly retarded; lot 5, however, was again but slightly retarded in regenerating. From the figures it seems reasonable to conclude that in *Bdellocephala* the inhibition from the median part reaches the lateral parts earlier than in *Dendrocoelum*; this is in agreement with the fact that eye regeneration proceeds at a higher rate in *Bdellocephala*. But how can the recovered regeneration rate in lot 5 be explained? We know that the time-graded field is restored during regeneration. Thus, in lot 5, the eyes in the

median segments have been formed already for several hours and therefore the regeneration rate of the lateral segments increases, at least nearly, to that of the normal, intact animal.

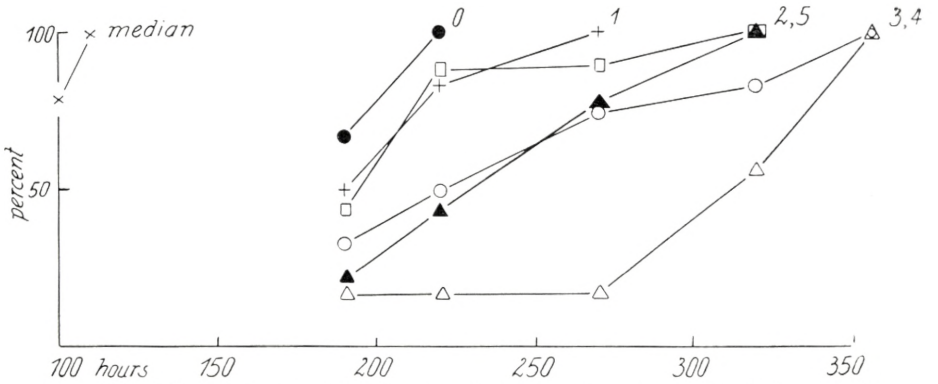


Fig. 23. *Bdellocephala punctata*. Results of the experiments represented in Fig. 22.

Furthermore, when the eyes are regenerated, the intactness of the animal is restored and only slight inhibitory forces are in action, i. e. those still present from the period when the blastema released such forces. The faster regeneration rate of lot 5 may therefore indicate that normalcy is restored in the animals after 120 hours of regeneration, as illustrated schematically in Fig. 24.

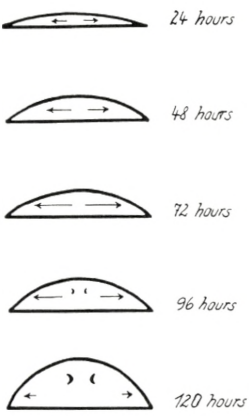


Fig. 24. Migration of inhibition in the blastema, the effect being greatest from 72—96 hours of regeneration (at an anterior level in the time-graded field), declining to zero when head formation has been fully established. The arrows indicate the direction and force of the inhibitory influence emanating from the median high-point at various times after decapitation.

V. The Rate of Regeneration Expressed by Differentation is Dependent on Factors in the Time-Graded Field; the Later Growth of Differentiated Parts is Dependent on Available Neoblasts.

It has earlier been shown (A. and H. V. BRØNDSTED, 1954) that the first discernible eyespots appear at the same rate in short segments and in whole decapitated animals, provided that

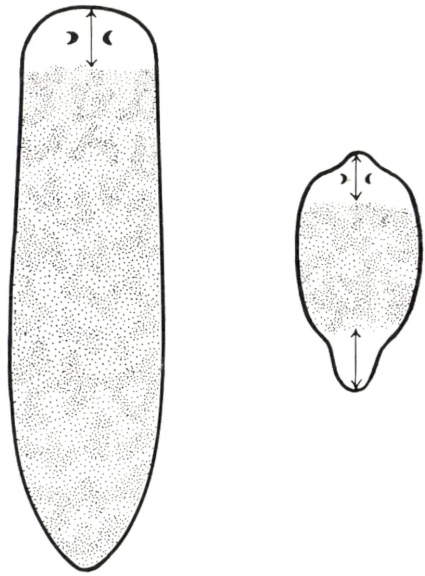


Fig. 25. *Euplanaria lugubris*. Regeneration from identical levels starts at the same time in decapitated animals and in short transverse sections; in the bigger piece with more neoblasts the bulk of regenerated parts is larger.

the cuts were inserted at the same level of the time-graded field; they appear also at the same rate even if large lateral pieces are cut away from the animal. This suggests the assumption that neither the size of a regenerating segment nor the extension of the wound is decisive for the rate of eye regeneration, the decisive factor rather being inherent in the various levels of the time-graded field. When blastema organization is terminated, the organs develop and continue to grow until they have reached a size conforming to the remnant body. This assumption has been confirmed by measurements performed on animals used in our earlier experiments (1954).

Fig. 25 shows two types of regenerating pieces from *Euplanaria lugubris*, viz. on the left side, I, the decapitated animal, on the right side, II, a short segment in which the anterior cut is inserted at the same level as in the decapitated animal. Eyespots regenerated at the same time in I and II. Four days after decapitation all eyespots could be seen, indicating that organization of the blastema was terminated. Four days later, the animals were fixed in formalin in order to enable exact measurements. At that time, the specimens in group II had regenerated short tails. We have measured the longitudinal diameters of the left and right eyespots in the 20 specimens of group I, and the distances between the anterior tip of the blastema and the foremost brim of the old pigmented part of the body. In II, also the distances between the tail tip and the hindmost brim of the pigmented part were measured.

Fig. 26. *Bdellocephala punctata*. The lines indicate the cutting intending to diminish the flow of neoblasts to the transverse anterior wound (BRØNDSTED, A. and H. V., J. Embr. exp. Morphol. 2, 1954, fig. 2b.)

As longitudinal diameters of the eyespots we found, in I, $23.12 \pm 0.59\ \mu$, in II, $15.52 \pm 0.53\ \mu$. The distance between the anterior tip of the blastema and the forebrim of the pigmented part of the body was found to be, in group I, 1.44 ± 0.04 mm, in group II, 0.96 ± 0.04 mm. The distance from the tail tip to the pigmented part was measured to 1.44 ± 0.07 mm in group II. Statistical treatment showed the significance of the differences in the values.

An experiment on *Bdellocephala punctata* was carried out in order to find out whether large wounds retard the regeneration of eyes. Fig. 26 shows that the eyes were regenerated at the same rate in animals without lateral wounds, but decapitated at the level of the time-graded field, and in animals with large wounds. The animals were fixed in formalin when all pieces had regenerated eyes. The values found are as follows. Average longitudinal diameter of the eyes in lot I (without lateral wounds) $41.9 \pm 1.82\ \mu$, in lot II (with lateral wounds) $35.22 \pm 2.11\ \mu$. Also this difference proved to be significant. The measurements seem to indicate that the growth of the eyes, after first differentiation, depends on the supply of neoblasts.

The difference between the values found for eye size in *Euplanaria lugubris* and in *Bdellocephala*, in groups I and II, is due to the fact that *Euplanaria lugubris* had been allowed four days of growth before fixation and measurement.

VI. Remoulding of the Time-Graded Regeneration Field during Regeneration in Polycelis nigra.

Abeloos (1930) observed that, when regenerated tissue is induced (by a new cut) to undergo a second regeneration, the rate of regeneration corresponds to that of the new-formed level in the segment in which the cut was made. Sivickis (1933) stated that, when anterior parts of the body have accomplished regeneration and thus restored the whole animal, the rate of regeneration of the various segments of the new body corresponds closely to that in an untreated animal and not to that of the original segment from which second regeneration has started. This finding is in agreement with the results obtained by Child and Watanabe (1935).

Some authors seem to have regarded this phenomenon as an indication of rejuvenation of the whole animal caused by the regeneration process proper. I have also frequently observed this phenomenon in experiments on planarian regeneration. After having detected the time-graded regeneration field (Brøndsted 1946), I suggested that this field might be remoulded during regeneration at the same time during which observable morphological regeneration occurs.

A cut *in the blastema or the regenerated tissue* of course traverses embryonic or at least young tissue; hence it is plausible that repeated regeneration proceeds here at a higher rate. Now, the problem arises whether the *old parts* of the body are influenced by the regeneration process in such a way as to alter the regeneration rate at a certain level. Since it is well known that the regeneration rate is a physiological property characteristic of every part of the planarian body, we may now ask: is this physiological property of adult tissue changed by regeneration processes? A quantitative investigation is necessary before this feature of the time-graded regeneration field can be elucidated in greater detail.

Polycelis nigra was chosen for our experiments. This planarian

species is known to have a caudally declining head frequency curve, and it may be assumed that, correspondingly, the time required for head formation increases caudally.

For the regeneration of eyes at six consecutive levels of the body, SIVICKIS (1931) found the following rates (in hours): 104, 124, 139, 151, 181, 175. Although our experimental animals had been collected in another locality than SIVICKI'S—both in our earlier studies (BRØNDSTED 1942) and in the present investigations, we used planarians from the Furesø, a lake in the vicinity of Copenhagen—the head frequency curves are largely similar.

Despite the fact that, in 1942, I had not yet been fully aware of the existence of a time-graded regeneration field in planarians, I was able to show that the regeneration along the main axis of the forepart of the body of *Polycelis nigra* follows the laws of such a field, presumably on almost the same lines as that of *Euplanaria lugubris* (A. and H. V. BRØNDSTED, 1953).

So far, the exact shape of the time-graded regeneration field of *Polycelis nigra* has not yet been worked out. However, SIVICKI's results, as well as my earlier experiments (BRØNDSTED 1942) and the findings of my control series 0 and I in the present paper, indicate that the time required for eye regeneration after a transverse cut increases the more, the more caudally the cut is inserted. This makes it probable that an antero-caudal axis of a time-graded regeneration field declining caudally does exist in *Polycelis nigra*; for the present investigations, it is not necessary to know the lateral extension of this field.

We have chosen *Polycelis* not only because this species possesses an antero-caudal time-graded regeneration field, but in addition because the great number of eyes in this species is convenient for estimating the degree of regeneration, and the position of the eyestrings makes it possible to perform sectioning rather accurately at a certain level.

Method.

115 specimens were cut transversely just behind the eyestring, as indicated by *a* in Fig. 27. Fifteen were kept for regeneration (control series 0), fifteen were immediately cut again at the level indicated by *b*, about 0.5 mm behind cut *a* and anterior to the

pharynx (control series I). The animals in the experimental groups were cut a second time at the same level as control series I, but at varying times after the initial operation, viz. 2 days (group I), 4 days (group II), 6 days (group III), 7 days (group IV), and 8 days (group V).

The aim of these experiments was to find whether, after removal of the regenerated part at a certain level of the old tissues at different periods after the first cut, regeneration started earlier from the new cut than in control series I in which the cut had been inserted at the same level. If this were true, a clear proof had been provided for the remoulding of the time-graded field in its antero-caudal axis.

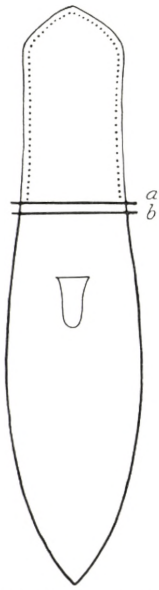


Fig. 27. *Polycelis nigra*. Decapitation *a*. Cutting away the forthcoming blastemata at *b* after varying times.

The experiments involve certain difficulties, insofar as no eye regeneration takes place if the transverse cut is closed by a strong contraction of the body. If the wound closes partially, eye regeneration proceeds at a slightly lower rate than with an open wound. For the estimation of the regeneration rate, animals with closed cuts had to be discarded. A further difficulty arises from the movements of the worms, which make it hard to insert the cut at exactly the desired place. Narcotization, to prevent movements, in general causes a strong contraction. If the worms are placed in very little water and put on a wax plate, a fair degree of exactitude can be reached in the operation. Nevertheless, slight deviations from the desired level cannot be avoided, differences in the level appearing as differences in the time required for eye regeneration. Therefore, a certain minimum number of animals in each group—at least 10 to 15—must be operated in order to secure a reliable mean value of the interval between the operation and the appearance of the eyes. Since counting of the eyes is very time-consuming, the handling of larger groups is not feasible.

The eyes were counted by means of a binocular dissecting microscope, magnification 25 ×, with standard illumination; light

from above proved to be most suitable for providing reliable counts. The animals were kept in Petri dishes filled with 50 ml tap water at a temperature of 20° C ± 0.5°; during the experiment, the animals in the Petri dishes were placed on white paper as a background and kept in the dark during regeneration.

The number of eyes formed in each specimen was counted daily. For each day, the total number of eyes in each group was divided by the number of living animals, giving a mean value of the number of eyes regenerated per animal.

Results.

The curves drawn in Fig. 28 show our results. The ordinates represent the average number of eyes per animal, the abscissae the time (in hours) elapsed until their regeneration.

In spite of the irregularity of the curves, they exhibit one main feature, viz. the new-formation in the adult tissues of the antero-caudal axis of the time-graded regeneration field during regeneration. The curve for control series 0 represents the regeneration rate of eyes at a transverse level just behind the eyestring; the curve of control series I at a level a little more caudally. Curves I—V show that the time required for the regeneration of a certain number of eyes decreased by about 80 hours from

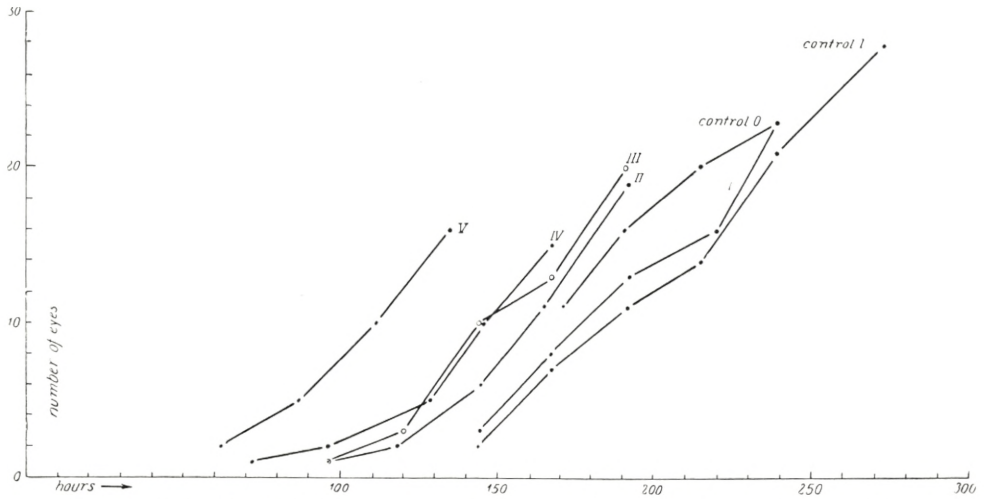


Fig. 28. *Polycelis nigra.* Eye formation in various groups. Explanation in the text.

control series I to the experimental group V. If no change in the antero-caudal axis of a time-graded regeneration field had occurred in the original tissue, eye formation should proceed at the same rate in the five groups as in control group I, e. g. in the course of 220 hours. However, our experiments show that when a new cut *b* is made after 8 days regeneration from a cut *a*, level *b* requires only 135 hours to regenerate the same number of eyes as had regenerated by level *b* in control I after 220 hours. It is therefore obvious that not only does a morphologically discernible regeneration occur from cut *a* of the removed part, but in the original tissues also a process of remoulding takes place of the structures and functions underlying the phenomenon of the antero-caudal axis of the time-graded regeneration field. In the course of 2—8 days regeneration in the various groups, level *b* has adapted its rate of regeneration to that of a far more anterior level; during regeneration of a forepart, the morphological level *b* acquires the physiological properties characteristic of more anterior levels. This indicates that the morphological level *b* is physiologically adapted to the much shortened main axis and, thus, the main axis acquires its property of being evenly time-graded.

In another investigation performed on *Bdellocephala punctata*, it could be shown that six animals which had been cut transversely, rather caudally in the time-graded field, had developed eyes in the blastemata only 11 days later. When a new transverse cut separated the blastemata with the newly formed eyes, already after five days eyes could be seen in the new blastemata of 5 of the 6 animals. This fact indicates that the time-graded field was reconstructed during restitution of the head by the blastemata.

VII. Discussion.

All our experiments indicate that time is the decisive factor in morphogenetic processes. The adult planarian is provided with a finely balanced trigger mechanism just like a ripe oocyte not yet activated. Activation is first set to work when a piece of the body is removed—just as embryogenesis in an egg starts when fertilization has occurred.

From the papers by Wolff and Dubois (1948) and Dubois (1949) it is known that the wound calls forth a migration of neoblasts to the wound place. The chemical mechanism involved in this migration is however unknown so far. Needham (1952) has discussed some factors which possibly are responsible for this process. In a marvellous way, the neoblasts building up the blastema participate in an organization process which in due time not only restores the missing parts, but by means of morphallaxis transforms some of the size of the adult tissues to such a degree that harmonious proportions of the animal are recovered in agreement with its reduced size. Thus, the adult planarian body may be conceived as an analogue to an egg of the regulation type, however possessing one advantage over the latter: whereas the egg in the course of time crystallizes into an organism losing more and more of its regulatory capacity, the planarian body retains this capacity.

In the study of planarian regeneration, we are thus dealing with highly embryonic organisms, in spite of the fact that we are concerned with adult animals. The neoblasts in the planarian body correspond in a certain way to the blastomeres in the blastula, with the difference, however, that in the blastula some of the blastomeres themselves are the determinators, whereas in the planarian body the totipotent neoblasts are determined by the adult tissues, at least in their main polarity; they must adapt to the existing body parts.

The elucidation of this phenomenon must be reserved to distant biochemical studies. The established fact of the time-graded field may nevertheless be a first step towards this goal.

The time-graded field is a physiological property firmly inherent in some fixed structure of the body, a fact which seems to be proved definitely by our transplantation experiments (cf. Section I). This property is specific of the species and is restored during regeneration according to the principles of restoration (regeneration) valid for all other species-specific characteristics of the animal; this seems to be proved by the experiments discussed in Section VI. No explanation of this phenomenon is available at the present time; the assumption may be put forward that the restitution of the relative number of neoblasts is responsible. For future regeneration, the restoration of the field is im-

perative, since a harmonious regeneration is secured just by the properties of the field.

This interpretation of the results recorded in Section VI appears to me much more plausible on the basis of our actual knowledge about planarian regeneration than a hypothesis of rejuvenescence of the regenerating animal.

When forming the blastema, the neoblasts start as totipotent cells, e. g. their capacity of regenerating a head is the same. In the ensuing competition, it is the time-graded field which decides the result. We still do not know its mechanism, but we assume that the activities in the area of this field are the more lively, the more densely the neoblasts are packed; this means, the earlier will the differentiation processes start. However, this fact alone cannot explain the finding that other parts of the body might differentiate in just the same way, although at a lower rate. An inhibitory action emanating from the most vigorously differentiating part must be assumed. The existence of such inhibitory forces is, to my view, proved by the experiments discussed in Section III.

In broad outline, we now realize the mechanism which secures a harmonious regeneration, although the differentiation processes themselves cannot yet be understood. A future comparison of the biochemical processes in the gastrula with those in the blastema may provide further evidence. As a first approach, the following hypothesis is suggested.

We know that the prospective ventral ectoderm in the amphibian gastrula can be induced to form neural tissue: it has neural potencies. Why are these potencies not developed in the normal gastrula? In the normal gastrula, inhibitory influences emanate from the prospective neural area in step with the determination and differentiation of the nervous system. Just the same mechanism—a time-graded morphogenetic field—is exhibited by the amphibian egg as by the planarian blastema. To my view, the formation of the axis in the oocytes is in fact a building up of the time-graded field.

In the planarian body, the time-graded field conveys its properties, so-to-say, to the blastema. Therefore, both in the egg-blastula-gastrula and in the blastema a high-point starts morphogenesis by organizing *and* inhibiting.

Another analogy may be mentioned: Ribonucleic acid (RNA) plays an important part in morphogenesis. In the amphibian egg, the high-points (dorsal parts) are characterized by a higher content of RNA. In the blastema, the neoblasts are charged with RNA (own investigations, unpublished) and it could be shown (A. and H. V. BRØNDSTED, 1953) that RNA accelerates regeneration in planarians.

The concept of the time-graded field may also lend some new perspective to the problem of bilaterality. Two symmetrical halves of a bilateral organism react in fact like two animals working harmoniously together. Only if they check each other's latent ability to produce their own mirror image, they are able to do so. A latent inhibition is thus continuously at work. Experiments have been performed in order to support this assumption. When separating an animal into two halves, each half will regenerate its symmetrical half. If, however, after separation the two halves are reunited in their former position, no regeneration occurs. If two reunited animals are decapitated, both halves develop a confluent anterior blastema in which each half regenerates an eye belonging to its own side, in spite of the fact that each half blastema has the power to regenerate a symmetrical eye, and irrespective of the amount of old tissues transplanted together (Fig. 29; BRØNDSTED 1942). A cooperation between the forces built up in the symmetrical parts of the common blastema is possible only if their rates of regeneration are not too different (cf. Section II). This finding again underlines the vital importance of the time-graded field. Theoretically, the reaction of the two time-graded fields may occur in three different ways. (1) The two fields are shifted very slightly so that one of the fields, that with the highest rate of inhibitory forces, did not have time enough to inhibit the regeneration of the other half's eye. If both halves have started in due time to inhibit each other's ability to develop a symmetrical eye of its own, a bilaterally symmetrical head is regenerated. The eye of that half which started regeneration somewhat earlier is of course somewhat larger in the beginning; later, however, full harmony is restored. Thus, the two fields are still able to cooperate. (2) The two halves have been shifted very severely so that the rate of regeneration in one of them is much higher than in the other. Then, its half

blastema will have time enough to regenerate not only its own eye, but also a new symmetrical one belonging to its own system. Simultaneously, the symmetrical part of the blastema is prevented from regenerating eyes (cf. Section III). Time is the factor determining this process. Only if the "weak" part of the blastema is so slow in regenerating that inhibitory forces are activated before it starts its own independent regeneration, the two fields cannot cooperate. (3) The fields are shifted so slightly that both halves of the common blastema, because of their sufficient regeneration rate, start redeveloping their own eyes. Actually, inhibitory forces emanate from both fields. If the shift, however, is made in such a way that the inhibitory forces from one half of the blastema are not allowed to reach the other half *before* the latter has started regenerating its own symmetrical eye, two heads will be regenerated.

These three theoretical conditions are corroborated by experiments. According to CHANDEBOIS (1950, 1952, 1953), a time-graded regeneration field exists also in marine Polyclads. She states that, in her experiments, regeneration proceeded after partial cytolysis in hypertonic seawater, resulting in a certain independence in the regeneration rate of the symmetrical halves. Her results are in good agreement with our above interpretation.

It would be interesting to investigate whether a time-graded field can also be observed in other organisms. This could be done by experimenting on a blastula or a young amphibian gastrula and, in a somewhat similar way, on chick blastoderm in its early stage. In these higher organisms, the time-graded fields may be so complicated that spectacular results might be expected.

The notion of time-graded fields differs, in my opinion, from the usual notion of morphogenetic fields in that the emphasis of morphogenetic differences is laid on the time factor in morphogenesis, although I am, of course, quite aware of the fact that the time factor has been drawn into consideration both in the comparison between, e. g., eggs of the regulation and mosaic type, and the formation and crystallization of a specific morphogenetic field. Our concept of the time-graded regeneration field, however, includes immanently the phenomenon of inhibition towards neighbouring fields which have the same possibilities, but lower rates of their realization.

In the beginning of this century, two eminent scientists, working with planarian regeneration, arrived at very diverging conclusions concerning morphogenesis. On the one hand, MORGAN held the view that proper regeneration is due to structural (qualitative) differences; CHILD, on the other hand, postulated that regeneration is determined by quantitative metabolic processes, a hypothesis which he extended to a comprehensive principle covering all morphogenesis: his well known gradient hypothesis.

To me it appears improbable that pure quantitatively graded metabolic processes should produce qualitative differences, such as those observed in all morphogenesis. Structural differences must first be created in the blastema, where they are given as *possibilities* immanent in every cell. In other, somewhat old-fashioned words, we may say: the differences are *preformed*, they possess a sum of reactive possibilities, hence a structure based on inheritance. So far I agree with MORGAN. But I think that the structural possibilities ought to be set to work by some kind of quantitatively graded influences, possibly by different levels of metabolic activities, according to CHILD's postulate. The concept of the time-graded field may be useful in providing means for a reconciliation of MORGAN's with CHILD's views.

In his brilliant studies on planarian regeneration, CHILD arrived at the conclusion that the head dominates and organizes. I was able to show that CHILD's opinion is not right (BRØNDSTED, 1939, 1942). It is true that it is a head which appears first in the blastema on a cranial wound, and analogously it is a tail which first appears at a caudal blastema. The bilateral time-graded field determines only the rate at which regeneration sets in. This evidence induced CHILD to the formulation of his hypothesis. Morphogenesis proper, however, is determined by quite other forces of much more subtle nature, which are intimately connected with molecular structures and functions and are independent of the metabolic level. These are the forces which led MORGAN to the statement of his views.

The elucidation of the molecular structures involved in the processes mentioned above is a very distant goal; it is conceivable, however, that a concept of the coarser material basis of the time-graded field may be found. To this purpose, two factors

present themselves immediately to consideration, viz. (1) the amount of neoblasts, and (2) the structure of the nervous system.

CURTIS and SCHULZE (1934) advanced the view that the smaller regeneration power in *Procotylon* and *Dendrocoelum* (lack of head regeneration from cranial wounds at levels behind the anterior $^1/_3$ and to $^2/_5$ of the body) is due to the smaller number of neoblasts in the body of these species compared to those with head regeneration powers throughout the body. The number given is not convincing to me. It is true that heads are not regenerated by posterior levels at cranial wounds, while tails are newly formed at the same levels from caudal wounds. Thus, it is plausible that not only neoblasts, but also other forces must be lacking in the hind parts. Moreover, the ratio of neoblasts in *Procotylon* and *Planaria* is given to 1:8 and, nevertheless, heads from anterior levels are regenerated at almost the same rate.

These incongruencies induced us to reinvestigate the problem. Thorough countings of neoblasts stained by pyronin-methyl green, toluidin blue, etc. were carried out in our laboratory. The results of these countings (shortly to be published) do not show any significant differences in the number of neoblasts in species with greatly varying time-graded regeneration fields.

The nervous system in planarians consists primarily of symmetrical head ganglia which give off nerve trunks running longitudinally from head to tail and are connected by transverse commissures. It might well be conceived that this system is responsible for the time-graded field. Against this view it might be objected that the time-graded field is not conform with the amount of nervous tissue, and the question may be raised why head regeneration should be restricted to the forepart of the body, despite the fact that the nervous system extends over the whole body.

In brilliant studies WOLFF and LENDER (1950) and LENDER (1951, 1952, 1954) were able to show that in *Polycelis* eye formation is dependent on the presence of head ganglia. But this does not solve the problem, it only shifts it from eyes to ganglia. The head ganglia must be regenerated, at least their rudiments must be differentiated before eye formation can start. Thus, we revert to our earlier statement that the rate of morphogenesis is dependent on some factor in the time-graded field. And again MORGAN's

view suggests itself: we have to deal with some hidden morphogenetic structure.

The question of polarity in connection with the time-graded field has been mentioned in my review of planarian regeneration (BRØNDSTED 1955). A more comprehensive discussion has to be postponed until the investigations in progress in our laboratory will be concluded.

Finally, the inhibition phenomenon may be touched upon (cf. Section III). Although the time required by the inhibitory force to traverse the body from the high-point to the lateral parts is roughly known, we are quite ignorant of the nature of these forces.

We know that these forces do not move freely through adult tissues; if they did, supernumerary heads could not be regenerated from other parts of an adult worm with an intact head (cf. BRØNDSTED 1939, 1942). The forces migrate only through embryonic tissue, the blastema. Thus, they only manifest themselves while organization is in progress. Therefore, I assume that they should be regarded as chemical stimulants in contact with one another from cell to cell. When a cell (*a*), responding to a given stimulus, is in the process of determination in a certain direction, it assumes biochemical properties of a very subtle serological kind; consequently, it becomes refractive towards its neighbouring cells (*b*) which may have received the same stimulus. Since, however, cell (*a*) has started first, it is also the first to gain the serological properties mentioned and, thus, it prevents its neighbouring cells (*b*) from acquiring exactly the same serological properties; these cells can therefore only reach differentiations of a "lower" kind.

This crude sketch is meant to indicate a possible way of approaching the inhibition problem. Elsewhere (BRØNDSTED 1954) it was suggested that the notion of the time-graded field may provide evidence for a reconciliation of the genetic totipotency of the cells with their diversification during morphogenesis.

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WOLFF, E. & LENDER, TH. (1950). Sur le rôle organisateur du cerveau dans la régénération des yeux chez une Planaire d'eau douce. C. R. Acad. Sci., Paris, **230**, 2238—9.

Added in proof:

LENDER, TH. (1956). Recherches expérimentales sur la nature et les propriétés de l'inducteur de la régénération des yeux de la planaire *Polycelis nigra*. J. Embr. Exp. Morphol. **4**, 196—216.

In this very interesting work, it is shown that a soluble substance presumably emanating from the brain, an "organisine", induces eye formation. The question of competence is not discussed.

Indleveret til selskabet den 17. marts 1956.
Færdig fra trykkeriet den 26. oktober 1956.

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SOME MARINE ALGAE FROM MAURITIUS

FINAL PART

BY

F. BØRGESEN †

EDITED BY

TYGE CHRISTENSEN

København 1957
i kommission hos Ejnar Munksgaard

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SOME MARINE ALGAE FROM MAURITIUS

BY

F. BØRGESEN

København 1940-57
i kommission hos Ejnar Munksgaard

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udgivet af
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Bind **23**, no. 4

Biol. Medd. Dan. Vid. Selsk. **23**, no. 4 (1957)

SOME MARINE ALGAE FROM MAURITIUS

FINAL PART

BY

F. BØRGESEN †

EDITED BY

TYGE CHRISTENSEN

København 1957
i kommission hos Ejnar Munksgaard

Synopsis.

Supplementary information is given on various algae discussed in the parts previously published. The whole series of papers, comprising 15 parts, is indexed.

Printed in Denmark.
Bianco Lunos Bogtrykkeri A-S.

Editor's Preface.

At the beginning of this year I undertook to assist the late Dr. BØRGESEN in his work on the final part of the series of papers dealing with marine algae from Mauritius. BØRGESEN's eyesight had become very bad at that time, and he found himself unable to do alone what was left to be done. On two short visits at his home I was told about his plans and received parts of his manuscripts for a closer study. At the time appointed for a third meeting he felt too weak to proceed, and shortly afterwards went to hospital, where he died on March 22nd, aged 90. Before then I had promised him to take care of the publishing alone, if necessary, by issuing the last articles together with an index to the complete series.

The whole text was available in the form of manuscripts and notes, mostly dating, it seems, from the end of 1954. My task has therefore been confined to that of fitting parts together and giving a slight finish to them, which has been done, of course, without interfering with the author's personal mode of writing.

BØRGESEN had begun an extension of the index accumulated from the various parts, to make it include a greater number of names and synonyms. This extension I have carried through, omitting only some very obsolete synonyms and such references where the algae in question are mentioned in the text without being either discussed or compared with species discussed, for instance when figuring as hosts of epiphytes.

The photographs are due to myself. In addition to those showing herbarium specimens I have taken leave to give two more, one to replace an unfinished verbal description of a cross section of *Spathoglossum asperum*, the other instead of a drawing referred to in BØRGESEN's text on *Vidalia fimbriata*, but apparently never made.

I hope I have done acceptably to this last product of a great authorship, which started nearly 67 years ago.

Copenhagen
Botanical Laboratory of the University

October 11th, 1956

TYGE CHRISTENSEN

PHAEOPHYCEAE

ISOGENERATAE

I. Dictyotales.

Fam. 1. ***Dictyotaceae.***

Spathoglossum (Kütz.) J. Ag.

1. **Spathoglossum asperum** J. Ag.

Alg. Mauritius, II, 1941, p. 48.

In 1954 I received some fine specimens of this species from Dr. VAUGHAN, having formerly seen only two small specimens which the late much regretted Dr. TH. MORTENSEN had dredged at the shore off the island.

The specimens, the bases of which are wanting, are bushy, up to about 20 cm high or more. They are divided in a very irregular manner into a great number of larger and smaller lobes; some of these are undivided, elongate-lanceolate, tapering downwards into a more or less acute base, often with a short stipe, and upwards with a more or less acute, sometimes also rounded apex; other lobes become broadened out and irregularly, more or less deeply, bi-tri-multi-furcate (Fig. 1).

Along the margins many of the lobes are provided with short, more or less densely placed spines; this is especially the case in two smallish specimens.

The surface of the thallus is smooth in the specimens I have examined. The surface cells, when seen from above, are found to be arranged in more or less distinct rows; they are approximately quadratic, about 30—40 μ broad, being rather like those found in a specimen gathered in Ceylon by FERGUSON.

Fig. 1. *Spathoglossum asperum* J.Ag. (× 1/4).

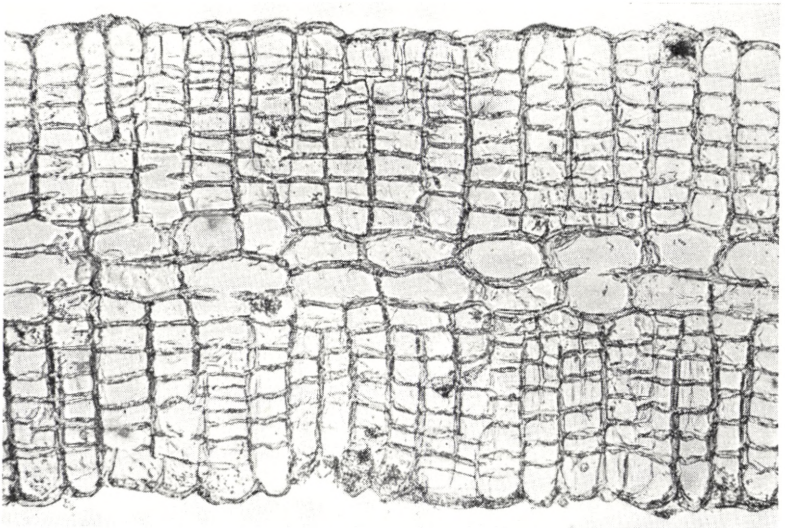

Fig. 2. *Spathoglossum asperum* J.Ag. Section through the lower part of the thallus (× 1000).

A transverse section of the lower part of the thallus is seen in fig. 2.

The specimens were found washed up by waves after storm.

Mauritius: La Cambuse, 3-1-54, G. Morin no. 1305 A.

Dictyopteris Lamouroux.

1. **Dictyopteris serrata** (Aresch.) Børgs.

Alg. Mauritius, Additional Lists, 1948, p. 47; Additions I, 1949, p. 25.

In the collection of algae received in the summer of 1954 two fine specimens of this species are found. One has a thallus about 2 cm broad. Its colour is yellowish brown. The other specimen has a thallus about 1—$1^1/_2$ cm broad, the colour of which is dark brown. Both plants are about 30 cm high.

As has been said earlier, *Dictyopteris serrata* is surely a sublittoral species growing in the rather deep sea; both specimens were found "washed into lagoon after storm."

Mauritius: La Cambuse, 3-1-54 and 20-1-54, G. Morin nos. 1306 and 1308.

RHODOPHYCEAE

FLORIDEAE

I. Nemalionales.

Fam. 1. ***Chaetangiaceae.***

Galaxaura Lamouroux.

1. **Galaxaura breviarticulata** Kjellm.

Alg. Mauritius, III, 1, 1942, p. 54, figs. 26-27.

In 1954 I received some beautiful specimens of this species from Mauritius, having formerly seen only a single sterile specimen collected by Jadin.

Kjellman placed *Galaxaura breviarticulata* in his sectio *Dichotomaria*, subsectio *Cameratae*. The species belonging to this group are always tetrasporic; the tetrasporangia are terminally placed upon a short stalk. Svedelius in his very valuable and detailed work: Zytologisch-entwicklungsgeschichtliche Studien über *Galaxaura*, Nov. Act. Reg. Soc. Sc. Ups. ser. IV vol. 13 no. 4, 1942, p. 139, places the species with this type of sporangia together in his sectio II: *Acrosporangiatae*.

The specimens form dense tufts up to about 10 cm high. The thallus has in a dried condition a greyish colour and a somewhat mealy surface. The joints are of a somewhat variable size, about 5 mm long and 2 mm broad.

As is said in the above-cited Part III, 1, 1942, p. 58, this plant is surely the asexual form of the species which, in its sexual form, was named *Galaxaura corymbifera* by Kjellman.

The specimens were found washed up by waves after storm.

Mauritius: La Cambuse, 20-1-54. G. Morin no. 1312.

II. Gigartinales.

Fam. 1. Nemastomaceae.

Titanophora (J. Ag.) Feldm.

1. Titanophora mauritiana Børgs.

Børgesen, F., On the Genus Titanophora (J. Ag.) Feldm. and Description of a new Species. Dansk Bot. Arkiv vol. 13, no. 4, 1949, p. 5, figs. 2—3 and pl. II.

In 1954 I received, besides a small specimen preserved in formalin and seawater, some very fine dried material of this species (Fig. 3) showing that it is a large stately plant.

The base, which was missing in the material formerly examined, is partly present in the large dried specimen, and in the small specimen preserved in formalin it is entire. It consists of a cartilaginous and very tough disc, from which several erect fronds of various sizes are given off. In the dried specimen, besides the large frond only one smaller frond issues from what is left of the disc.

The large frond has a thick flattened cuneate base, from both edges of which a number of erect, very polymorphic lobes are given off, the largest one reaching a length of about 40 cm. The large lobes are deeply subdivided into smaller very irregular ones, more or less narrowed at their base. Along the margins of these lobes proliferations issue, near the base simple and small, but upwards gradually larger and lobed themselves, each being a replica of the mother lobe. The proliferations are in most cases densely placed, separated by incisions which are rounded at the bottom; they are more or less curved and tapering towards their acute apex.

The colour of the specimens is a greyish rosy-red, and the surface is dull and mealy.

The specimens were found washed up by waves after storm.

Mauritius: La Cambuse, 3-1-54, G. Morin no. 1304 A.

Fig. 3. *Titanophora mauritiana* Borgs. (× 1/3).

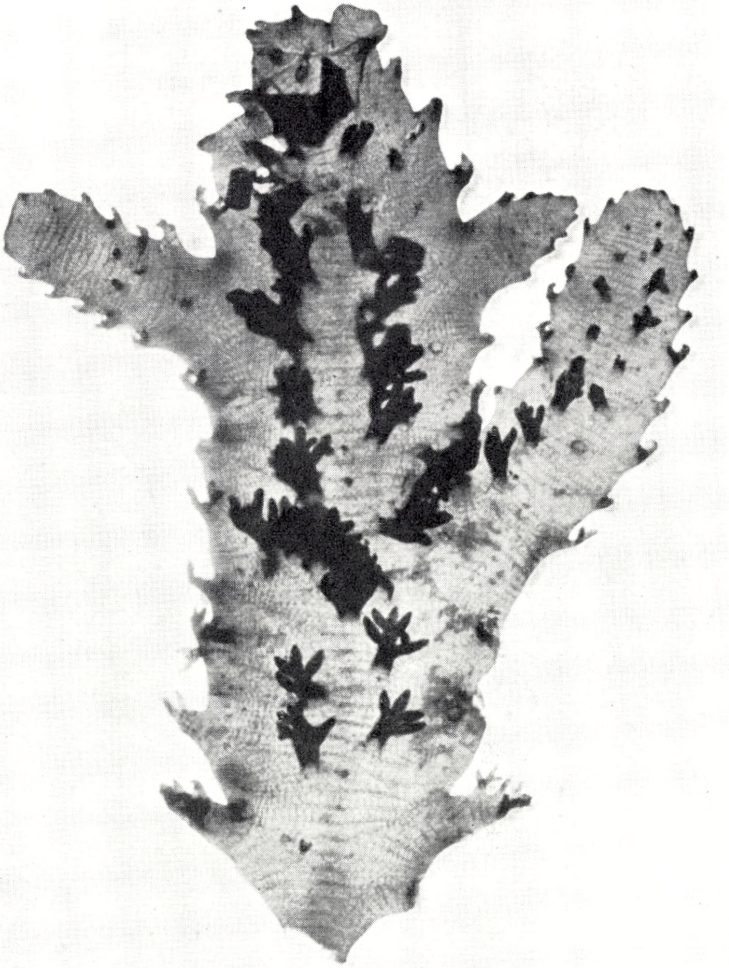

Fig. 4. *Vidalia fimbriata* (R.Br.) J.Ag. Part of the thallus (× 8).

III. Ceramiales.

Fam. 1. ***Rhodomelaceae.***

Subfam 1. Amansieae.

Vidalia Lamouroux.

1. **Vidalia fimbriata** (R. Br.) J. Ag.

Falkenberg, P., Die Rhodomelaceen, Fauna u. Flora d. Golfes v. Neapel, 26. ("25.") Monographie, 1901, p. 430, pl. 7, fig. 19; Alg. Mauritius, III, 4, 1945, p. 44; Additions, VI, 1954, p. 47.

In the collection received in 1954 from Dr. Vaughan there are some fine specimens of this species. As appears from fig. 4, the plants are in good agreement with the description and figure given by Falkenberg *l. c.*

The specimens are tetrasporic. The stichidia are developed on the branchlets, on the lateral ones as well as on those given off from the flat side of the thallus; they are rather short, about twice as long as broad, and a single or up to 3—4 stichidia are found on each branchlet.

As to the locality it is said: "growing on reef exposed to strong surf".

Mauritius: La Cambuse, 20-1-54, G. Morin no. 1313.

Subfam. 2. Laurencieae.

Laurencia Lamouroux.

1. **Laurencia subcolumnaris** Børgs.

Børgesen, F., Two New Species of *Laurencia* from Mauritius. Bot. Tidsskr., vol. 51 (Professor Knud Jessen Festskrift), 1954, p. 48, figs. 1—3.

Mauritius: On rocks exposed to strong surf, Pointe aux Roches, 22-9-52, G. Morin no. 1286 p. p.

2. **Laurencia verruculosa** Børgs.

Børgesen *l. c.* p. 50, figs. 4—5.

Mauritius: On rocks exposed to strong surf, Pointe aux Roches, 22-9-52, G. Morin no. 1286 p. p.

Index of Species.

Synonyms printed in italics.

The present series of papers, with the common title Some Marine Algae from Mauritius, includes 15 parts, which are referred to in the index by their respective years of publication:

1940.	I Chlorophyceae	Biol. Medd. Dan. Vid. Selsk.	15, no. 4
1941.	II Phaeophyceae	—	16, no. 3
1942.	III Rhodophyceae, Part 1: Porphyridiales, Bangiales, Nemalionales	—	17, no. 5
1943.	III Rhodophyceae, Part 2: Gelidiales, Cryptonemiales, Gigartinales	—	19, no. 1
1944.	III Rhodophyceae, Part 3: Rhodymeniales	—	19, no. 6
1945.	III Rhodophyceae, Part 4: Ceramiales	—	19, no. 10
1946.	An additional list of species to Part I Chlorophyceae	—	20, no. 6
1948.	Additional lists to the Chlorophyceae and Phaeophyceae	—	20, no. 12
1949.	Additions to the parts previously published	—	21, no. 5
1950.	Additions to the parts previously published. II	—	18, no. 11
1951.	Additions to the parts previously published, III	—	18, no. 16
1952.	Additions to the parts previously published, IV	—	18, no. 19
1953.	Additions to the parts previously published, V	—	21, no. 9
1954.	Additions to the parts previously published, VI	—	22, no. 4
1957.	Final part	—	23, no. 4

Four smaller papers are connected with this series, but have been published outside it, and therefore are not included in the index:

1942. *Griffithsia Weber-van-Bosseae*, nov. spec. *Blumea*, Suppl. II (Dr. A. A. Weber-van Bosse Jubilee Vol.): 15—20.

1949. On the genus *Titanophora* (J. Ag.) Feldm. and Description of a New Species. Dansk Bot. Arkiv 13, no. 4. (*Titanophora Pikeana* (J. Ag.) Feldm., *T. incrustans* (J. Ag.) Børgs., *T. Weberae* Børgs., *T. mauritiana* Børgs., *T. calcarea* (Okamura) Børgs.).

1950. *Vaughaniella*, A New Genus of the Dictyotaceae. Biol. Medd. Dan. Vid. Selsk. 18, no. 8.

1954. Two New Species of *Laurencia* from Mauritius. Bot. Tidsskr. 51 (Professor Knud Jessen Festskrift): 48—52. (*Laurencia subcolumnaris* Børgs., *L. verruculosa* Børgs.).

Chlorophyceae.

Phaeophyceae.

[1] On reconsidering the figure given on p. 18 I have come to the conclusion that I have made a mistake by referring the fragments pictured to *Ectocarpus indicus*. Apart from the sporangia the appearance of the plant is that of *E. Mitchellae* and not that of *E. indicus* = *E. Duchassaingianus*, cp. my drawings in Mar. Alg. D.W. I. vol. I 1914, so now I think it is a form of *E. Mitchellae* with relatively long and somewhat irregular plurilocular sporangia.

Rhodophyceae.

[1] Occasioned by a letter from Dr. Y. Yamada, Hokkaido, Japan, in which he writes that in Formosa he has collected some specimens quite like those which I have named *Gelidiopsis scoparia*, but which he has determined to be *Gelidiopsis acrocarpa*, I have again taken up the question and have arrived at the result that I surely have made a mistake here: The specimens I have determined as *Gelidiopsis scoparia* (Mont. & Mill.) Schmitz in the parts referred to above, are not this species, but are referable to *Gelidiopsis acrocarpa* (Harv.) Schmitz.

Phycomycetes.

A map of Mauritius is found at the end of the 1948 part.

Indleveret til selskabet den 12. oktober 1956.
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Bind **23**, no. 5

Biol. Medd. Dan. Vid. Selsk. **23**, no. 5 (1957)

UNTERSUCHUNGEN ÜBER DETERMINATION UND DIFFERENZIERUNG

4. ÜBER DEN AUFBAU DES ZELLWANDGERÜSTES DER PFLANZEN UND DIE DETERMINATION DESSELBEN

VON

P. BOYSEN JENSEN

With a Summary in English

København 1957
i kommission hos Ejnar Munksgaard

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UNTERSUCHUNGEN ÜBER DETERMINATION UND DIFFERENZIERUNG

4. ÜBER DEN AUFBAU DES ZELLWANDGERÜSTES DER PFLANZEN UND DIE DETERMINATION DESSELBEN

VON

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With a Summary in English

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i kommission hos Ejnar Munksgaard

Synopsis.

Wenn junge Prothallien von *Pteris longifolia* auf Agar, der 2,4-D oder 2M-4Cl enthält, kultiviert werden, entstehen kallusähnliche Bildungen. Werden die Gifte durch Nährlösung ersetzt, treten Differenzierung und Bildung normaler Prothallien ein.

Da man somit die Wirkung des determinierenden Faktors ausschalten kann, während Wachstum und Zellteilung fortgesetzt werden, muss der erstere Vorgang den letzteren übergeordnet sein. Der determinierende Faktor wird durch Gifte nicht verändert.

Es ist nicht möglich die Wirkung des determinierenden Faktors auf Stoffe, Strukturen oder elektrische Potentiale zurückzuführen. Soweit man es beurteilen kann, sind in dem anorganischen Bereich keine Elemente vorhanden, mit welchen der determinierende Faktor verglichen oder aus welchen er abgeleitet werden kann.

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1. Einleitung.

Sowohl bei Tieren als bei Pflanzen wird die Gestaltung durch das Plasma oder durch einen an das Plasma geknüpften Faktor geregelt. Weil aber bei den Pflanzen das Plasma von Zellwänden wie in einem Kerker eingeschlossen ist, muss es, um selbst wachsen zu können, erst ein Wachstum der Zellwände oder Teile derselben hervorrufen. Wachstum einer Pflanze ist primär Wachstum der Zellwände.

Dies geht besonders deutlich aus der Betrachtung von *Caulerpa* hervor. Obschon dieser Organismus einzellig ist, ist er doch im Besitze einer reichen, morphologischen Differenzierung, indem er ausser einer Hauptachse assimilierende Thalluslappen und Rhizoiden besitzt. Diese Teile entstehen dadurch, dass an bestimmten Orten an der Hauptachse ein Wachstum der Zellwand einsetzt, so dass Ausstülpungen gebildet werden, die sich zu flachen, blattähnlichen Assimilatoren oder verzweigten Rhizoiden entwickeln. Gleichzeitig mit der Vergrösserung des Zellvolumens tritt ein Wachstum des Plasmas ein.

Bei höheren Pflanzen ist der Pflanzenkörper durch die Epidermis nach aussen abgegrenzt. Die Epidermis der oberirdischen Teile einer einjährigen Keimpflanze ist dieselbe, die diese Teile der Pflanze das ganze Leben hindurch bekleidet. Gleichzeitig damit, dass sie wächst, werden in mannigfaltigster Weise Ausstülpungen und Faltungen der Epidermis gebildet, so dass sie alle Pflanzenorgane, Achsenorgane, Laubblätter, Perianthblätter, Staubfäden und Fruchtblätter überzieht.

In ähnlicher Weise wie bei *Caulerpa* wird auch bei höheren Pflanzen das Wachstum durch Wachstum der Zellwände eingeleitet. Soll z. B. ein Blatt gebildet werden, so geschieht dies dadurch, dass die Zellwände der Epidermis und einiger der darunterliegenden Schichten an einem begrenzten Ort in der Nähe der Stengelspitze zu wachsen anfangen. Die Epidermis wölbt

sich empor, es entsteht ein kleiner, kreisförmiger Höcker. Gleichzeitig damit, dass das Volumen der Zellen durch das Wachstum der Zellwände vergrössert wird, tritt auch ein Wachstum des Plasmas der Zellen ein. Ferner treten Zellteilungen und Bildung neuer Zellwände ein, die neuen Zellwände sind in der Epidermis vorwiegend Antiklinen, in den inneren Zellen in dem Höcker anfangs Periklinen. Unter fortwährendem Wachstum der Zellwände und Zellteilungen mit Bildung neuer Zellwände wächst der Höcker zu einem fingerförmigen Körper aus, an den Flanken desselben entsteht später durch Marginalwachstum eine Blattfläche. Die neuen Zellwände in dem Mesophyll werden so orientiert, dass der künftige histologische Bau der Blätter, der durch die folgenden Vorgänge, Zellstreckung und Differenzierung der gestreckten Zellen, erreicht werden soll, vorbereitet wird.

Da die Epidermis die Pflanze nach aussen abgrenzt, taucht die Frage auf, ob es das Wachstum der Epidermis oder dasjenige der inneren Gewbe ist, das die äussere Gestalt der Pflanzen bestimmt. Eine Antwort auf diese Frage ergibt sich, wenn man die Blattformen der Chimaeren betrachtet. Bei *Solanum tübingense* besteht die Epidermis aus Tomatenzellen, die inneren Gewebe sind dagegen aus *Solanum nigrum*zellen gebildet. Die Blätter sind nicht wie bei *Solanum* ganzrandig, sondern schwach gelappt, und auch die Kelchblätter und die Früchte sind grösser und mehr tomatenähnlich als bei *Solanum*. Man muss daher schliessen, dass die Epidermis nicht einen passiven Überzug der inneren Gewebe bildet, sondern dass sie an der Gestaltung der Blätter und der Früchte, wenn auch in beschränktem Umfang, aktiv beteiligt ist.

Es geht aus dem Angeführten hervor, dass die Gestaltung der Pflanzen sowie auch die innere Struktur derselben durch den Bau des Zellwandgerüstes bestimmt wird. Der Aufbau desselben kann auf ein gleichmässiges oder ungleichmässiges Flächen- oder Dickenwachstum der Zellwände und auf die Bildung neuer Zellwände zurückgeführt werden.

Hinsichtlich des Wachstums der Zellwände unterscheidet man zwischen Flächen- und Dickenwachstum.

Flächenwachstum. Nach Untersuchungen über das Flächen-

wachstum in der Spitze der Wurzelhaare von *Phleum* (Boysen Jensen 1954) ragt die Plasmaoberfläche, die mit Zellulosenbildnern besetzt ist, als Papillen oder Kämme in die Zellwand hinein. Die Zellwand wird durch den Turgordruck gedehnt, und die Zellulosenbildner erzeugen Zellulosenfibrillen, die zwischen den schon vorhandenen eingelagert werden. Namentlich in der Spitze der Membrankuppe werden unaufhörlich neue Plasmapapillen in die Zellwand hineingeschoben.

Auch in anderen Zellen dürfte das Flächenwachstum durch eine Zusammenwirkung von Turgordruck und Intussusceptionswachstum zustandekommen. In den jüngsten meristematischen Zellen müssen auch neue Papillen in die Zellwand hineingeschoben werden, ob dasselbe während der Zellstreckung der Fall ist, muss vorläufig dahingestellt bleiben.

Das Flächenwachstum kann gleichmässig über die ganze Zellwand verteilt sein, ist aber am häufigsten ungleichmässig. Ein Beispiel eines sehr starken lokalen Wachstums ist die Bildung eines Wurzelhaares, die häufig an dem apikalen Ende einer Epidermiszelle stattfindet. Dieses lokale Wachstum kommt dadurch zustande, dass ein mehr oder weniger grosser Teil der Zellulosenbildner, die ursprünglich gleichmässig über die Zellwand verteilt sind, sich an dem apikalen Ende der Zelle anhäuft (Boysen Jensen 1955).

Auch in den prismatischen oder zylindrischen Zellen, die in Stengeln und Wurzeln bei der Zellstreckung entstehen, ist das Wachstum nicht gleichmässig, es wachsen nur die tangentialen und radialen Wände, die transversalen dagegen nur wenig. Und nicht einmal in den tangentialen und radialen Wänden ist das Wachstum gleichmässig. Sinnott and Bloch (1939) haben in einer schönen Untersuchung gezeigt, dass in der Wurzelepidermis von *Pleum*, deren Zellreihen aus wechselweise kurzen und langen Zellen zusammengesetzt sind, die Anpassung der verschiedenen Zellreihen aneinander unter anderem dadurch zustande kommt, dass der Teil der Zellwand einer langen Zelle, der mit einer kurzen Zelle in Berührung steht, langsamer wächst als der übrige Teil der Zellwand. Auch dieses ungleichartige Wachstum der Zellwand dürfte wahrscheinlich in ähnlicher Weise wie die Bildung der Wurzelhaare durch eine Verschiebung der Zellulosenbildner zustandekommen.

Die Verschiebung der Zellulosenbildner geschieht in einigen Fällen mit grosser Schnelligkeit. Wenn Wurzeln von *Phleum* in eine Lösung von IES gelegt werden, wachsen die Wurzelhaare bisweilen wellenförmig (Abb. 1). Diese Wachstumsweise muss dadurch zustandekommen, dass das System der Zellulosenbildner, das sich in der Spitze des Wurzelhaares befindet (vgl. Boysen Jensen 1955), hin und her pendelt, so dass der Neuzuwachs wechselweise an der linken und rechten Seite der Spitze zu liegen kommt. Es kann dann entweder das Pendeln der Zellulosenbildner aufhören und das Wurzelhaar gerade weiter wachsen (Abb. 1,2) oder das System der Zellulosenbildner kann in zwei Teile zerreissen. Es entsteht dann eine Gabelung (Abb. 1,3).

Mit welcher Geschwindigkeit die Verschiebung der Zellulosenbildner stattfindet, vermag ich nicht zu sagen, weil ich nicht Gelegenheit gehabt habe, das wellenförmige Wachstum eines Wurzelhaares unter dem Mikroskop zu verfolgen. Wenn man aber von der Wachstumsgeschwindigkeit eines normalen Wurzelhaares ausgeht, kann man schätzen, dass das Hin- und Herpendeln etwa eine Stunde dauert.

Dickenwachstum. Wenn eine Wurzel von *Phleum* in eine Lösung von Kongorot gelegt wird, wird die Adhäsion zwischen Plasma und Zellwand aufgehoben; das Plasma zieht sich aus der Zellwand heraus, die Zellulosenbildner in der Spitze der Wurzelhaare setzen ihre Wirksamkeit fort und bilden eine Verdickung an der inneren Seite der Zellwand. Man darf annehmen, dass die normalen Verdickungen in ähnlicher Weise entstehen. Wenn die Zellulosenbildner gleichmässig über die Plasmaoberfläche verteilt sind, entsteht eine gleichmässige Verdickung (Steinzellen), häufiger aber ordnen sie sich zu einem bestimmten Muster an, es entstehen dann ring-, netz- oder schraubenförmige Verdickungen (Trakeiden und Gefässe).

Die Anordnung der neuen Zellwände. Dieselbe wird durch die Lage der Zellkerne und die Orientierung der Kernspindeln bestimmt.

In den meristematischen Zellen liegt der Zellkern ungefähr in der Mitte der Zelle, und bei der Zellteilung werden zwei gleich grosse Zellen gebildet. In *Equisetum*sporen, in den Zellen des *Sphagnum*blattes (Zepf 1952) und in vielen anderen Fällen liegt der Zellkern dagegen exzentrisch, und bei der Zellteilung

entstehen zwei Zellen von ungleicher Grösse und oft mit verschiedenen Entwicklungsmöglichkeiten.

Die Orientierung der Kernspindel kann durch äussere Faktoren bestimmt sein. Bei der ersten Teilung der *Equisetum*sporen

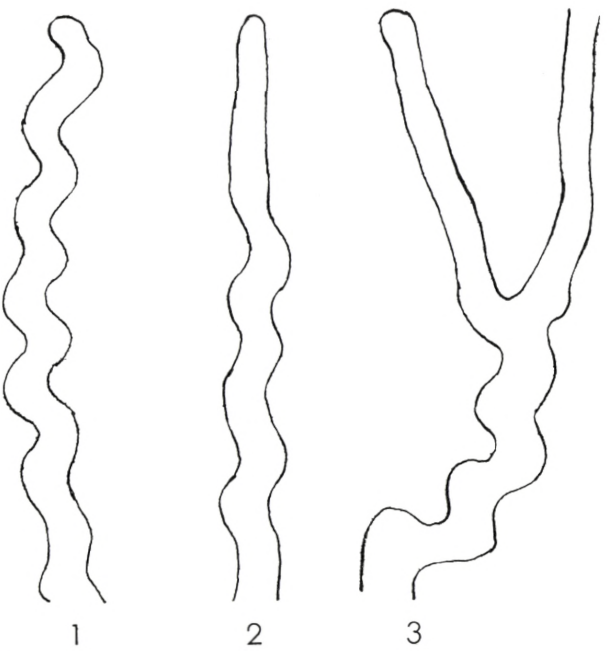

Abb. 1. Wellenförmiges Wachstum bei Wurzelhaaren von *Phleum*, 1) permanentes, 2) transitorisches wellenförmiges Wachstum, 3) Gabelung. Zeichenapp. 400/1.

steht die Längsachse der Kernspindel in der Richtung des einfallenden Lichtes.

In den meisten Fällen aber ist die Orientierung der Kernspindel autonom. Bei niederen Pflanzen findet sich im Plasma ein Körper, das Zentrosom. Vor der Kernteilung teilt dasselbe sich in zwei, diese beiden Teile rücken von einander aus, legen sich einander gegenüber an den Zellkern und umgeben sich mit einer Strahlensonne. Die Plätze, die sie einnehmen, sind die Pole der Kernspindel bei der folgenden Zellteilung.

Bei den höheren Pflanzen sind keine Zentrosomen vorhanden, aber auch bei diesen ist das Plasma für die Orientierung der Kernspindel massgebend. Das geht aus Untersuchungen von Sinnott und Bloch (1941) über die Zellteilung in vakuolisier-

ten Zellen hervor. Es werden von dem randständigen Plasma Stränge gebildet, die die Vakuole durchziehen. In diese wandert der Kern ein, so dass er in die Mitte der Zelle zu liegen kommt. Die Stränge, die von dem Kern zum Randplasma ausgehen, bilden einen Kreuz. Die beiden Achsen des Kreuzes sind jedoch nicht gleichwertig. Während die kürzere Achse aus Einzelsträngen gebildet wird, besteht die längere Achse dagegen aus einer Plasmaplatte, die aus anastomierenden Strängen zusammengesetzt ist. In diese kommt die künftige Zellwand zu liegen.

Die Orientierung der Kernspindel und die Lage der Zellwand ist somit durch Vorgänge im Plasma bestimmt, ein neuer Beweis dafür, dass das Plasma und nicht der Kern für die Gestaltungsvorgänge bestimmend ist.

Das Wachstum der Zellwände und die Bildung neuer Zellwände verlaufen in gesetzgebundener, für jede Art spezifischer Weise. Das schliessliche Ergebnis derselben ist das Zellwandmuster der betreffenden Pflanzenart. Der Verlauf dieser Vorgänge wird vom Plasma geregelt, und es müssen somit in demselben oder an dasselbe geknüpft determinierende Faktoren vorhanden sein, die die Entstehung des Determinations- und Zellwandmusters hervorrufen.

Wie verwickelt die Vorgänge bei der Entstehung des Zellwandgerüstes sind, erhellt, wenn man sich die Entwicklung einer sehr einfach gebauten Pflanze, z. B. eines Mooses, vergegenwärtigt.

Es treten nacheinander eine Reihe verschiedener Zellwandmuster auf, das erste und zweite bei der Entwicklung des Protonemas, das aus Chloronemafäden mit quergestellten und aus Rhizoiden mit schiefen Querwänden besteht, das dritte und vierte bei der Entwicklung der Mosspflanze, die aus dem Stengel mit einer dreiseitigen, pyramidenförmigen Scheitelzelle und den Blättern mit einer zweischneidigen Scheitelzelle besteht. Das fünfte und sechste Zellwandmuster treten bei der Entwicklung der Archegonien und Antheridien auf. Aus der befruchteten Eizelle entsteht dann der Sporophyt mit etwa 10 verschiedenen Zellwandmustern, nämlich denjenigen des Haustoriums, der Seta, der Columella, des Archespors, des Assimilationsgewebes,

der Epidermis mit Spaltöffungen, des Peristoms, des Deckels, des Annulums und der Kalyptra.[1]

In jeder einzelnen Zelle wird das Wachstum der Zellwand so geregelt und bei der Zellteilung wird die Achse der Kernspindel so orientiert, wie es der Fall sein muss, wenn diese verwickelten Zellwandmuster zustande kommen sollen.

Untersuchungen über die Entstehung des Zellwandgerüstes sind einmal sehr wichtig, weil dieses Gerüst massgebend für die Gestaltung der Pflanzen ist. Ferner steht der Aufbau des Zellwandgerüstes einerseits in Beziehung zu relativ einfachen biochemischen Vorgängen, indem man die Zellulosenbildung in Analogie zu der Stärkebildung als einen enzymatischen Vorgang auffassen muss, andererseits steht dieser Aufbau in Beziehung zu den grundlegenden ontogenetischen Vorgängen, indem er ortsgemäss verläuft. Wir stehen somit dem Problem gegenüber: Wie ist es möglich, dass ein enzymatischer Vorgang ortsgemäss verlaufen kann? Vorläufig wird man annehmen müssen, dass zwei Möglichkeiten vorhanden sind: Entweder eine richtungsbestimmte Verschiebung der Zellulosenbildner, so dass diese an einem bestimmten Ort angehäuft werden, oder eine ortsbestimmte Neubildung oder Aktivierung von Zellulosenbildnern oder anderen Stoffen, die die Wirkung der Zellulosenbildner in der einen oder anderen Weise beschleunigen können.

Die Erhellung des Aufbaues des Zellwandgerüstes dürfte zu den einfachsten Problemen der Gestaltungsphysiologie gehören. Wenn es nicht möglich sein sollte, diese Aufgabe zu lösen, darf man es vermutlich als vollkommen ausgeschlossen ansehen, dass es gelingen sollte, die weit schwierigere Aufgabe der Erhellung des Aufbaues des Protoplasmas zu bewältigen.

Die Mittel, deren man sich bedienen muss, wenn man versuchen will, sich dem Verständnis des Aufbaues des Zellwandgerüstes zu nähern, sind einmal direkte Beobachtung des Zellwandwachstums und der Bildung neuer Zellwände in lebenden Pflanzen, und ferner Untersuchungen über die Einwirkung äus-

[1] Die verschiedenen Zellwandmuster in dem Gametophyten und dem Sporophyten sind nicht durch die Unterschiede in den Chromosomenzahlen der zwei Generationen bedingt. Es ist möglich, durch Regenerationsversuche aus der Seta Protonemafäden mit diploider Chromosomenanzahl zu entwickeln. Die Fäden bilden in gewöhnlicher Weise diploide Moospflanzen. Diese haben zwar grössere Zellen und grössere Blätter, sind aber im übrigen den gewöhnlichen haploiden Moospflanzen ähnlich (v. Wettstein 1927).

serer Faktoren auf diese Vorgänge. Man hat dabei den grossen Vorteil, dass man bei geeigneten Versuchsobjekten die Entstehung und das Wachstum der Zellwände in den lebenden Pflanzen direkt unter dem Mikroskop verfolgen kann.

Es ist früher gezeigt worden, dass man das normale Verteilungsmuster der Zellulosenbildner in der Spitze von Wurzelhaaren durch Gifte zerstören kann (Boysen Jensen 1955). Es ist gleichfalls bekannt, dass man durch Trijodbenzoesäure und andere Stoffe tiefgehende Veränderungen in der Gestaltung der Pflanzen hervorrufen kann (Zimmermann und Hitschcock 1942, Gorter 1949, 1951, Harder und Oppermann 1952, Linser, Frohner und Kirschner 1955 u. a.). Von besonderer Bedeutung für die folgenden Untersuchungen ist der Nachweis, dass man durch Gifte verschiedene Pflanzenteile in kallusähnliche Gewebe umwandeln kann. Das ist z. B. der Fall mit dem Chloronema von Moosen (Bünning und v. Wettstein 1953, v. Wettstein 1953), und mit Farnprothallien (Sossountzov 1953, Mohr 1956 u. a.). Bei höheren Pflanzen tritt als Folge der Einwirkung von 2,4-D im Stengel eine starke Vermehrung der Zellen in der Endodermis, in dem Pericycel und dem Kambium ein, so dass Schichten von proliferierendem Gewebe gebildet werden können. (Turkey, Hammer and Imhof 1945, Eames 1950 u. a.).

Um die Wirkung von Giften auf den Aufbau des Zellwandgerüstes und damit auf die Gestaltung einer Pflanze näher verfolgen zu können, wurde ein möglichst einfaches Versuchsobjekt, nämlich das Prothallium von *Pteris longifolia* gewählt. Über die Ergebnisse dieser Untersuchungen soll im folgenden berichtet werden.

2. Methodisches.

Die Prothallien wurden auf 0,6 %iger Agar gezüchtet.

1ste Methode. Wenn man die Entwicklung eines bestimmten Prothalliums verfolgen will, geht man in folgender Weise vor.

Auf einem Objektträger werden zwei Glasringe, wie sie für Herstellung feuchter Räume benutzt werden (Diameter 1,5 cm, Höhe 0,8 cm) mit Agar festgeklebt. Die Glasringe werden ganz mit 0,6 %iger Agar (in $^1/_2$ I_b + II (Boysen Jensen 1950 (1) gelöst) gefüllt. Soll die Einwirkung eines Giftes auf die Entwicklung

untersucht werden, wird dasselbe in der erwünschten Konzentration in dem Agarsubstrat gelöst. Wenn der Agar erstarrt ist, werden Sporen von einem Stück eines *Pteris*blattes direkt auf die Agaroberfläche ausgeschüttelt. Die *Pteris*blätter dürfen nicht in allzu trockener Luft aufbewahrt werden, weil dann gleichzeitig mit den Sporen zu viele Unreinheiten auf die Agaroberfläche gelangen. Gelegentlich findet man auf der Oberfläche leere Sporangien, was jedoch für die Versuche ohne Belang ist. Der Objektträger mit den Agarringen wird in einer Petrischale (10 × 2 cm), deren Boden mit feuchtem Filtrierpapier bedeckt ist, untergebracht. Die Schale wird mit einem Ring von schwarzem Papier umgeben und mit einer oder zwei Schichten von rotem Zellopfanpapier oder mit einer Schicht Pergamentpapier, je nachdem ob man mit rotem Licht oder mit Tageslicht arbeiten will, und ferner mit einer Glasplatte bedeckt. Wenn das Licht schwach wird, arbeitet man am besten ohne Pergament. Das Präparat wird in ein Nordfenster bei Zimmertemperatur (18—22°) gestellt.

Wenn die Prothallien untersucht werden sollen, legt man das Objektglas unter das Mikroskop und untersucht die Agaroberfläche mit schwachen Vergrösserungen (etwas $8 \times 10 \times 1^1/_2$). Es bildet sich häufig an der Oberfläche der Prothallien eine dünne Luftschicht, die eine zellenähnliche Kammerung besitzt und daher zu Täuschungen Anlass geben kann. Man tröpfelt daher 2—3 Tropfen derselben Flüssigkeit, in die der Agar gelöst ist, auf die Oberfläche, wobei die Luftschicht verschwindet.

Man kann dann die Lage eines bestimmten Prothalliums mit Hilfe der Masstäbe des Objektführers fixieren und es später wiederfinden. Mit Hilfe eines Zeichenapparates ist man gleichfalls imstande, ein bestimmtes Prothallium zu zeichnen, und kann in dieser Weise die Veränderungen desselben von Tag zu Tag verfolgen.

2*te Methode.* Wenn man dagegen die Einwirkung verschiedener Lösungen auf das Wachstum der Prothallien untersuchen will, bedient man sich am besten der folgenden Methode.

Die Glasringe werden um die Hälfte mit 0,6 %iger Agar (in $^1/_2$ I$_b$ + II gelöst) beschickt; im übrigen werden sie ganz wie oben behandelt. Am dritten oder vierten Tag, nachdem die Sporen ausgesät sind, wird ein Glasring mit Agar und Sporen von dem Objektträger auf den Boden einer kleinen Petrischale (4 × 2 cm)

hinübergeschoben. Die Petrischale wird ganz wie oben mit einem schwarzen Papierring umgeben und mit Zellophan oder Pergamentpapier bedeckt. Von der Flüssigkeit, deren Wirkung auf das Wachstum man untersuchen will, wird so viel in die Petrischale ausserhalb des Glasringes gegossen, dass die Oberfläche der Flüssigkeit 1—2 mm unter dem Meniscus der Agaroberfläche liegt. Zwei oder dreimal täglich werden mit einer Pipette 2—3 Tropfen der äusseren Flüssigkeit auf die Agaroberfläche übertragen; diese Flüssigkeit soll im Laufe von 5—6 Stunden durch den Agar ablaufen. Sollte das nicht der Fall sein, saugt man etwas von der Flüssigkeit ausserhalb des Glasringes ab. Die Flüssigkeit, die bei Beginn des Versuches in dem Agar vorhanden war, ist bei dieser Anordnung bald durch die Versuchsflüssigkeit ersetzt.

Wenn man die Entwicklung der Prothallien unter dem Mikroskope untersuchen will, wird die Petrischale auf einen Objektträger gestellt. Mit Hilfe des Objektführers kann man dann die Petrischale hin und her bewegen. Auch bei dieser Methode kann man meistens leicht die Entwicklung bestimmter Prothallien verfolgen, wenn man einen Fremdkörper, z. B. ein Sporangium, als Fixpunkt benutzt.

Alle Flüssigkeiten, Glasgeräte und Pipetten (in Reagensgläsern untergebracht) werden sorgfältig sterilisiert. Dagegen habe ich nicht gewagt, die Sporen selbst zu sterilisieren, weil die sterilisierende Flüssigkeit leicht eine unkontrollierbare Wirkung auf die Sporen üben könnte. Die Kulturen sind daher mehr oder weniger infiziert. Wenn die Infektion zu stark ist, wird die Kultur weggeworfen. Schwache Infektionen schaden den Kulturen nicht.

Wenn ein Versuch beendet ist, kann man die Prothallien in folgender Weise konservieren. Es wird eine Giletteklinge unter dem Agarring eingeschoben, worauf man ihn dann leicht emporheben kann. Auf den Agarring legt man ein Deckglas und darüber einen dünnen Karton und dreht das ganze um. Man lässt nun den Agarzylinder auf das Deckglas hinuntergleiten, entfernt den Glasring, schneidet mit einer Giletteklinge den grössten Teil des Agars weg und legt einen Objektträger auf den Agar. Wenn man dann das ganze noch einmal umkehrt, liegen die Prothallien unmittelbar unter dem Deckglas auf einer dünnen Agarschicht. Man setzt nun mehrere Tage lang nach und nach 5 %iges Glycerin

zu; schliesslich wird das Präparat mit einem Deckglas-Umrandungslack verschlossen.

Die Flüssigkeiten, die für die Untersuchungen benutzt werden, sind die folgenden:

$^1/_2$ Ib + II (zur Untersuchung der normalen Entwicklung)
0.08—0,2 % Colchicin
0,01 % 2,4-D (2-4-Dichlorphenoxyessigsäure, Natriumsalz)
0,01 % 2 M-4 Cl (2-Methyl-4-chlorphenoxyessigsäure)

Alle Stoffe werden in $^1/_2$ Ib + II gelöst. Bei der Zubereitung der 2 M-4 Cl lösung muss man zu 100 ccm etwa 5 Tropfen 1/10 n NaOH hinzusetzen, um denselben p_H Wert wie bei den übrigen Versuchsflüssigkeiten zu erhalten.

Ausserdem ist noch die Wirkung einer Reihe anderer Flüssigkeiten, Indolylessigsäure, Trijodbenzoesäure und Chloralhydrat untersucht worden. Es wurden durch diese Versuche keine neuen Erfolge erzielt, und sie sollen daher nicht näher besprochen werden.

Es wurden soweit möglich folgende Versuchsreihen durchgeführt:

normale Entwicklung $^1/_2$ Ib + II

Colchicin
- 0,2 % Colchicin
- 0,2 % Colchicin + $^1/_2$ Ib + II
- $^1/_2$ Ib + II + 0,2 % Colchicin

2,4-D
- 0,01 % 2,4-D
- 0,01 % 2,4-D + $^1/_2$ Ib + II
- $^1/_2$ Ib + II + 0,01 % 2,4-D

2 M-4 Cl
- 0,01 % 2 M-4 Cl
- 0,01 % 2 M-4 Cl + $^1/_2$ Ib + II
- $^1/_2$ Ib + II + 0,01 % 2 M-4 Cl

In jeder Versuchsreihe wurde soweit möglich die Entwicklung eines bestimmten Prothalliums verfolgt und abgebildet; in einigen Fällen sind ferner verschiedene Typen in dem Schlussstadium der Versuchsreihe dargestellt. Mit Ausnahme der normalen Versuche ist die Formmannigfaltigkeit in den meisten Versuchen überwältigend gross, häufig sind nicht zwei Prothallien

einander gleich. Es war eine schwierige Aufgabe, bei dieser Mannigfaltigkeit repräsentative Typen für die Zeichnungen auszuwählen.

3. Die Wirkung von Giften auf das Zellwandmuster und die Gestaltung der Prothallien von *Pteris longifolia*.

Wie KLEBS (1916, 1917) und andere nachgewiesen haben, wird die Gestaltung der Prothallien sehr stark durch Licht beeinflusst; jedoch ist die Wirkung ziemlich kompliziert, indem sowohl die Lichtstärke als die Lichtfarbe von Bedeutung ist. Bei einer Lichtstärke von 27 MK entstehen Fäden, die entweder aus einer einzelnen Zelle gebildet sind oder vereinzelte Querwände enthalten. Bei höherer Lichtstärke, etwa 250 MK, entstehen schmale, flächenförmige Prothallien mit terminalem Wachstum. Bei höherer Lichtstärke bilden sich normale, herzförmige Prothallien. In ähnlicher Weise erhält man in rotem Licht schlauchförmige, in weissem Licht dagegen herzförmige Prothallien.

Bei meinen Versuchen verwendete ich, wie oben erwähnt, teils rotes, teils weisses Licht.

a. Versuche mit rotem Licht.

Die Entwicklung der Prothallien in rotem Licht auf Agar, der mit $^1/_2$ I_b + II getröpfelt wurde, ist in Abb. 2, 1,2 dargestellt. Die Prothallien sind schlauchförmig mit Spitzenwachstum. Wenn Zellteilungen auftreten, sind die Achsen der Kernspindeln alle gleichgerichtet, und die Zellwände stehen senkrecht auf der Längsachse der Schläuche. Man darf diese Wachstumsweise wohl als eine Etiolementserscheinung auffassen. Wie verschiedene Forscher gezeigt haben (vgl. BOYSEN JENSEN 1950 (2)) kann man durch Kongorot das Flächenwachstum in der Spitze der Wurzelhaare in Dickenwachstum umwandeln. Ich habe festzustellen versucht, ob sich dieses Verfahren auch auf die schlauchförmigen *Pteris*prothallien anwenden lässt. Das Ergebnis war negativ. Die Zellwände der Prothallien lassen sich nicht durch Kongorot färben.

1. *Wirkung von Colchicin.* Wenn die Prothallien kurz nach der Keimung mit 0,08—0,2 %iger Colchicinlösung getröpfelt wur-

den, entstand bisweilen, nachdem ein schlauchförmiger Basalteil gebildet worden war, eine Aufblähung in der Spitze. Gleichzeitig wurden die Zellteilungen unregelmässig. Es entwickelten sich keulenförmige, mehrschichtige Bildungen, in welchen die Zellwände in allen möglichen Ebenen zu liegen kamen.

Wurden nun solche keulenförmige Prothallien anstatt mit Colchicin mit $^1/_2$ Ib + II getröpfelt, kehrten sie zu ihrer normalen Wachstumsweise zurück. Es entstanden, wie aus Abb. 2, 3, 4 hervorgeht, Schläuche mit Querwänden. Die zahlreichen Querwände in dem linken Schlauch in Abb. 2, 3 sind durch Belichtung mit blauem Licht hervorgerufen.

2. *Wirkung von 2,4-D.* Die Entwicklung eines Prothalliums, in einem Präparat, das bald nach der Keimung mit einer 0,01 %igen Lösung von 2,4-D getröpfelt wurde, ist in Abb. 2, 6, dargestellt. Es entstand erst ein langer Schlauch mit Querwänden, der schliesslich einen Kopf mit unregelmässiger Anordnung der Zellen in mehreren Schichten bildete. Als das Präparat dann mit $^1/_2$ Ib + II getröpfelt wurde, wurde ein kurzer Schlauch gebildet, nach erneuter Behandlung mit 2,4-D entstanden in der Spitze desselben einige unregelmässige Zellteilungen, die wahrscheinlich als der Anfang eines zweiten Kopfes aufzufassen sind.

3. *Wirkung von 2M-4Cl.* Die Entwicklung eines Prothalliums in einem Präparat, das mit einer 0,01 %igen Lösung dieses Stoffes getröpfelt wurde, ist in Abb. 2, 5 dargestellt. Es entstand eine Reihe von grossen, runden Zellen, in der Spitze mit unregelmässigen Zellteilungen in mehreren Ebenen. Nach 10 Tagen wurde mit $^1/_2$ Ib + II getröpfelt. Es entwickelte sich dann von einer der basalen Zellen aus ein langer Schlauch. Nach weiteren 16 Tagen wurde wieder mit 2M-4Cl getröpfelt, es bildete sich dann in der Spitze des Schlauches ein zweiter Kopf mit unregelmässigen Zellteilungen.

Es geht aus dem Angeführten hervor, dass die Prothallien, wenn sie mit Giften behandelt werden, eine abnorme Gestalt annehmen. Es werden Keulen oder Köpfe mit unregelmässiger Anordnung der Zellen gebildet. Wenn die Gifte durch Nährlösung, $^1/_2$ Ib + II, ersetzt werden, kehren die Prothallien zu der normalen, schlauchförmigen Wachstumsweise zurück. Die durch die Gifte hervorgerufene abnorme Gestaltung der Prothallien ist somit reversibel.

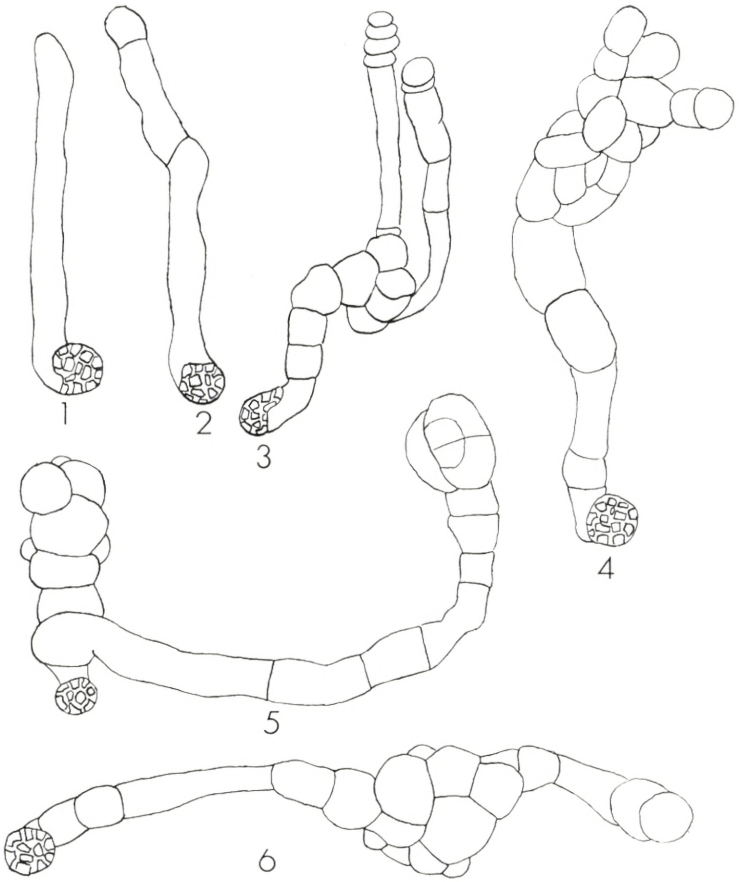

Abb. 2. Wirkung von Colchicin, 2,4-D und 2M-4Cl auf die Entwicklung der *Pteris*-prothallien in rotem Licht, 1), 2) normale Prothallien, 10 Tage alt, 3), 4) Wirkung von Colchicin. Saat 19/7 0,2 % Colchicin 25/7, $^1/_2$ Ib + II 29/7, blaues Licht 1/8 gez. 2/8, 5) Wirkung von 2M-4Cl, Saat 3/2, 0,01 % 2M-4Cl 14/2, $^1/_2$ Ib + II 24/2, 0,01 % 2M-4Cl 12/3, gez. 21/3, 6) Wirkung von 2,4-D, Saat 30/1, 0,01 % 2,4-D 9/2, $^1/_2$ Ib + II 25/2, 0,01 % 2,4-D 29/2, gez. 9/3. Zeichenapp. 110/1.

Es ist jedoch häufig mit Schwierigkeiten verbunden, die Versuche in rotem Licht zu reproduzieren.

b. Versuche mit weissem Licht.

In Gegensatz zu den Versuchen in rotem Licht verlaufen die Versuche in weissem Licht immer in derselben Weise, und sie lassen sich leicht reproduzieren.

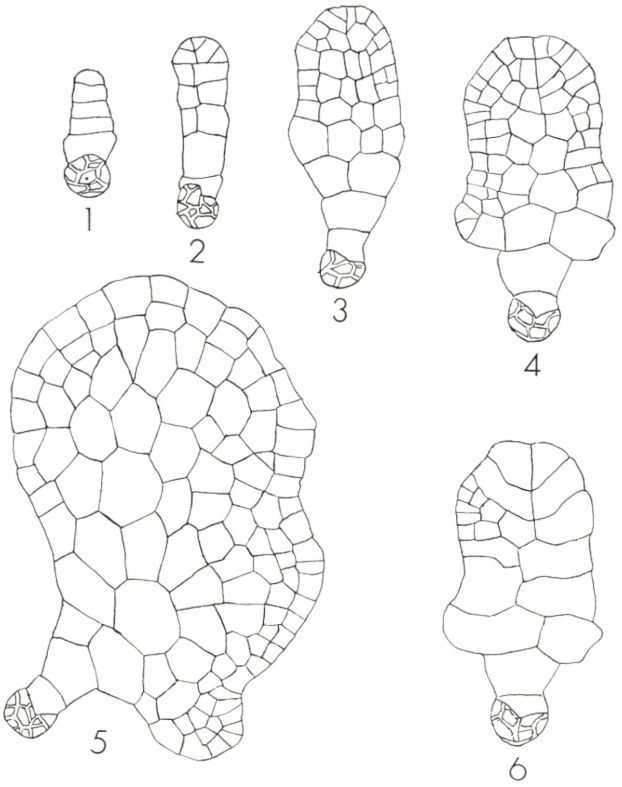

Abb. 3. Normale Entwicklung der *Pteris*prothallien in weissem Licht, 1) 9 Tage, 2) 11 Tage, 3) 13 Tage, 4) 16 Tage, 5) 24 Tage alt. 6) Schematische Darstellung der Entwicklung des in 4) dargestellten Prothalliums. In einem der Sektoren sind die Periklinen und Antiklinen eingetragen. Zeichenapp. 110/1.

1. *Normale Entwicklung.*

Bei der Keimung entsteht zuerst eine Zellreihe, die aus 6—8 Zellen besteht (Abb. 3,1). Die erste und zweite Zelle teilen sich nicht. Die übrigen Zellen teilen sich durch Längswände, die jedoch nicht immer in Verlängerung zueinander liegen. Bisweilen entwickelt sich die apikale Zelle als eine Scheitelzelle (Abb. 3,2). Die Zellen der zweigeteilten Zellreihe in Abb. 6,2 wachsen sodann zu Sektoren, Trapezen oder unregelmässigen Vierecken aus, die sich zu dem flachen, einschichtigen, ovalen, horizontalen Prothallium zusammenfügen (Abb. 3,6). Gleichzeitig teilen sie sich wie gewöhnlich durch perikline und antikline Zellwände (Abb. 3, 3, 4). Die periklinen Zellwände sind mit der Peripferie parallel,

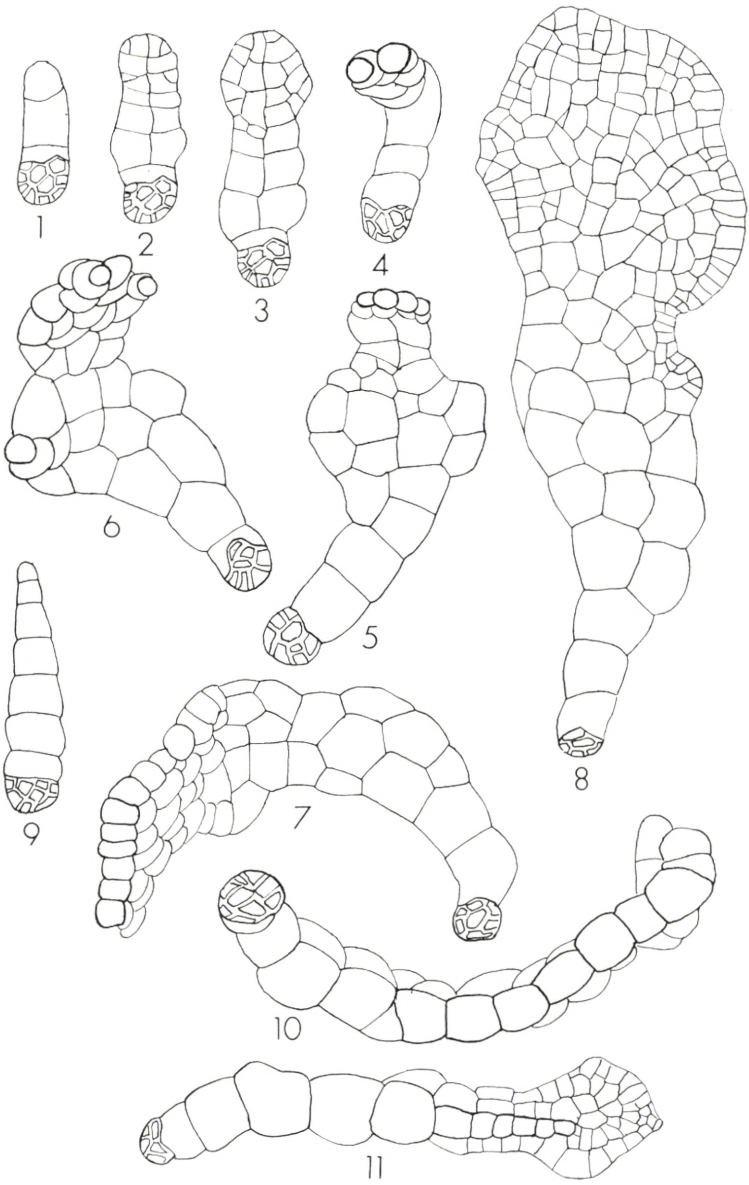

Abb. 4 a.

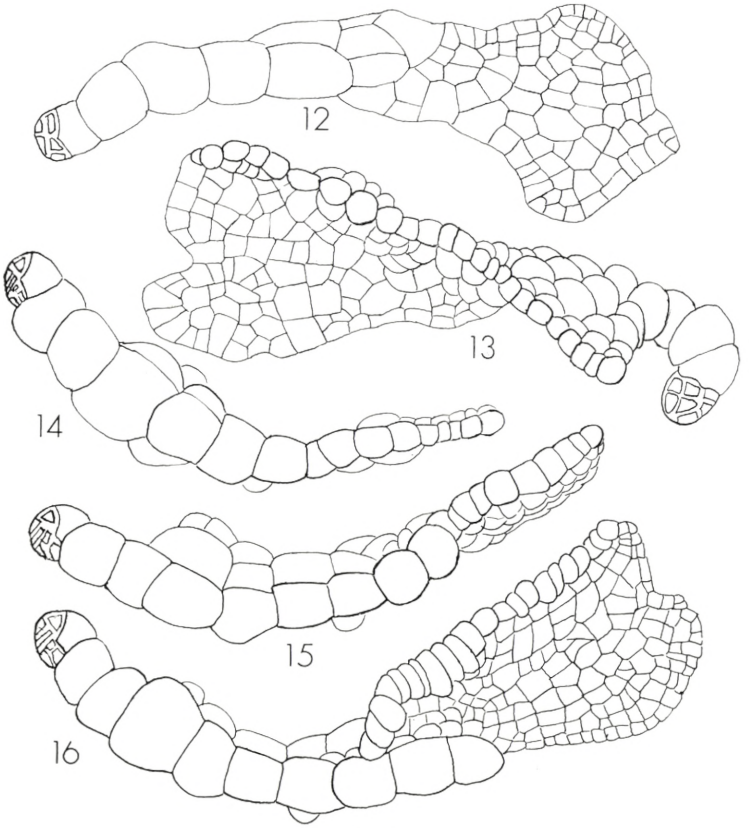

Abb. 4 b.

Abb. 4. Wirkung von Colchicin auf die Entwicklung von *Pteris*prothallien in weissem Licht. 1)—8) Pseudonormale Entwickl. 1), 2), 3) Entw. eines Proth. Saat 25/7, 0,2 % Colchicin 28/7 1) gez. 4/8, 2) gez. 6/8, 3) gez. 8/8, 4) neg. geotr. Krümmung, Saat 18/8, 0,2 % Colchicin 22/8, gez. 3/9, 5), 6), 7) ältere Proth. 6) und 7) mit vertik. Proth.fläche, Saat 9/8, 0,2 % Colchicin 13/8, 5) gez. 28/8, 6), 7) gez. 1/9, 8) Regen. eines norm. Proth. Saat 9/8, 0,2 % Colchicin 13/8, $^1/_2$ I_b + II 23/8, gez. 31/8, 9)—16) Kegelförmige Proth. 9), 10), Saat 9/8, 0,2 % Colchicin 13/8, 9) gez. 23/8, 10) gez. 1/9, 11), 12) Regen. eines symm. Proth., Saat 8/8, 0,2 % Colchicin 13/8, $^1/_2$ I_b + II 23/8, 11) gez. 28/8, 12) gez. 30/8, 13) Regen. einer horizont. (einseitigen) und einer vertik. Proth.fläche, Saat 25/7, 0,2 % Colchicin 28/7, $^1/_2$ I_b + II 11/8, gez. 20/8, 14), 15), 16) Regener. einer horizont. (einseitigen) und einer vertikalen Proth.fläche, Saat 9/8, 0,2 % Colchicin 13/8, $^1/_2$ I_b + II 23/8, 14) gez. 28/8, 15) gez. 30/8, 16) gez. 3/9. Zeichenapp. 110/1.

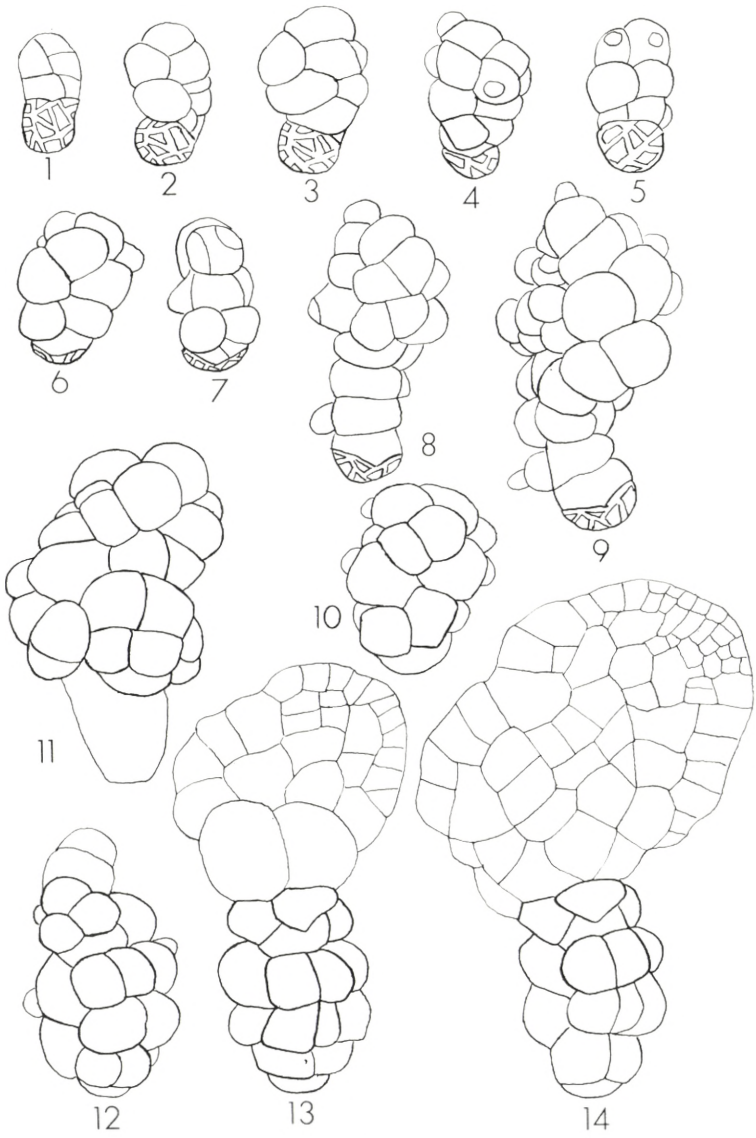

Abb. 5 a.

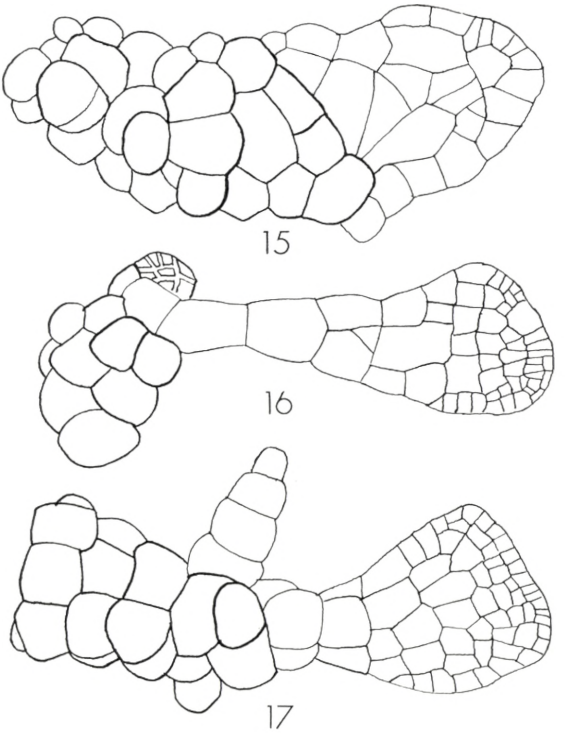

Abb. 5 b.

Abb. 5. Wirkung von 2,4-D auf die Entwicklung von *Pteris*prothallien in weissem Licht 1), 2), 3) Entw. eines Proth., Saat und 0,01 % 2,4-D 27/7, 1) gez. 9/8, 2) gez. 11/8, 3) gez. 13/8, 4), 5), 6), 7), vier Typen aus demselben Präp. gez. 15/8, 8), 9), Entw. eines älteren Proth., Saat 9/8, 0,01 % 2,4-D 13/8, 8) gez. 29/8, 9) gez. 1/9, 10), 11) (= 4) Bildung einer Zunge, Saat und 0,01 % 2,4-D 27/7, $^1/_2$ I_b + II 13/8, 10) gez. 18/8, 11) gez. 25/8, 12), 13), 14), Regen. eines norm. Proth. (dasselbe Präp. wie 10), 12) gez. 21/8, 13) gez. 27/8, 14) gez. 29,8, 15) Regen. eines Proth. aus vielen Zellen (dasselbe Präp. wie 10) gez. 31/8, 16) Regen. eines Proth, aus einer Zelle, Saat 9/8, 0,01 % 2,4-D 13/8, $^1/_2$ I_b + II 25/8, gez. 6/9, 17) Entw. einer Zunge und eines norm. Proth. (dasselbe Präp. wie 16) gez. 6/9. In den Präparaten sind die regenerierten, normalen Prothallien hellgrün, die Kallusprothallien dagegen dunkelgrün, weil in den letzteren viele Zellen übereinander liegen und der Chlorophyllgehalt grösser ist. Dieser Unterschied ist in den Zeichnungen dadurch wiedergegeben, dass die Zellwände in den Kallusprothallien dicker gezeichnet sind, was eigentlich unrichtig ist. Zeichenapp. 110/1.

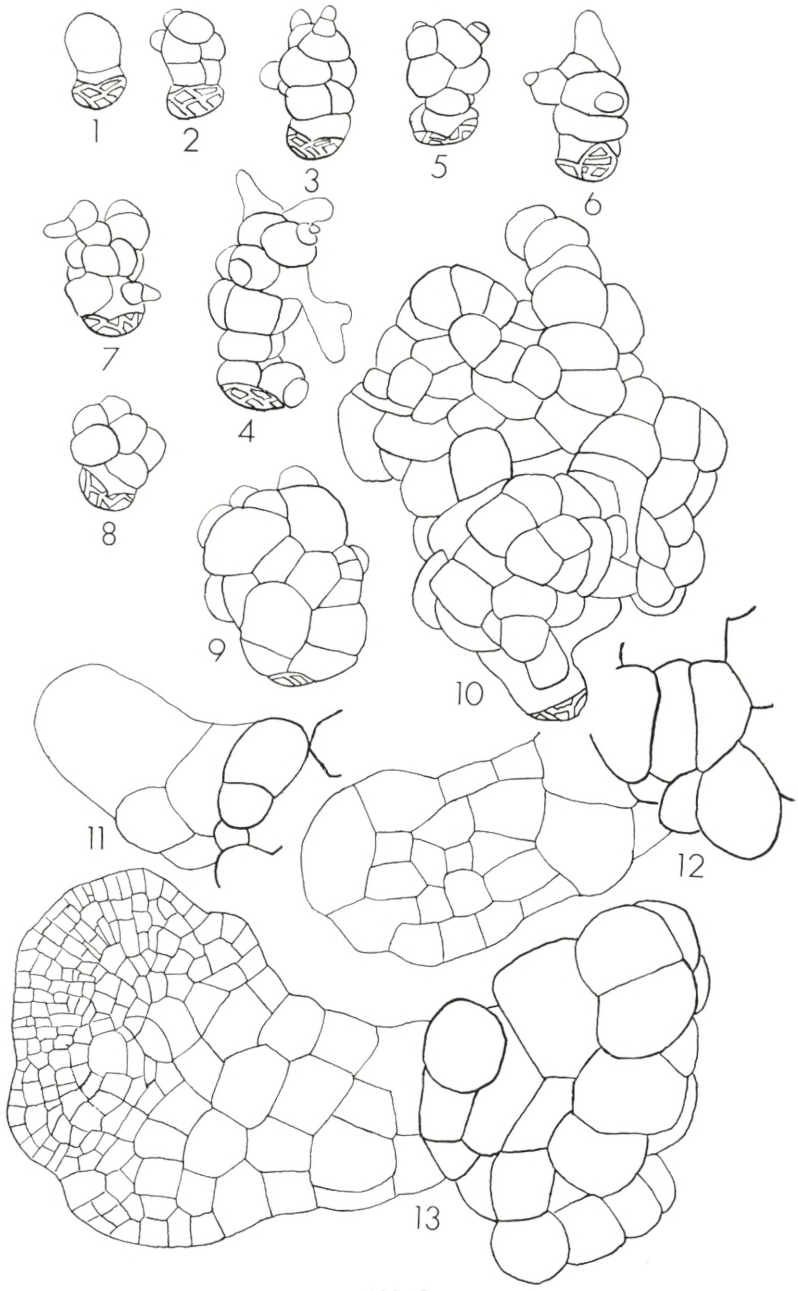

Abb. 6.

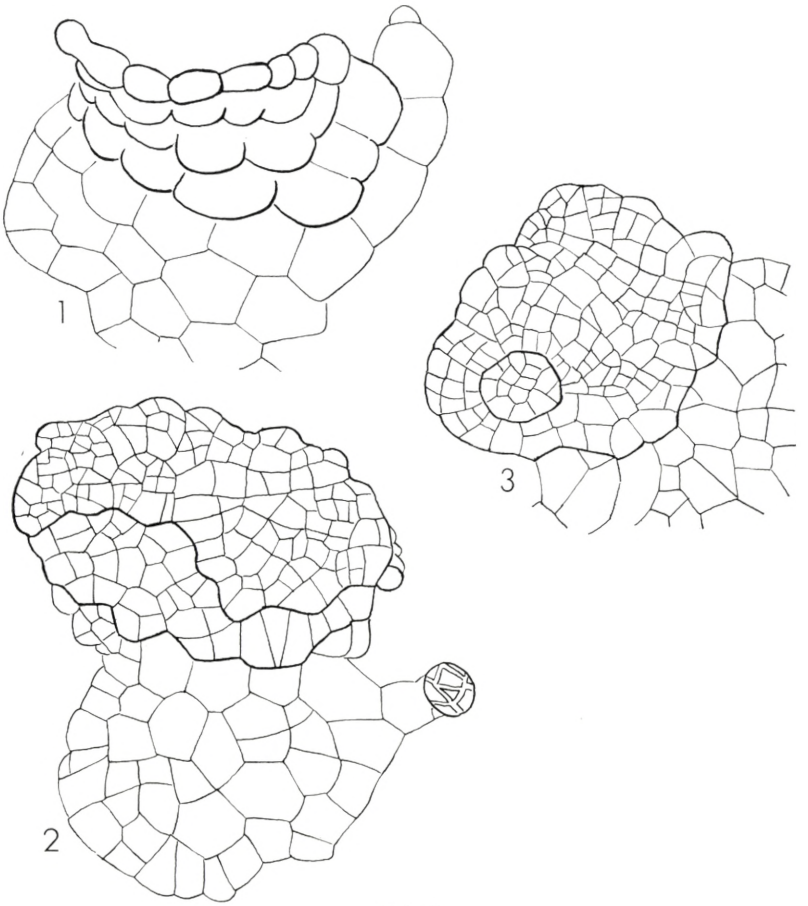

Abb. 7.

Abb. 6. Wirkung von 2M-4Cl auf die Entwicklung von *Pteris*prothallien in weissem Licht. 1), 2), 3), 4), Entw. eines Proth., Saat 9/8, 0,01 % 2M-4Cl 13/8, 1) gez. 23/8, 2) gez. 25/8, 3) gez. 27/8, 4) gez. 31/8, 5), 6), 7), drei Typen aus demselben Präp. gez. 27/8, 8), 9), 10), Entw. eines Kallusproth. in Nährl., keine Regen., Saat und 0,01 % 2M-4Cl 17/7, $^1/_2$ I_b + II 30/7, 8) gez. 1/8, 9) gez. 8/8, 10) gez. 21/8, 11), 12), 13), Entw. eines norm. Proth. (dasselbe Präp. wie 8—10), 11) gez. 25/8, 12) gez. 27/8, 13) gez. 29/8. Zeichenapp. 110/1.

Abb. 7. Wirkung von Colchicin, 2,4-D und 2M-4Cl auf ältere normale Prothallien. 1) Saat und $^1/_2$ I_b + II 25/7, 0,2 % Colchicin 11/8, gez. 24/8, 2) Saat und $^1/_2$ I_b + II 24/4, 0,01 % 2,4-D 9/5, gez. 18/5, 3) Saat und $^1/_2$ I_b + II 24/4, 0,01 % 2M-4Cl 9/5, gez. 20/5. Die dicken Linien sind Grenzen verschiedener Stockwercke in den Zellenpolstern. Zeichenapp. 110/1.

und da die letztere mehr oder weniger gekrümmt ist, sind auch die Periklinen häufig schwach bogenförmig, sie liegen namentlich in älteren Zellen nicht in Verlängerung zueinander; die antiklinen Zellwände divergieren gegen die Peripferie. Das Wachstum ist marginal, später entsteht an der einen Seite des Flügels ein Meristem (Abb. 3,5), das sich zu einem zweiten Flügel entwickelt, das Prothallium erhält dann die für die Polypodiaceen charakteristische, herzförmige Gestalt.

Die Grundelemente, aus denen die oben erwähnten Sektoren namentlich in den peripferen Teilen aufgebaut sind, sind Zellen, die von 6 Zellwänden umgeben sind. Die zwei Zellwände sind horizontal und wachsen nach allen Richtungen aus. Die 4 senkrechten Wände wachsen nur in die Länge, dagegen nicht in die Höhe. Die vordere und hintere vertikale Zellwand, die Periklinen, stellen zwei gerade Linien oder konzentrische Kreisbögen dar, die zwei seitlichen, die Antiklinen, zwei Radien. Hinsichtlich des Wachstums der Zellwände ist somit in dem normalen Prothallium eine ausgesprochene Differenzierung vorhanden. Eine jede Zelle ist von zwei Typen von Zellwänden mit verschiedener Wachstumsweise umgeben, den vertikalen und den horizontalen, die sich unter rechten Winkeln schneiden.

2. *Wirkung von Colchicin.*

Wenn man ein Präparat mit Prothallien, die sich auf Colchicinagar entwickelt haben, oder die mit einer 0,2 %igen Colchicinlösung getröpfelt worden sind, unter dem Mikroskop untersucht, erhält man ein sehr buntes Bild. Es scheint jedoch, dass man die Prothallienformen in zwei Hauptgruppen einteilen kann, die pseudonormalen und die kegelförmigen.

a. Die pseudonormalen Prothallien. 1. *0,2 %iger Colchicin.* Es entsteht eine Reihe von etwa 6—8 kurzen, zylindrischen Zellen, die basalen Zellen teilen sich durch Längswände, die apikalen entwickeln sich zu einer einschichtigen, ovalen, kleinzelligen Prothalliumfläche. Diese ist somit gestielt (Abb. 4,1,2,3). Anscheinend ist eine gewisse Ähnlichkeit mit dem normalen Prothallium vorhanden. Eine nähere Analyse zeigt jedoch, dass gewisse Unterschiede vorhanden sind. Die Zellteilungen sind unregelmässiger. Häufig werden aus grösseren Zellen durch bogenförmige Wände kleine Zellen herausgeschnitten.

Das endständige ovale Prothallium hört bald zu wachsen auf. Es kann dann der Stiel eine Prothalliumfläche bilden, so dass man zwei horizontale Zellflächen erhält, die durch Einbuchtungen von einander getrennt sind (Abb. 4,5). Ferner können die Prothalliumzellen sich durch horizontale Zellwände teilen, so dass das Prothallium stellenweise mehrschichtig wird.

Es waltet in den Colchicin-Prothallien ein Bestreben, die periklinen oder die antiklinen Zellwände, die in den normalen Prothallien senkrecht stehen, in horizontaler Lage unterzubringen. Dies kann auf zweierlei Weise erreicht werden. Einmal sieht man junge Prothallien, die nur aus einer Zellreihe bestehen, sich negativ geotropisch krümmen (Abb. 4,4). Die weitere Entwicklung führt dann zu einem Prothallium, das aus zwei Zellflächen besteht, die senkrecht aufeinander stehen, einer basalen horizontalen und einer distalen vertikalen (Abb. 4,6). Ferner können in dem apikalen Teil einer Zellreihe wiederholt horizontale Längswände gebildet werden. Es entsteht dann gleichfalls eine senkrechte Prothalliumfläche (Abb. 4,7).

Wenn die Prothallien auf dem Colchicinagar verbleiben, hören sie allmählich zu wachsen auf. Die basalen Zellwände färben sich braun.

2. *0,2 % Colchicin* + $^1/_2$ *Ib* + *II*. Wenn das Colchicin aus dem Agar durch Tröpfeln mit Nährlösung auf einem frühen Stadium entfernt wird, entwickeln sich aus den pseudonormalen Prothallien normale Prothallien mit Marginalwachstum. Die Prothallien sind jedoch bisweilen gestielt (Abb. 4,8). Häufig teilen sich jedoch auch die basalen Zellen, so dass die Prothallien vollkommen normal werden.

b. Die kegelförmigen Prothallien. 1. *0,2 % Colchicin*. Neben den pseudonormalen Prothallien werden mehr oder weniger häufig Prothallien gebildet, die nur aus einer Reihe von Zellen bestehen, deren Grösse allmählich abnimmt. Auf diese Weise entsteht ein Kegel, der sehr spitz und häufig etwas gebogen ist (Abb. 4,9). Diese kegelförmigen Prothallien setzen das Wachstum eine Zeitlang fort. Die Zellen können sich durch horizontale Längswände teilen (Abb. 4,10).

2. *0,2 % Colchicin* + $^1/_2$ *Ib* + *II*. Auch die kegelförmigen Prothallien können zu normalen umgebildet werden. Häufig gehen jedoch die Zellteilungen, die zur Bildung der Prothalliumfläche

führen, nicht von den kleinen Zellen in der Spitze aus, sondern von den grösseren Zellen, die hinter der Spitze liegen. Es kann dann, wie in Abb. 4,11,12 dargestellt, eine Prothalliumfläche, die symmetrisch zu der Achse liegt, gebildet werden. Die Spitze der Achse verkümmert.

In Abb. 4,13 ist dagegen eine einseitige Prothalliumfläche gebildet worden. Daneben haben die Zellen in dem Basalteil der Achse sich mehrmals durch horizontale Längswände geteilt. Es ist dadurch ein Kamm entstanden, der von etwa 7 Zellreihen, die übereinander liegen, gebildet wird. Das Prothallium besteht somit aus zwei senkrecht aufeinander stehenden Zellflächen, einer horizontalen und einer vertikalen.

In ähnlicher Weise hat sich das in Abb. 4,14,15,16 dargestellte Prothallium entwickelt. Die Achse besteht, was sich in der Zeichnung nicht darstellen lässt, aus drei Stockwerken, von denen das apikale das höchste ist. Von den oberen Zellen des letzteren hat sich eine einseitige Prothalliumfläche entwickelt, die sich schräg nach unten senkt.

c. $^1/_2\ I_b + II + 0{,}2\ \%$ Colchicin. Wenn ältere, normale Prothallien mit 0,2 % Colchicin getröpfelt werden, beginnen in vielen Prothallien eine Reihe von Zellen, die an dem Rande liegen und die daher jung und teilungsfähig sind, sich durch horizontale Zellwände zu teilen. Es entsteht daher ein Kamm von Zellen, der sich senkrecht über die horizontale Prothalliumfläche erhebt. Die Zellengrösse der Zellreihen nimmt nach oben ab (Abb. 7,1).

Die Wirkung des Colchicins auf das Wachstum der *Pteris*-prothallien kann in folgender Weise zusammengefasst werden.

Die Wirkung von 2,4-D und 2M-4Cl, die unten besprochen werden soll, führt zu Kalluswachstum der Prothallien. Ein solches kann auch durch Colchicin hervorgerufen werden, ist aber relativ selten. Am häufigsten bleibt bei dem Wachstum der colchicinbehandelten Prothallien eine gewisse Gesetzlichkeit bewahrt. Aber zwei Unregelmässigkeiten können häufig beobachtet werden.

1. Die Zellen, die bei den Zellteilungen entstehen, nehmen allmählich an Grösse ab. Diese Erscheinung ist sehr auffällig bei den spitzen, kegelförmigen Prothallien, kann aber auch bei den vertikalen Prothalliumflächen beobachtet werden.

2. In normalen Prothallien liegen die Achsen der Kernspindeln in der Horizontalebene. In den colchicinbehandelten Prothallien tritt häufig entweder während (Abb. 7,1) oder teilweise nach (Abb. 4,14,15,16) der Behandlung eine Drehung der Kernspindeln um 90° ein, so dass entweder ausschliesslich horizontale Zellwände oder sowohl horizontale als senkrechte Zellwände gebildet werden. Es entstehen dadurch senkrechte Prothalliumflächen.

Die Wirkung des Colchicins auf die Orientierung der Achsen der Kernspindeln ist jedoch wahrscheinlich eine sekundäre. Die primäre Wirkung besteht vermutlich darin, dass eine Umlagerung des Wachstums stattfindet. Während in den normalen Prothallien die vertikalen Zellwände nur in die Länge, indessen nicht in die Höhe wachsen, wachsen sie in den colchicinbehandelten Prothallien dagegen in die Höhe, gleichzeitig hören die horizontalen Zellwände zu wachsen auf. Es entstehen auf diese Weise vertikale, prismatische Bildungen, die sich durch horizontale Zellwände teilen, die Achsen der Kernspindeln müssen dann vertikal zu liegen kommen.

3. *Wirkung von 2,4-D.*

1. *0,01 % 2,4-D.* Wenn die *Pteris*sporen mit einer 0.01 %-igen Lösung von 2,4-D behandelt werden, treten schon, wenn eine Reihe von 3 Zellen gebildet worden ist, verschiedene Veränderungen ein. Die Grösse der Zellen nimmt apikalwärts zu, sie runden sich ab und es entstehen abnorme Teilungen. Die Endzelle kann sich durch eine horizontale Längswand in zwei, oder durch zwei Längswände in vier Zellen teilen, es können aus den grösseren kleinere Zellen herausgeschnitten oder es können durch Sprossung auf der Oberfläche der Zellen neue Zellen gebildet werden. Das Prothallium wird kallusähnlich, die Achsen der Kernspindeln liegen in allen möglichen Richtungen. Trotzdem ist die äussere Oberfläche jedoch ziemlich eben, das ganze Prothallium ist meistens keulenförmig. Die Entwicklung eines Prothalliums ist in Abb. 5,1,2,3 und verschiedene Typen sind in Abb. 5,4,5,6,7 dargestellt.

Die Prothallien setzen oft das Wachstum lange Zeit fort und können eine bedeutende Grösse erreichen. Die Keulenform wird

oft bewahrt, die Oberfläche aber wird häufig sehr uneben (Abb. 5, 8, 9).

2. *0,01* $^0/_0$ *2,4-D* + $^1/_2$ *Ib* + *II*. Wenn auf einem frühen Stadium der Entwicklung eines Kallusprothalliums die 2,4-D lösung durch eine normale Nährlösung ersetzt wird, setzt sich das Kalluswachstum zunächst einige Tage fort. Nach etwa 10 Tagen kommt aus den meisten Prothallien eine Zunge hervor (Abb. 5, 10 11), und diese entwickelt sich zu einem normalen, einschichtigen, horizontalen Prothallium mit Marginalwachstum. Die Geschwindigkeit, mit der sich das normale Prothallium entwickelt, ist sehr bedeutend. Es findet etwa in zwei Tagen eine Verdopplung der Prothalliumfläche statt; die Entwicklung ist somit exponentiell (Abb. 5, 12, 13, 14). In einigen Fällen ist der Neuzuwachs jedoch zylindrisch, offenbar weil die Giftwirkung nicht ganz überwunden ist.

Da man annehmen muss, dass alle Zellen in einem Kallusprothallium gleichartig sind, wäre zu erwarten, dass von jeder derselben ein normales Prothallium gebildet würde. Merkwürdigerweise ist das nicht der Fall. Mit vereinzelten Ausnahmen entsteht aus jedem Kallusprothallium nur ein normales Prothallium. Zwar habe ich wiederholt beobachtet, dass zwei bis drei anscheinend gleich starke Zungen herauskamen. Bald traten aber vermehrte Zellteilungen in einer derselben ein, gleichzeitig übernahm diese die Führung und entwickelte sich zu einem normalen Prothallium, während die andere zu wachsen aufhörte (Abb. 5, 17). Man wird wohl daher schliessen müssen, dass das Kallusprothallium, wenn das Gift entfernt ist, trotz seinem zufälligen Aufbau eine Ganzheit bildet.

Die normalen Prothallien können sich anscheinend an einem beliebigen Ort des Kallusprothalliums entwickeln, bisweilen entstehen sie in der Spitze (Abb. 5, 15), in anderen Fällen an der Basis desselben (Abb. 5, 16). In einigen Fällen gehen sie von einer einzelnen Zelle aus, es entsteht dann zuerst eine Reihe zylindrischer Zellen, die sich in ähnlicher Weise wie bei normalen Prothallien zu einem flächenförmigen Prothallium entwickeln (Abb. 5, 16). In anderen Fällen sind die regenerierten Prothallien so breit angesetzt, dass eine Mehrzahl von Zellen bei der Ausbildung der Prothallien beteiligt sein muss (Abb. 5, 15). Der determinierende Faktor, der die Prothalliumbildung hervorruft, wirkt in dem letzteren Falle schon von Anfang an überzellulär.

3. $^1/_2$ *Ib* + *II* + *0,01* % *2,4-D.* Werden ältere, normale Prothallien mit 0,01 % 2,4-D getröpfelt, fangen die Zellen stellenweise an sich zu teilen, es entstehen dann Polster von unregelmässig angeordneten Zellen auf der Prothalliumfläche (Abb. 7, 2).

Zusammenfassung: 1. Wenn *Pteris*sporen mit 2,4-D lösung getröpfelt werden, verschwindet der Unterschied zwischen der Wachstumsweise der vertikalen und horizontalen Zellwände und das Wachstum wird gleichmässig über die Zellwand verteilt, es entstehen dann, statt den flachen prismatischen oder zylindrischen Zellen des normalen Prothalliums, kugelförmige Zellen. Es können sich auch die Zellulosenbildner an bestimmten Stellen der Zelloberfläche anhäufen, es werden dann neue Zellen durch Sprossung gebildet.

2. Die Achsen der Kernspindeln, die in den normalen Prothallien horizontal sind, kommen unter der Einwirkung der 2,4-D lösung in allen möglichen Richtungen zu liegen. Die Anordnung der Zellen wird, namentlich in älteren Prothallien, daher mehr oder weniger zufällig, es entsteht ein kallusähnliches Prothallium.

3. Wenn die 2,4-D lösung durch eine Nährlösung ersetzt wird, können die kallusähnlichen Prothallien normale Prothallien erzeugen. Von jedem Prothallium wird normalerweise nur ein normales Prothallium gebildet; dasselbe kann entweder an der Basis oder an der Spitze entstehen, es kann von einer einzelnen Zelle oder von einer Mehrzahl von Zellen ausgehen.

4. *Wirkung von 2M-4Cl.*

Die Wirkung von 2M-4Cl auf die *Pteris*prothallien ist eine ähnliche wie diejenige von 2,4-D; doch ist die erstere Verbindung viel giftiger.

1. *0,01* % *2M-4Cl.* Schon die zweite Zelle, die bei der Keimung entsteht wird häufig abnorm gross, und es treten bald unregelmässige Teilungen ein. Es entsteht ein kurzes, dickes, zylindrisches Prothallium mit einem sehr unregelmässigen Bau und einer unebenen Oberfläche. Die Entwicklung eines solches Prothalliums ist in Abb. 6, 1, 2, 3, 4, abgebildet. Verschiedene Typen sind in Abb. 6, 5, 6, 7 dargestellt. Auffallend sind namentlich kleine warzenförmige Zellen, die an der Oberfläche grösserer

Zellen entstehen, und die sehr lange, dünne, farblose Stäbchen tragen können.

Die Prothallien setzen das Wachstum lange Zeit fort, und sie nehmen oft eine grünschwarze Färbung an.

2. *0,01 % 2M-4Cl + ½ Ib + II.* Wenn Kallusprothallien, die 8 Tage alt sind, mit Nährlösung getröpfelt werden, bilden die meisten derselben normale Prothallien. Mit zunehmendem Alter nimmt die Regenerationsfähigkeit stark ab. Mit einem 13 Tage alten Kallusprothallium wurden folgende Ergebnisse erzielt.

Die meisten Prothallien setzten in der Nährlösung das Kalluswachstum fort und erreichten eine sehr bedeutende Grösse (Abb. 6, 8, 9, 10). Es scheint, dass sie die Fähigkeit zur Chlorophyllbildung verloren hatten. Die Prothallien wurden bleicher und bleicher.

Neben diesen Prothallien fanden sich aber einige wenige, die langsam wuchsen und die grüne Farbe behielten. Aus diesen kam, wie oben geschildert wurde, eine Zunge hervor, die sich zu einem normalen Prothallium entwickelte. Die Wachstumgeschwindigkeit des normalen Prothalliums war sehr gross; das in Abb. 6, 11, 12, 13 dargestellte Prothallium wurde in 4 Tagen entwickelt.

Ganz wie in dem Versuche mit 2,4-D kamen bisweilen 2—3 Zungen zum Vorschein. Es entwickelte sich aber stets nur ein normales Prothallium aus jedem Kallusprothallium.

3. *½ Ib + II + 0,01 % 2M-4Cl.* Wenn normale Prothallien mit 0,01 % 2 M-4 Cl getröpfelt werden, findet, ganz wie in den Versuchen mit 2,4-D, stellenweise ein Kalluswachstum mit Bildung von Zellenhaufen an der Oberfläche der Prothallien statt (Abb. 7, 3).

4. Schlussfolgerungen.

a. Die undifferenzierte Wachstumsweise.

Wenn eine Kalluszelle wächst, geschieht es in der Weise, dass die verschiedenen Teile, aus denen das Protoplasma aufgebaut ist, Zytoplasma, Plastiden, Gene u. s. w., sich selbst reproduzieren oder (und) verschiedene andere Stoffe, die für den Aufbau notwendig sind, erzeugen. Die neugebildeten Elemente werden somit zwischen den schon vorhandenen eingelagert, es ist dies

jene Wachstumsweise, die man Intussusception nennt. So lange die normale Verteilung von Reaktions- und Bildungszentren beibehalten wird, behält auch das Protoplasma während des Wachstums seine Struktur unverändert.

Wenn eine solche Zelle ihr Wachstum in dieser Weise forsetzen würde, würde schnell ein Gebilde entstehen, das nicht existenzfähig wäre. Während des Wachstums findet zweifellos eine nähe Zusammenarbeit zwischen Plasma und Zellkern statt; damit dieselbe stattfinden kann, darf der Abstand zwischen den verschiedenen Plasmateilen und dem Kern nicht zu gross sein. Wenn daher die Zelle eine gewisse Grösse erreicht hat, tritt ein Sprung in der Entwicklung ein, eine Kernteilung, deren Bedeutung darin zu suchen ist, dass der nahe Kontakt zwischen den zwei Protoplasmakomponenten aufrecht erhalten werden kann. Während der Kernteilung werden die Chromosomenhälften jede nach ihre Seite bewegt, sie sammeln sich in zwei Gruppen, von denen jede zu einem Tochterkern umgebildet wird. Nachher tritt dann auch eine Teilung des Zytoplasmas ein, und es entstehen in dieser Weise zwei Tochterzellen, die mit der Mutterzelle vollkommen identisch sind. Wir benennen eine solche Teilung eine äquale Zellteilung.

Die Zellteilung ist ausserordentlich kompliziert, sie besteht aus einer Reihe von Vorgängen, die zwangsläufig aufeinander folgen. Dieser Komplex liegt im Protoplasma bereit, und kann entweder autonom eintreten oder durch verschiedenen Stoffe in Gang gesetzt werden. Wir bezeichnen einen solchen Komplex als einen Bereitschaftskomplex.[1]

b. Die differenzierte Wachstumsweise.

Im Gegensatz zu dem Kalluswachstum hat man eine andere Wachstumsweise, die sich darin äussert, dass Zellteile, Zellen oder Zellgruppen, die ursprünglich gleichartig sind, gegeneinander ungleichartig werden. Das Ergebnis solcher Wachstumsvorgänge nennt man eine Differenzierung. Die Gestaltung einer höheren

[1] Andere Bereitschaftskomplexe in dem Protoplasma sind die Zytoplasmabewegungen, die durch Histidin, IES oder andere Stoffe hervorgerufen werden, und die Chloroplastenbewegungen, durch welche die Chloroplasten sich an bestimmten, für ihre Tätigkeit optimalen Orten ansammeln. Ferner stellt vielleicht auch die Bildung von Tracheiden einen Bereitschaftskomplex dar.

Pflanze ist die Summe von Differenzierungsvorgängen während der Entwicklung.

Als Beispiele solcher ontogenetischen Diffenzierungstypen sollen einige möglichst einfache erwähnt werden.

Die Bildung der Trichoblasten in der Wurzelepidermis von *Phleum* ist dadurch ermöglicht, dass durch eine inäquale Zellteilung zwei ungleichartige Zellen gebildet werden, eine plasmareichere (der Trichoblast) und eine plasmaärmere, die kein Wurzelhaar bildet.

Die Wurzelhaare bei *Phleum* werden dadurch gebildet, dass an dem apikalen Ende der Aussenwand der Trichoblasten eine Ausstülpung entsteht, die zu dem Wurzelhaar auswächst.

Das flächenförmige Prothallium der Farne entwickelt sich, wie oben beschrieben, dadurch, dass die Zellen in einer zweigeteilten Zellreihe durch ungleichartiges Wachstum der vertikalen und horizontalen Zellwände zu flachen Sektoren oder Trapezen auswachsen, die sich durch antikline und perikline Zellwände teilen und sich zu dem Prothallium zusammenfügen.

Alle die erwähnten Vorgänge, die man unmittelbar entweder mit blossem Auge oder mit dem Mikroskope wahrnehmen kann, bezeichnen wir als Differenzierungsvorgänge, und das Ergebnis derselben als ein Differenzierungsmuster. Bevor ein Differenzierungsvorgang eintritt, muss aber ein nicht unmittelbar sichtbarer Vorgang eingetreten sein, der den betreffendenden Differenzierungsvorgang hervorruft. Diesen Vorgang nennt man einen Determinationsvorgang, und das Ergebnis der Determinationsvorgänge nennt man ein Determinationsmuster. In den Zellen oder Zellgruppen, in welchen ein Determinationsmuster entstehen soll, müssen alle die für die Entstehung desselben notwendigen materiellen Anlagen vorhanden sein, aber ferner muss auch, wie unter anderem aus den oben erwähnten Versuchen mit Farnprothallien hervorgeht, ein determinierender Faktor mitwirken, der die mit der Bildung des Determinationsmusters verknüpften Umlagerungen hervorruft.

Als Beispiel sei erwähnt, dass die Bildung eines Wurzelhaares ein Differenzierungsvorgang ist. Die Anlagen in den Trichoblasten, die für die Wurzelhaarbildung notwendig sind, sind die Zellulosenbildner, die ursprünglich gleichmässig in der Zellwand verteilt sind. Das Determinationsmuster entsteht nun dadurch,

dass die Zellulosenbildner verschoben werden, so dass sie sich an dem apikalen Ende der Trichoblasten anhäufen. Der Faktor, der diese Verschiebung hervorruft, ist der determinierende Faktor.

c. Die Eigenschaften des determinierenden Faktors.

Wir kehren nun zu den Versuchen über die Wirkung der Gifte auf die Farnprothallien zurück.

Wenn die Prothallien mit den Giftlösungen behandelt werden, setzen sich der Aufbau der Protoplasmabestandteile, das Wachstum der Zellwände und die Zellteilungen fort. Alle Anlagen, die für diese Vorgänge notwendig sind, müssen somit in den Zellen vorhanden sein. Während aber in den normalen Prothalliumzellen die horizontalen und vertikalen Zellwände ungleichartig wachsen, ist das Wachstum in den mit Giften behandelten Prothallien gleichmässig über die Zellwände verteilt. Die Zellen werden daher ungefähr kugelförmig, und es entsteht statt des flächenförmigen Prothalliums ein mehr oder weniger unregelmässiger Zellhaufen, d. h. die normale gesetzgebundene Zusammenfügung der Zellen zu einer Ganzheit, das Prothallium, wird durch eine gesetzlose Anordnung der Zellen ersetzt. Es ist somit die Wirkung des determinierenden Faktors ausgeschaltet worden. Die Ausschaltung ist transitorisch, indem, wenn die Giftwirkung entfernt wird, ein normales Prothallium gebildet wird.

Man wird aus den Versuchen die folgenden Schlüsse ziehen können:

1. Da man die Wirkung des determinierenden Faktors ausschalten kann, während Wachstum und Zellteilung fortgesetzt werden, müssen die zwei Gruppen von Vorgängen von verschiedener Ordnungshöhe sein. Die erstere, die Determination, muss der letzteren, Wachstum und Zellteilung, übergeordnet sein.

2. Da die Ausschaltung des determinierenden Faktors transitorisch sein kann, wird dieser Faktor durch die Giftwirkung nicht zerstört, wahrscheinlich gar nicht beeinflusst, sondern nur gelähmt oder betäubt. Richtiger ist es vielleicht zu sagen, dass die Zellen durch die Giftwirkung in einen Zustand versetzt werden, in welchem der determinierende Faktor seine Wirkung nicht entfalten kann.

Man ist somit nicht imstande, den determinierenden Faktor

durch Gifte zu verändern. Man hat ihn oder man hat ihn nicht. Und ebensowenig vermag man diesen Faktor in anderen Fällen für dauernd zu beeinflussen.

Das schliessliche Ergebnis der Wirkung des determinierenden Faktors, der an dem Aufbau des Zellwandgerüstes beteiligt ist, ist das Zellwandmuster der fertigen Pflanze und die durch dieses Muster bedingte Gestalt derselben. Indem wir von den Veränderungen der Gestalt der Arten, die während der Evolution eingetreten sind, absehen, kann man feststellen, dass die Gestaltung der Individuen einer Art in der Natur von Generation zu Generation hinsichtlich aller wesentlichen Eigenschaften unveränderlich ist. Es muss somit auch der determinierende Faktor unveränderlich sein. Wir verfügen überhaupt nicht über Mittel, diesen Faktor für dauernd zu verändern, wie daraus hervorgeht, dass wir nicht imstande sind, eine Art in eine andere umzuwandeln.

Da die lebenden Organismen nicht statische, sondern dynamische Gebilde sind, ist die Konstanz der Arten eine sehr sonderbare Tatsache. Nach der üblichen Auffassung besteht das Lebensgetriebe ausschliesslich aus einer Unmenge verschiedener chemischer und physikalischer Vorgänge, die in einander eingreifen. Diese können unzweifelhaft jede für sich leicht durch äussere Faktoren, Temperatur, Licht, chemische Stoffe u. s. w. in sehr verschiedener Weise beeinflusst werden. Man könnte daher erwarten, dass es leicht möglich sein würde, dauernde Veränderungen in dem schliesslichen Ergebnis dieser Vorgänge, der artspezifischen Entwicklung, hervorzurufen. Das ist aber nicht der Fall. Ob wir die Pflanzen unter allen möglichen extremen Bedingungen wachsen lassen, ob wir sie mit Giften oder Strahlenwirkungen behandeln, immer erhalten wir, wenn die Pflanzen überhaupt zu wachsen und sich zu differenzieren im Stande sind, Individuen von derselben Art, mit der wir arbeiten.

Es geht aus dem Angeführten hervor, dass man die Wirkungsweise und die Eigenschaften des determinierenden Faktors des Zellwandmusters in folgender Weise kennzeichnen kann.

1) Dieser Faktor ist imstande, die orientierten Stoffumlagerungen, die mit der Bildung eines von Ort zu Ort qualitativ verschiedenen Determinationsmusters verknüpft sind, hervorzurufen. (Ein Determinationsmuster kann jedoch vielleicht auch durch lokale Neubildung von bestimmten Stoffen erzeugt werden).

2) Der determinierende Faktor kann z. B. durch Gifte transitorisch oder permanent ausgeschaltet werden. Dabei wird dieser Faktor nicht verändert.

3) Wir verfügen überhaupt nicht über Mittel, den determinierenden Faktor für dauernd zu verändern.

Daneben hat der determinierende Faktor unzweifelhaft auch andere Eigenschaften, die hier nicht besprochen werden sollen.

Bei der folgenden Untersuchung soll vorzugsweise die erstere dieser Eigenschaften berücksichtigt werden.

d. Kann die Wirkung des determinierenden Faktors bei der Bildung des Zellwandmusters auf die Wirkung von Stoffen, Strukturen oder Potentialen zurückgeführt werden?

In dem anorganischen Bereich sind zwei Wirklichkeiten vorhanden, die Materie und das Feld. Beide sind benutzt worden, um die Entstehung des Differenzierungsmusters zu erklären.

Am ältesten sind die Gedanken und Versuche, die Differenzierungsvorgänge auf Stoffe zurückzuführen. Schon Sachs sprach von organbildenden Stoffen. Diese Auffassung wird dadurch gestützt, dass man nachweisen kann, dass von aussen zugeführte Stoffe in das Entwicklungsgeschehen eingreifen können, z. B. ist IES imstande, die Bildung von Seitenwurzeln hervorzurufen. Die Wirkung der betreffenden Stoffe ist indessen davon abhängig, dass ein Reaktionssystem mit einer bestimmten Struktur und mit gewissen Entwicklungsmöglichkeiten, d. h. einer gewissen Determination, vorhanden ist. Diese Entwicklungsmöglichkeiten können durch die betreffenden Stoffe in Gang gesetzt, dagegen nicht geschaffen werden.

Vielleicht könnten aber solche determinierende Stoffe in der Zelle, in dem Plasma oder in dem Kern, vorhanden sein.

Tatsächlich kann man leicht zeigen, dass von dem Zellkern Stoffe abgegeben werden, die für die Bildung der Zellwände notwendig sind. Wenn man ein Wurzelhaar, z. B. von *Cucurbita* plasmolysiert, vermögen nur Plasmakugeln, die einen Zellkern enthalten oder mit einer solchen Kugel durch einen Plasmafaden in Verbindung stehen, sich mit einer Zellwand zu umgeben, kernfreie Plasmakugeln dagegen nicht.

Ferner kann man feststellen, dass Genprodukte modifizierend

in die Ausformung eines Zellwandmusters eingreifen können. Durch Kreuzung von *Urtica pilulifera* × *U. Dodartii* kann man nachweisen, dass die Zacken des Blattes der ersteren Art durch ein Gen bedingt sind. Man darf sich jedoch nicht vorstellen, dass das betreffende Gen einen »zackenbildenden« Stoff erzeugt, sondern es wirkt mit bei einem Stoffumsatz, der in das Getriebe der Stoffwechselvorgänge eingreift, wodurch dasselbe verändert wird. Die Folge ist, dass die Determination, die in dem Blattrande vorgeht, in verschiedener Weise verläuft, je nachdem ob das betreffende Gen vorhanden ist oder nicht. Prinzipiell besteht kein Unterschied zwischen der betreffenden Genwirkung und der Wirkung eines von aussen zugeführten Stoffes, z. B. von Indolylessigsäure.

Es geht hieraus hervor, dass von aussen zugeführte oder von Genen erzeugte Stoffe modifizierend in die Entwicklung des Zellwandmusters eingreifen können, und dass sie, wenn ein latentes Determinationsmuster vorhanden ist, die Entwicklungsmöglichkeiten in Gang setzen können, so dass ein Zellwandmuster gebildet wird. Dagegen ist es ausgeschlossen, dass Stoffe Determinations- und Zellwandmuster sollten schaffen können. Man ist nicht imstande, durch Stoffe andere organisierte Zellwandmuster hervorzurufen als diejenigen, die für die betreffende Pflanze normal sind. Von dieser Regel gibt es nur eine interessante Ausnahme, nähmlich die Bildung der prosoplasmatischen Gallen (vgl. Küster 1911). Die Galle von *Mikiola fagi* wird dadurch erzeugt, dass der Gallenbildner, die Insektenlarve, von ihrem Instinkte geleitet, wuchsstoffähnliche Stoffe in spezifischer Weise auf der Unterseite des Buchenblattes und an der inneren Wand der sich entwickelnden Galle verteilt (Boysen Jensen 1953). Eben diese Gallenbildung bietet eine Bestätigung der oben angeführten Regel, dass Stoffe an sich nicht ein artfremdes, organisiertes Gebilde erzeugen können; nur in dem Fall, dass sie von einem lebenden Organismus in gesetzmässiger Weise verteilt werden, können sie ein solches Gebilde hervorrufen. Ein »toter Organisator« ist, wie Spemann (1936) bemerkt, ein Widerspruch in sich selbst.

Aber vielleicht könnte die Determination durch ein bestimmtes Strukturgefüge in dem Protoplasma hervorgerufen werden; man

wird daher untersuchen müssen, ob eine Struktur, die für die Entwicklung verantwortlich sein könnte, in der Zygote vorhanden ist.

In dem Kern ist ein solches Strukturgefüge nicht vorhanden, wie daraus hervorgeht, dass die Gene bei dem Aufbau des Zellwandgerüstes nur indirekt beteiligt sein können. Das Wachstum der Zellwände und die Orientierung der neuen Zellwände wird, wie oben dargelegt, durch das Plasma geregelt.

Es muss sodann untersucht werden, ob in dem Plasma ein Strukturgefüge vorhanden ist, dass die Entstehung des Determinationsmusters ermöglichen könnte. Das scheint nicht der Fall zu sein. Wie HÄMMERLING und seine Schule zeigen konnten, sind kernlose *Acetabularia*stiele und Teile derselben imstande, Apikaldifferenzierungen (Haarwirtel und Hüte) und Rhizoidregenerate zu bilden. Es konnte ferner gezeigt werden, dass die Fähigkeit zur Bildung der Apikaldifferenzierungen von der Länge der Stielstücke und dem Abstand derselben von dem Apikalende abhängig ist, dass somit ein Konzentrationsgefälle von Apikaldifferenzierungsstoffen in dem *Acetabularia*stiel vorhanden sein muss. Man wird aus diesen Versuchen schliessen müssen, dass eine unbegrenzte Anzahl potentieller Hüte in dem *Acetabularia*stiel vorhanden ist, es wird aber im allgemeinen nur ein Hut gebildet. Die Entstehung desselben kann somit nicht auf eine bestimmte, in dem *Acetabularia*stiel vorhandene Struktur zurückgeführt werden. Die Struktur, die mit der Hutbildung verknüpft ist, entsteht erst während der Entwicklung des Hutes.

Aber diese Ausführungen sind wohl eigentlich überflüssig. Die Präformationstheorien des 17ten und 18ten Jahrhunderts und die spätere von WEISMANN sind längst ins Grab gesunken. In der Zelle sind zwar Strukturen vorhanden, die sich selbst reproduzieren können, z. B. Chromosomen, Zellkerne, Plastiden u. s. w., aber keine *Acetabularia*hüte, Blütenteile u. s. w. Das Zellwandmuster dieser letzteren wird an Ort und Stelle unter Mitwirkung von in einem Determinationsmuster verteilten Enzym aus niedrig molekularen Stoffen aufgebaut.

Was im vorhergehenden über die Möglichkeit gesagt wurde, die Wirkung des determinierenden Faktors auf Stoffe oder Strukturen zurückzuführen, kann folgenderweise zusammengefasst werden:

In der Zygote findet man nur diskrete Einzelelemente, An-

lagen, Gene, Plastiden, Mitochondrien, Zellulosenbildner u. s. w., die die Stoffe, die für den Aufbau der Konstitutionsstoffe notwendig sind, erzeugen. Diese Elemente stellen zwar eine notwendige, dagegen nicht eine hinreichende Bedingung für die Entwicklung dar. Es muss noch ein Faktor vorhanden sein, der diese Elemente verteilt und ihre Wirkungen koordiniert, so dass eine Ganzheit, ein Determinationsmuster und später ein Differenzierungsmuster, entsteht. Dieser determinierende Faktor kann nicht stofflicher Natur sein. Z. B. sind die Zellulosenbildner nicht imstande, sich selbst so zu verteilen, dass das Zellwandmuster in einer Blüte entstehen kann. Ebensowenig vermögen andere Stoffe oder Strukturen durch Verteilung oder durch lokale Neubildung von Zellulosenbildnern ein Determinationsmuster hervorzurufen, dass die Entstehung eines solchen Zellwandmusters ermöglichen könnte.

Ist dann der determinierende Faktor ein Feld im physikalischen Sinne des Wortes, so dass das Determinationsmuster durch ein System physikalischer Kräfte hervorgerufen wird?

Von Gurwitch, Weiss und anderen (vgl. Spemann 1936) ist der Begriff: das embryonale Feld in die Gestaltungsphysiologie eingeführt worden. Darüber, was man unter dem Feldbegriff zu verstehen hat, sind die Meinungen geteilt. Die Einführung dieses Begriffes ist wohl ein Versuch, die ortsgemässse Entwicklung, die die Grundlage der gesamten Ontogenese bildet, auf ein Potentialmuster irgend einer Art zurückzuführen. Es soll nun gezeigt werden, dass der determinierende Faktor nicht mit einem physikalischen Feld identifiziert werden kann.

Wenn man sich überlegt, welche Potentiale imstande sein könnten, die mit der Ausbildung eines Determinationsmusters verknüpften Stoffbildungen und Stoffumlagerungen hervorzurufen, kommt man zu dem Ergebnis, dass wohl nur elektrische Potentiale in Frage kommen können. Tatsächlich sind solche Potentiale in den lebenden Organismen allgemein verbreitet, und es soll nicht geleugnet werden, dass eine gewisse Relation zwischen diesen Potentialen und den Lebensvorgängen beobachtet werden kann. Z. B. ist in den Wurzeln die Wachstumszone (in dem abgeleiteten Strom) negativ im Verhältnis zu der Oberfläche in den übrigen Teilen der Wurzeln. Es ist auch sicher, dass gewisse

Bewegungsvorgänge in den Pflanzen ebenso wie die Reizleitung in Nerven von Potentialschwankungen begleitet sind, und dass man durch elektrische Ströme Krümmungen, z. B. in Wurzeln, hervorrufen kann.

Bioelektrische Potentiale können in verschiedener Weise entstehen (vgl. Bull 1948, Höber 1945). Am wichtigsten sind wohl die Potentiale, die an Phasengrenzflächen geknüpft sind, man nimmt an, dass sie entweder durch Jonenkonzentrationsdifferenzen oder durch Oxydations-Reduktionssysteme (Rosene and Lund 1953) hervorgerufen werden. Sicher ist auch, dass äussere Faktoren, namentlich Schwerkraft, Potentialdifferenzen erzeugen können. Z. B. wird in Koleoptilen, die in horizontaler Lage untergebracht werden, die Unterseite positiv im Verhältnis zur Oberseite. Wichtig ist es, dass in nicht differenzierten Zellen, beispielsweise in einer Eizelle, zwar eine Struktur, aber keine Potentialdifferenzen zwischen den einzelnen unversehrten Punkten der Oberfläche vorhanden sind.

Elektrische Potentialdifferenzen sind natürlich imstande, Verschiebungen von Jonen hervorzurufen. Man hat vermutet, dass die Verschiebung des Wuchsstoffes, die bei der phototropischen und geotropischen Krümmung stattfindet, und die das ungleichartige Wachstum auf den beiden Seiten des sich krümmenden Organs hervorruft, durch Potentialdifferenzen erzeugt wird. Es ist doch ziemlich sicher, dass die polare Leitung des Wuchsstoffes in der Avenakoleoptile mit den in derselben vorhandenen elektrischen Potentialen nichts zu tun hat (vgl. Söding 1952).

Damit nun aber Potentialdifferenzen imstande sein sollten, ein Determinationsmuster hervorzurufen, damit sie z. B. imstande sein sollten, die Zellulosenbildner so zu verteilen, dass sie das Zellwandmuster in einer Blüte bilden könnten, müsste vor der Bildung des komplizierten Determinationsmusters der Zellwände ein Potentialmuster von ebenso komplizierter Beschaffenheit wie das Determinationsmuster vorhanden sein.

Die erste Frage lautet dann, ob ein solches kompliziertes Potentialmuster überhaupt in dem Plasma vorkommen kann. Man ist wohl kaum imstande, diese Möglichkeit im voraus zu verneinen. Es finden sich unzweifelhaft in dem Plasma viele Phasengrenzflächen, z. B. sind alle Organellen, Mitochondrien und Sphaerosomen von solchen Grenzflächen umgeben, die der

Sitz elektrischer Potentiale sein könnten. Zwar werden viele von diesen mit den Strömungen herumgeführt, und sind somit jedenfalls nicht imstande, ein interzellulares Muster zu bilden. Aber auch in dem ruhenden Plasma und in der Hautschicht des Plasmas und in den verschiedenartigen Zellen können sicher komplizierte Strukturen und komplizierte Potentialmuster vorhanden sein.

Die zweite Frage lautet nun, ob die wechselnden Determinationsmuster der Zellwände, die während der Entwicklung auftreten, durch ein solches kompliziertes Potentialmuster hervorgerufen werden können.

Wenn man z. B. von embryonalen Zellen in der Wurzelspitze ausgeht, darf man annehmen, dass dieselben gleichartig sind, und das sie alle dasselbe Potentialmuster enthalten. Aus diesen gleichartigen embryonalen Zellen sollen nun verschiedene Zelltypen, Trichoblasten, Gefässe, Siebröhren u. s. w. gebildet werden. Jede dieser Typen müsste natürlich ein spezifisches Potentialmuster haben. Die Voraussetztung dafür, dass solche Potentialmuster entstehen könnten, müsste sein, dass vorher stoffliche Umlagerungen eingetreten wären, wodurch die notwendigen Phasengrenzflächen geschaffen würden.

Auf dieselbe Schwierigkeit stösst man in noch höherem Grade, wenn neue und mehr komplizierte Organe, z. B. Blüten, gebildet werden sollen. Hierzu würden natürlich ganz andere Potentialmuster als diejenigen, die in den vegetativen Organen auftreten, notwendig sein. Auch in diesem Falle müssten vor der Entstehung dieser neuen Potentialmuster tiefgehende Veränderungen der Phasengrenzflächen, d. h. stoffliche Veränderungen, stattgefunden haben.

Ob elektrische Potentiale in der einen oder anderen Weise bei den orientierten Stoffumlagerungen beteiligt sind, muss man der Wissenschaft der Zukunft zu entscheiden überlassen. Jedoch scheint es sicher, dass der determinierende Faktor als solcher mit einem System von elektrischen Potentialen nicht identifiziert werden kann. Das Potentialmuster, falls ein solches vorhanden ist, ist eine Folge des Determinationsmusters und nicht die Ursache desselben.

Es ist somit nicht möglich, die Entstehung des Determinations- und Differenzierungsmuster der Zellwände mit Hilfe der Wirkung

von Stoffen, Strukturen oder elektrischen Potentialen zu erklären. Dieses Ergebnis steht im Einklang mit der oben erwähnten Tatsache, dass es nicht möglich ist, den determinierenden Faktor zu verändern.

e. Wie wirkt, was ist der determinierende Faktor des Zellwandmusters?

Bisher haben wir uns mit der negativen Seite des Problems, des determinierenden Faktors, beschäftigt, wir haben versucht zu erhellen, was der determinierende Faktor nicht ist. Wir wollen nun versuchen, uns der positiven Seite zuzuwenden. Wir fragen, wie wirkt, was ist der determinierende Faktor, und wir wählen, um diese Frage zu beleuchten einen speziellen Differenzierungstypus, die Entstehung des Zellwandmusters bei der Blütenbildung.

Das Kennzeichnende bei der Ontogenese der lebenden Organismen ist, dass eine Strukturerhöhung stattfindet, dass aus einem weniger komplizierten ein komplizierterer Organismus entwickelt wird (Boysen Jensen 1939, p. 9). Eine solche Strukturerhöhnung tritt z. B. ein, wenn Blüten gebildet werden. Die Entwicklung der Blüten ist ziemlich kompliziert. Auf der Blütenachse einer zyklischen Blüte entstehen Wirtel von ursprünglich anscheinend gleichartigen Höckern. Die Höcker der einzelnen Wirtel werden simultan gebildet. Die Entstehung der Wirtel geschieht nicht immer in aufsteigender Folge. Die apikalen Teile eilen bisweilen voran, und die Anlagen der Staubblätter können dann in absteigender Folge gebildet werden. Später entwickeln sich die Höcker in ungleichartiger Weise zu Kelch- und Kronblättern, und zu dem Androzeum, dass aus mehreren bis zahlreichen Staubblättern besteht. An dem Scheitel der Blütenachse entsteht das Gynäzeum. Im einfachsten Falle besteht dasselbe aus einem oder mehreren freien Fruchtblättern; diese entstehen als schiefe, becher- oder kapuzenförmige Gebilde, deren Ränder später verwachsen, so dass geschlossene Fruchtblätter, die die Samenanlagen umschliessen, gebildet werden. Wenn mehrere Fruchtblätter mit den Rändern oder mit den randlichen Teilen der Aussenseite verwachsen, entsteht ein synkarpes, einräumiges oder mehrräumiges Gynäzeum. In den Hohlräumen desselben

entwickeln sich die Samenanlagen (vgl. Abb. 1463, E, F, 1499,5, 1502, 1505, 1507 in GOEBEL 1923).

Das schliessliche Ergebnis dieser komplizierten Entwicklungsvorgänge sind Blüten, deren Baupläne in allen Beziehungen genau identisch sind. Die Blüten sind, wie es aus den Blütendiagrammen hervorgeht, ein ganz besonders schönes Beispiel der ortsgemässen Entwicklung während der Ontogenese.

Nach dieser kurzgefassten Beschreibung der Entwicklung der Blüten, soll untersucht werden, was man aus derselben folgern kann.

Nach einer viel verbreiteten Auffassung, wird die ortsgemässe Entwicklung, d. h. die Entwicklung bestimmter Zellen und Zellgruppen zu einem bestimmten Gebilde, durch innere Anlagen und durch die Entwicklungsbedingungen, d. h. die Einwirkung der umgebenden Zellen, bestimmt.

Die verschiedenartige Entwicklung der Höcker zu den Blütenteilen kann nicht darauf beruhen, dass die inneren Anlagen der Zellen verschieden sind, sie entstehen nämlich alle aus gleichartigen, embryonalen Zellen.

Man kann sich auch nicht vorstellen, dass die Anlage der einzelnen Blütenteile durch eine Wirkung anderer früher angelegter Organe sollte hervorgerufen werden können. Zwar ist es unmittelbar einleuchtend, dass eine starke morphologische Koordination zwischen den verschiedenen Organen und Organteilen der Blüte vorhanden ist. Diese Koordination muss dadurch erreicht werden, dass die Wachstumvorgänge der verschiedenen Zellen oder Zellgruppen während der Entwicklung der Blüte mit der grössten Genauigkeit koordiniert werden. Man wird aber diese Koordination der Wachstumsvorgänge nicht durch die Annahme erklären können, dass eine Kausalverknüpfung zwischen den einzelnen Stufen der Entwicklung vorhanden sein sollte, so dass jede Stufe zwangsläufig die folgende Stufe hervorgerufen habe. Es müssten dann nämlich während der Entwicklung einer bestimmten Stufe Stoffe oder Potentiale gebildet werden, die die folgende Stufe hervorrufen könnten. Wie oben gezeigt wurde, können Stoffe zwar in die Determinations- und Differenzionsvorgänge modifizierend eingreifen, aber nicht diese Vorgänge schaffen.

Es kann somit, was eigentlich schon aus dem vorigen Ab-

schnitte hervorgeht, die Entstehung des Determinations- und Differenzierungsmusters während der Blütenbildung weder durch innere Anlagen noch durch eine koordinierende Wirkung der älteren Blütenteile auf die Bildung der jüngeren erklärt werden.

Wir müssen daher versuchen, das Wesen des determinierenden Faktors von seiner Wirkungsweise aus zu erhellen.

Weil die ursprünglich gleichartigen Zellen, die z. B. bei der Bildung eines synkarpen Gynäzeums beteiligt sind, von Anfang an gleichzeitig in ungleichartiger Weise oder überhaupt nicht wachsen, wird man annehmen müssen, dass die Einheitlichkeit der Wachstumsvorgänge, die gesetzgebundene und unveränderliche Entwicklung des Gynäzeums, und übrigens auch der anderen Blütenteile, dadurch hervorgerufen wird, dass vor dem Beginn der Gestaltungsvorgänge der Bauplan der ersten Entwicklungsstadien des Gynäzeums und der Blüte überhaupt vorhanden ist. Dieser Bauplan ist in dem Determinationsmuster der beginnenden Entwicklung verwirklicht. Jedenfalls hinsichtlich des Aufbaues der Zellwandgerüstes in der Blüte ist das Determinationsmuster nicht etwas Hypothetisches, sondern ein durchaus reales Gebilde. Es besteht aus Stoffen, besonders Zellulosenbildnern, Wuchsstoffen u. s. w., die bei dem Wachstum der Zellwände beteiligt sind, und die durch Verteilung oder lokale Neubildung in einer bestimmten, während der Entwicklung wechselnden Struktur angeordnet werden. Durch das Determinationsmuster wird dann die Wachstumweise der einzelnen Zellen koordiniert, so dass die Ganzheit, die in der Blüte zutage tritt, verwirklicht wird. Ebenso wie die Blüte muss auch das Determinationsmuster ein Ganzheitsgepräge besitzen.

Man muss nun weiter schliessen, dass ein etwas vorhanden ist, das überzellulär wirkt, und das Determinationsmuster hervorruft. Dieses etwas, das nicht auf Stoffe, Strukturen oder elektrische Potentiale zurückgeführt werden kann, nennen wir den determinierenden Faktor. Da dieser Faktor imstande ist, ein ganzheitsgeprägtes Determinationsmuster hervorzurufen, wird man zu der Auffassung gedrängt, dass das Determinationsmuster, das geschaffen werden soll, in der einen oder anderen Weise in dem determinierenden Faktor enthalten sein muss. Man kann daher sehr wohl den determinierenden Faktor als ein ontogenetisches Feld auffassen, das von Ort zu Ort qualitativ ver-

schiedene Wirkungen hervorzurufen vermag. Ein physikalisches Feld stellt der determinierende Faktor aber nicht dar.

Die Entwicklung einer Pflanze ist zwar gesetzmässig in dem Sinne, dass man sie sich beliebig oft wiederholen lassen kann, wobei zugleich eine Determination und Gleichmässigkeit auftritt, die der anorganischen Natur ganz fremd ist. Sie ist aber nicht gesetzmässig im physikalischen Sinne, weil man auf physikalischer Grundlage nicht ein späteres Stadium aus einem früheren Stadium ableiten kann.

Wir kommen somit zu dem Ergebnis, dass, soweit man es beurteilen kann, in dem anorganischen Bereich keine Elemente vorhanden sind, mit welchen der determinierende Faktor verglichen oder aus welchen er abgeleitet werden kann.

Dem Carlsbergfond, der mir die für die Untersuchungen notwendigen Instrumente zur Verfügung gestellt und mich auch in anderer Weise unterstützt hat, spreche ich meinen besten Dank aus.

Meiner Tochter, Frau Margrete Ehlers, möchte ich auch an dieser Stelle für ihre gewissenhafte Hilfe bei der Ausführung der Versuche herzlich danken.

Summary.

Spores of *Pteris longifolia* were germinated on 0.6 % agar in small glass-rings (1.5 × 0.8 cm) placed in petri dishes (4 × 2 cm). The bottom of the dishes was covered with the solution, the effect of which on the growth of the prothalli was to be investigated. With a pipette 2—3 drops of the solution were placed on the agar surface 2—3 times a day. The greater part of the experiments were carried out in white light.

The development of prothalli in a nutrient medium is represented in fig. 3.

When spores are germinated on agar with 0.2 % colchicine, prothalli of many different types arise. In fig. 4, 9, 10, one of these types is drawn, a pointed cone-shaped prothallus, consisting of a row of cells, the size of which decreases towards the tip. If the colchicine solution is replaced by a nutrient medium, a normal, horizontal prothallus plate grows out from the row of cells, either unilaterally or symmetrically (fig. 4, 12). If cell divisions in

the axial cell row occur, the new-built cell walls are horizontal so that besides the horisontal plate also a vertical plate is formed (fig. 4,13,16). The development and regeneration of other types is represented in fig. 4, 1–8.

On agar with a solution of 0.01 % 2.4-D club-shaped parenchymatous callus develop (fig. 5, 1–9). If the 2.4-D solution is replaced by a nutrient medium, a regeneration takes place. From one or more cells of the callus a normal horisontal prothallial plate grows out. Each callus only regenerates one prothallus (fig. 5, 17).

The effect of 2M-4Cl on the growth of the *Pteris* spores is similar to that of 2.4-D.

It appears from the experiments that in cultures of *Pteris* spores on agar containing colchicine, 2.4-D or 2M-4Cl, growth and cell division can be continued, whereas differentiation, the fusion of the cells to an organized entity, a normal prothallus, is discarded, but it begins when the poison is removed from the agar.

We must therefore conclude that the last process, the differentiation, is of a higher order than cell growth and cell division. The factor achieving the formation of a normal prothallus is called the determining factor. As to the nature of this factor it is concluded that in the physical world no elements are found with which it can be compared or from which it can be derived.

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CYTOLOGICAL AND EXPERIMENTAL STUDIES ON THE GENUS *ERODIUM* WITH SPECIAL REFERENCES TO THE COLLECTIVE SPECIES *E. CICUTARIUM* (L.) L'HER.

BY

KAI LARSEN

København 1958
i kommission hos Ejnar Munksgaard

Synopsis.

The paper gives the chromosome number of 34 strains of *Erodium*, all of wild origin, belonging to the species: *E. hirtum* (Forsk.) Willd. subsp. *maroccanum* Maire, $2n = 20$; *E. maritinum* (Brum. f.) L'Her., $2n = 20$; *E. glutinosum* Dumort., $2n = 20$; *E. cicutarium* (L.) L'Her., $2n = 40$, and a new-described species, *E. danicum* K. Larsen, $2n = 60$. This *E. danicum* was found in five localities in Denmark. It is suggested to be an amphidiploid between *E. glutinosum* and *E. cicutarium*. Biological observations and the results of experimental cultivations are communicated.

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I. Introduction.

The genus *Erodium* belonging to the Geraniaceae consists of about 60 species, the majority of which have a Mediterranean distribution. Though the taxonomy of this group and particularly *E. cicutarium* (L.) L'Her. and its allies have been treated by various authors from an experimental point of view in recent times, several problems of distribution and cytology still remain.

The present material has been produced by collection of seeds in nature, partly by the author, partly through the kindness of other botanists. The author owes his sincerest thanks to everybody who has contributed to these studies by collecting seeds and placing material at his disposal, and to the Carlsberg Foundation and the Dansk Botanisk Rejsefond, which have provided grants to cover travelling expenses.

The chromosome countings have been carried out on root tips fixed in Navashin-Karpechenko's fluid, where nothing else is stated, embedded in paraffin, and sectioned at about 13 μ. The staining for some few early slides has been done with Gentian Violet, the majority of slides, however, being stained according to Feulgen.

All experimental cultivations have been carried out in the experimental fields of the Botanical Gardens of the University of Copenhagen (HBH). Herbarium studies all refer to the Herbarium of the University of Copenhagen (Herb. C), where also this material will be placed after these studies have been finished.

The writer is indebted to Prof. Dr. T. W. Böcher for help and advices during the work, and to Mr. Tyge Christensen, M.Sc., for latinizing the diagnosis.

II. Cytotaxonomy.

A. Discussion of previous studies in *E. cicutarium* (L.) L'Her. and its allies.

The basis of taxonomic studies in the family Geraniaceae is the monograph compiled by Knuth (1912) on orthodox taxonomic lines. It has not been possible to follow this work as far as

TABLE I.
Chromosome counts of previous authors (only species of known origin mentioned).

Origin	Authors	Obs.
Erodium glutinosum Dum. $2n = 20$		
England:		
Blakeney Point, Norfolk	WARBURG 1938	
Merthyr Mawr, Glam.	—	
Sweden:		
Scania: Kungsmarken near Lund	LÖVE & LÖVE 1942	It is subject to doubt whether this specimen belongs to *E. glutinosum* (see discussion in the text on p. 23).
Holland:		
Without further statement of the locality	ANDREAS 1946	
Erodium cicutarium (L.) L'Her. $2n = 40$		
England:		
Freckenham, Suffolk	WARBURG 1938	
Borth, Cardigan	—	
Mendip Hills, Somerset (near Wells)	—	
N. Ireland:		
Newcastle, Co. Down	—	
Scotland:		
Aberdeen	—	
Holland:		
Without further statement of the locality	ANDREAS 1946	
Sweden:		
Scania at Åhus	LÖVE & LÖVE 1942	
Germany:		
Schleswig-Holstein	GAUGER 1937	Var. *immaculatum* KOCH $2n = 36$?; var. *pimpinellifolium* Sm. $2n = 40$
U. S. A.:		
California	HEISER & WHITAKER 1948	

the *E. cicutarium*-group is concerned since experimental studies have revealed a more natural classification. Thus the diploid West European dune species is here treated as *E. glutinosum* Dum., while KNUTH regards it as a variety under *E. bipinnatum* (Cav.) Willd., viz. var. *sabulicolum* (Jord.) Brumh. The other species belonging to the subsection *Cicutario* Willk. et Lange, *E. cicutarium* (L.) L'Her. is by KNUTH divided into several varieties and forms. It is evident that Knuth was in doubt about

the classification, as three other systems are quoted. In the present treatment I have followed ANDREAS (1946), who divides it into two interfertile subspecies.

E. glutinosum Dum. — The chromosome number $2n = 20$ has been stated by the following authors in *E. cicutarium* coll., viz. WARBURG (1938), LÖVE & LÖVE (1944), and ANDREAS (1946). The last-mentioned author was the first to treat the problem concerning the morphology and distribution of the diploid cytotype in a serious way. From a study of Dutch material of *E. cicutarium* coll. ANDREAS reached the conclusion that two chromosome races exist in Holland, one diploid with $2n = 20$, and one tetraploid with $2n = 40$. ANDREAS concluded that the proper botanical terminology of these two types, worthy of specific rank, is *E. glutinosum* Dum. (Fig. 13) and *E. cicutarium* (L.) L'Her.

The two species differ in a number of quantitative characters, but the best distinguishing character is found in the morphology of the fruits. *E. cicutarium* has fruits with a conspicuous pit surrounded by a furrow at the apex. The fruits of *E. glutinosum* has a small pit without a furrow and the beak is usually shorter than 24 mm (15—24) while *E. cicutarium* has a beak of 22—40 mm.

E. cicutarium (L.) L'Her. — According to TISCHLER (1950) the following authors have found the chromosome number $2n = 40$ in *E. cicutarium* coll., viz. GAUGER (1937), WARBURG (1938), LÖVE & LÖVE (1942), ANDREAS (1946), STEBBINS (in HEISER & WHITAKER 1948), MATTICK (in TISCHLER *loc. cit.*), and ROTTGARDT (ibid.). Furthermore $2n = 36$ has been reported by HEITZ (1926), GAUGER (1937), NEGODI (1937), and ROTTGARDT (in TISCHLER *loc. cit.*). It is not possible at the present time to decide whether the number $2n = 36$ really does exist in the species or whether it is due to incorrect countings from bad preparations. All cultures studied by me have shown $2n = 40$. Experience has shown that only the vigorous root tips, however, contain mitosis in which the chromosomes lie sufficiently well spaced so that it is possible to count the chromosome number with certainty. On the other hand it is not inconceivable that aneuploid types do exist in Central and South Europe.

According to ANDREAS (*loc. cit.*) *E. cicutarium* (L.) L'Her. can be divided in two subspecies, viz. subsp. *arvale* Andr.,

which is the common weed distributed over the greater part of Europe (and may be regarded as nearly a cosmopolitan), and subsp. *dunense* Andr., which is found in the dunes and on sandy fields near the coast (Figs. 9—10).

WARBURG (1938) studied British material and established that all the inland strains examined and some of the maritime ones were tetraploid, but that two diploids, both maritime, also occurred. These results are not in contradiction with the results of ANDREAS. Much more difficult to explain are the results of LÖVE & LÖVE (1942, 1944), two papers not considered by ANDREAS These authors found that in Sweden a spring type with $2n = 20$ and an autumn type with $2n = 40$ occur. The diploid plant originated from Lund (Scania) and the tetraploid from Åhus (Scania)., the authors therefore conclude that in Sweden the diploid type is found inland, the tetraploid on the coast, i. e. just contrary to what is the case in England.

These conclusions of LÖVE & LÖVE seem a little premature, as only two strains have been studied, one of "the autumn type" and one of "the spring type". Nor has it been possible for the present author to confirm this on Danish material (cp. p. 23). Furthermore the conclusion that the species react in the opposite way in England and Sweden, is not sufficiently substantiated.

In Table I the author has surveyed the previous counts in which the origin of the material studied could be established. Chromosome counts made on material from botanical gardens are not of much interest any more as far this species is concerned.

B. Present Investigations.

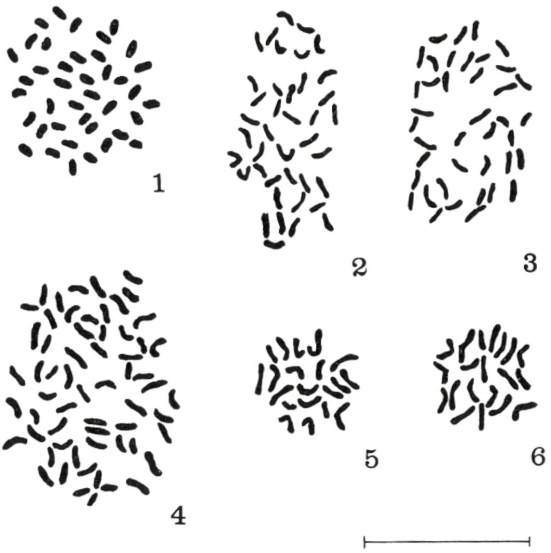
All strains of *Erodium* studied cytologically by the author are surveyed in Table II. The distribution of the European ones is illustrated on the maps Fig. 7 and Fig. 8 together with previously published, localized chromosome counts.

E. glutinosum Dum. — A single sample of this species originating from the British Isles showed the chromosome number $2n = 20$; this is in accordance with the results of ANDREAS (*loc. cit.*).

E. cicutarium (L.) L'Her. — Several strains of this very polymorphous species have been studied. They include the subsp. *arvale*, the typical inland type and field weed, as well as subsp.

dunense, the dune type. In all collections with the exception of some Danish ones further dealt with below, the chromosome number $2n = 40$ has been counted (cp. Fig. 1—3). The chromosomes are nearly isoshaped with a median constriction.

It is a question whether the strain from Portugal (2451) is to be referred to subsp. *arvale* without taking it in an unusually



Figs. 1—6. Root tip mitosis. Fig. 1: *E. cicutarium* subsp. *dunense*, $2n = 40$ (Cult. No. 3040). The chromosomes are contracted in consequence of a pretreatment with 8-hydroxychinoline for 4 hours before fixation. — Fig. 2: *E. cicutarium* subsp. *dunense*, $2n = 40$ (4451). — Fig. 3: *E. cicutarium* subsp. arvale, $2n = 40$ (4238). — Fig. 4: *E. danicum*, $2n = 60$ (2749). — Fig. 5: *E. maritimum* var. *apetala*, $2n = 20$ (4455). — Fig. 6: *E. hirtum* subsp. *maroccanum*, $2n = 20$ (55—18). — The scale is 10 μ.

wide sense. But it is beyond the scope of this work to make a classification for all European forms.

As mentioned in the introduction, the species has got a nearly world-wide distribution. In order to ascertain which type has become the common weed in the new world, two South American strains were studied, one from Chile and one from Argentina. Both showed the chromosome number $2n = 40$ and could without hesitation be referred to subsp. *arvale*.

Besides the tetraploid species to which the majority of the strains seems to belong, a hexaploid chromosome type constituting a new species was found in Denmark (Fig. 4). Now poly-

TABLE
Species, origins and chromosome

Species	Localities of collection
E. cicutarium subsp. *dunense*	Denmark : Lakolk, Römö
— —	— : Southern Römö
— —	— : Kandestederne, N. Jutland
— —	Wales : St. Davis (No. 2).
— —	— : Ibidem (No. 3).
— —	— : Ibidem (No. 4).
— —	— : Tenby (No. 5).
— —	— : Ibidem (No. 6).
— —	— : Ibidem (No. 7).
— —	— : Dale (No. 8).
— —	— : Ibidem (No. 10).
— —	— : Morfa Duffryn, (No. 12).
E. cicutarium subsp. *arvale*	Denmark : N. of Hillerød, N. Zealand
— —	— : Hillerød, N. Zealand
— —	— : Nödebo, N. Zealand
— —	— : Sletten, N. Zealand
— —	— : Salling, Voldskrænten, N. Jutland
— —	France: : Gavarnie, in the Pyrenees
— —	— : Formiguere, in the Pyrenees
— —	Holland : W. of Hengelo
— —	— : Island of Terschelling
— —	Scotland : Nairn near Innvernes
— —	Austria : Seewinkel, E. of Neusiedlersee
— —	Portugal : Coimbra (wild origin)
— —	Chile : Huasco, N. of Valparaiso
— —	Argentina: Chos Malal (Dept. Neuouen)
E. danicum	Denmark : Frederikshavn, N. Jutland
—	— : Vaalse Vesterskov, Falster
—	— : Vesterø, Sigvej, Læsø
—	— : Vesterø, Læsø
—	— : Vesterø, Kirkevej, Læsø
E. glutinosum	Wales : Morfa Duffryn
E. maritimum	— : St. Davis
E. hirtum subsp. *maroccanum*	Marocco : Haha distr., S. of Mogador

ploid individuals are occasionally found when a sufficiently large material of a species is studied. Probably in every species such individuals occur sporadically (DARLINGTON 1956). In this case, however, in which five strains from different parts of the country

II.
numbers of the strains studied.

Habitat	Collector	Cult. No.	$2n$
sandy dune path	Author	3039	40
dunes influenced by sheep	—	3040	40
sandy dune path	—	4543	40
	Th. Sørensen	4446	40
	—	4447	40
	—	4448	40
dunes	—	4449	40
dunes	—	4540	40
dunes	—	4451	40
	—	4452	40
	—	4454	40
dunes	—	4456	40
sandy field	Author	2206	40
field	—	2212	40
dry slope	—	55—40	40
roadside	—	2298	40
	P. Kaad	4238	40
weed in field, alt. 1400 m.	Author	1533	40
weed in field, alt. 1600 m.	—	1667	40
roadside	—	1202	40
dune road	—	2432	40
roadside	Th. Sørensen	4457	40
dry field	T. W. Böcher	4098	40
	Bot. Gard.	2451	40
desert	T. W. Böcher & K. Rahn	A 513	40
rocks outside the town	—	A 1591	40
sandy hills	Author	2749	60
	K. Højgaard	2753	60
	P. Kaad	4239	60
sandy roadside	—	4240	60
	—	4243	60
dunes	Th. Sørensen	4455	20
dunes	—	4445	20
	Inst. Sci. Cherif.	55—18	20

show hexaploidy, the hexaploid is in my opinion to be interpreted as a separate genetic species which till now has been found in Denmark only.

It is possible of course that a closer study on West European

Erodiums will reveal that this hexaploid species also occurs outside Denmark. The finding of 5 Danish localities among 13 Danish samples collected at random points in the direction of the species having a northern distribution (for taxonomic treatment see p. 11).

E. maritimum (Burm. fil.) L'Her. — This species belongs to

Fig. 7. Map showing all European localities with known chromosome number of *Erodium cicutarium* (solid dots), $2n = 40$; and *E. glutinosum* (rings), $2n = 20$. The Danish finds are excluded and illustrated on Fig. 8. For extra-European finds see Table I and II.

the subsection *Chamaedryoidea* Brumh. It has two separate distribution areas, one Liguric-Tyrrhenic, and one in Atlantic Western Europe. The sample studied is referred to var. *maritimum* f. *apetala* Rouy (pro. subvar.) characterized by the absence of petals. The chromosome number $2n = 20$ was found (Fig. 5). This is the same number as observed in the two other species belonging to this group, viz. *E. corsicum* Leman, and *E. chamaedryides* (Cav.) L'Her. (Warburg 1938).

E. hirtum (Forsk.) Willd. — A perennial species distributed in the North African steppe region from South West Morocco to Syria. It belongs to the section *Plumosa* Boiss.

$2n = 20$ was found in material from Morocco: Haha district, South of Mogador (Fig. 6). This is the same number as that found by WARBURG (1938) in *E. glaucophyllum* (L.) L'Her., which belongs to the same section.

The material studied was received as seeds from the Institut Scientifique Chérifien, Rabat, through the kindness of Professor CH. SAUVAGE. The strain belonged to var. *maroccanum* Maire (1923), which is a glabrous variety found in Western Morocco.

III. Taxonomical Treatment of the Hexaploid Danish Erodium.

The hexaploid plants of *E. cicutarium* (L.) L'Her. differ from the tetraploid ones in a number of minor characteristics, most of which, however, are quantitative and biological.

The three strains from Læsø Nos. 4239, (Fig. 16), 4240 (Fig. 16) typus, and 4243, were almost identical in their behaviour when grown in the experimental field, as well as in the her-

TABLE III.

Diameters of pollen.

E. danicum 4239—4243, *E. cicutarium* subsp. *arvale* 1202—1591 A, *E. cicutarium* subsp. *dunense* 4452. Further explanation of the Cult. Nos.; see Table II.

Cult. No. 1	Pollen diam. in μ								Average pollen diam. in μ
	35	40	45	50	55	60	65	70	
4239 Denmark				7	32	*48*	10	3	59
4240 —				6	32	*54*	8		58
4243 —				7	29	*49*	10	5	59
1202 Holland			7	*59*	28	5	1		52
2206 Denmark		1	5	*65*	24	4			51
2212 —		1	4	*54*	35	6			52
2298 —	1	13	21	*47*	16	2			49
2451 Portugal			25	*56*	14	4	1		50
4098 Austria			4	*59*	29	8			52
4457 Scotland			9	*58*	28	5			51
1591A Argentina			1	19	*55*	21	4		55
4452 Wales			6	*53*	30	9	2		52

barium material preserved. The two previously cultivated strains, Nos. 2749 from N. Jutland and 2753 from Falster, behaved in nearly the same way under experimental conditions.

It is evident that the hexaploid is closely related with the tetraploid dune *Erodiums*, and might be referred to the *E. cicutarium* ssp. *dunense*. A measurement of the diameter of the pollen grains revealed small but significant differences; these measures

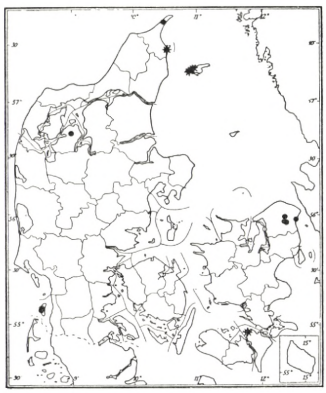


Fig. 8. Map showing Danish localities of *Erodium cicutarium* studied cytologically. Solid dots for tetraploids (subsp. *dunense* and subsp. *arvale*); * indicates hexaploids, *E. danicum*.

are summarized in Table III. From this it is seen that *E. danicum* has slightly larger pollen, with an average diameter of 58—59 μ, than *E. cicutarium*, in which average diameters from 49 to 56 μ were found.

The measurements of other structures of the hexaploid *E. danicum* are all in close accordance with those of the tetraploid *E. cicutarium* given by ANDREAS (1946), as far can be judged. Unfortunately too little fruiting material was available to give a clear picture. The measurements are: seeds about 3 mm in length beak 25—30 mm, fruit ÷ beak 4—6 mm. The furrow around the pit, however, is little accentuated. There are most frequently 5 flowers in the inflorescences. This is more than is generally met with in subsp. *dunense*. It seems, furthermore, to be a larger

Figs. 9—10. Danish *E. cicutarium* subsp. *dunense* collected at Kandestederne, N. Jutland, in Aug. 1955. The specimens Fig. 9 may be seedlings from the same summer, while Fig. 10 may be a hibernated specimen.

Fig. 9

Fig. 10

plant than subsp. *dunense*, with decumbent stems and very fine dissected leaves. The stems and in a still higher degree the petioles are densely covered with glandular hairs, in a degree seldom met with in such large forms. Thus the robust habit of subsp. *arvale* is combined with a glandular indumentum and fine dissected leaves. A characteristic biological feature seems to be that the leaves are falling off early in the autumn and new rosettes are formed in the leaf axils. This increased degree of vegetative reproduction is a feature often seen in polyploids.

This hexaploid type should be established as a new species.

Diagnosis.

Erodium danicum nov. sp.

Sectio *Barbata* Boiss., subsectio *Cicutaria* Willk. et Lange.

E. cicutaria satis affine, his notis ab eo diversum: Folia minora, magis dissecta, basalia mox marcescentia. Caules petiolique pilis et glandulis dense obsessi. Inflorescentia 5—6 flora. Plica concentrica foveae ad basim styli in fructum impressae circumjecta minus manifesta. Rosulae vegetativae axillares autumno profuse formatae. Numerus chromosomatum $2n = 60$. Pollen majus quam in *E. cicutario*, 58—59 μ diam.

Hab. in arenosis Daniae.

Typus juxta viam prope Vesterø insulae danicae Læsø lectus, in Horto Botanico Hauniensi cultus, postea siccus in Museo Botanico Hauniensi depositus.

Going through the Danish collections from Herb. C it was found that such large forms with proliferating shoots have been found elsewhere in Denmark. It is interesting that the oldest find of this species is just from *Læsø* (1870, coll. J. P. Jacobsen). *E. danicum* seems to prefer sandy localities near the coast. It is possible that a form without glutinous hairs (but with large inflorescences, fine dissected leaves and autumnal proliferation) exists within *E. danicum*.

The author is aware that the characters distinguishing *E. danicum* from some *E. cicutarium* (L.) L'Her. types are subtle, on the other hand *E. danicum* is a genetically distinct unit separated from *E. cicutarium* by a sterility barrier and may thus be regarded as a specific evolutionary unit quite different from subsp. *arvale* and subsp. *dunense*, between which gene exchange is possible. In such a case the only consistent solution is, in spite

of the slight morphological deviations from the tetraploid plant, to regard it as a separate species.

Another question which arises in this connection is: how has

Figs. 11. British *E. cicutarium* subsp. *dunense*. Collected in nature July 24, 1954.

the hexaploid species developed? Unfortunately meiosis has not been studied, but the pollen seems to be quite normal and dwarf pollen is completely absent. Furthermore, the fruit-setting is also normal. Both indicate a regular meiosis. A polyploid plant with normal meiosis might have developed through amphidi-

ploidy. Here it is obvious to think of a diploid and tetraploid parent, and as a working hypothesis, until further experimental studies have been procured, it may be natural to think of *E.*

Fig. 12. British *E. cicutarium* subsp. *dunense*, No. 4448 grown in the experimental field, coll. in the middle of Sept. 1955.

glutinosum Dum. and *E. cicutarium* (L.) L'Her. as the two parents:

E. glutinosum × *E. cicutarium* ssp, *arvale* → *E. danicum*

$2n = 20$ $2n = 40$ $2n = 60$

Therefore it cannot be taken for granted that *E. glutinosum* is to be found in Denmark. It is possible that *E. danicum* is much more widely distributed than known at present and may have invaded Denmark from the South. Further studies of the West European dune forms of *Erodium* therefore are highly needed. Also a synthetisation of the amphidiploid should be tried.

IV. Experimental Cultivations.

WARBURG (1938) tried to grow strains of *E. cicutarium* coll. from different habitats but without great success. More intensive

Fig. 13. *E. glutinosum* from nature, No. 4455.

cultivations were carried out by ANDREAS (1946). These show that subsp. *dunense* was rather difficult to grow on ordinary soil; the plants started flowering very late and some did not flower at all in the first summer, but hibernated in mild winters and flowered the following summer, while in severe winters (e.g. 1941/42) no strains were able to hibernate.

In the present experiments the greatest trouble has been the low germination percentage of the seeds, and the author is aware

of the insufficiency of the number of plants in the experiments; nevertheless some of the data have been put in tabular form.

The species have been grown in the experimental field of

Fig. 14. *E. cicutarium* subsp. *arvale* No. 2298 grown in the experimental field and showing typical appearance of the field weed.

the Botanical Gardens of the University of Copenhagen. The soil there is heavy clay, very dry in summer.

E. cicutarium (L.) L'Her. In the summer of 1956 a number of strains from the British Isles and Denmark were grown in this experimental field. In Table IV these plants have been arranged according to their ability to produce flowers. It is evident, in spite of the regrettably low number of individuals in the batches,

TABLE IV.

Cultivations 1956.

Material of *E. cicutarium* subsp. *dunense* (4459—4452), *E. cicutarium* subsp. *arvale* (4457—4238), *E. danicum* (4239—4243) and *E. glutinosum* (4455). The Cult. Nos. are explained in Table II.

Cult. No.	5. 8. 56			25. 9. 56		
	Rosette	Flowers + rosette	Flowers – rosette	Rosette	Flowers + rosette	Flowers – rosette
4459	1	—	—	1	—	—
4451	9	—	—	8[1]	—	—
4454	12	—	—	6	4	—
4450	10	—	—	4	6	—
4446	8	—	—	—	7	—
4447	13	—	—	—	11	—
4456	2	—	—	—	2	—
4448	4	2	—	—	5	—
4452	—	2	—	—	—	2[2]
Total	59	4	—	19	35	2
4457	—	13	—	—	—	11
4098	—	2	1	—	—	3
4238	—	—	1	—	—	1
Total	—	15	2	—	—	15
4239	—	—	2	—	—	1
4240	—	—	2	—	—	2
4243	—	—	1	—	—	1
Total	—	—	5	—	—	4
4455	2	—	—	—	—	2

[1] When, as in this case, there are fewer plants on the 25. 9. than the 5. 8., this is due to some specimens preserved for the herbarium.

[2] Perhaps the strain has hybridized with subsp. *arvale*.

that the tetraploid British dune types (Figs. 11—12) practically do not flower under the present conditions while the tetraploid British weed as well as the Danish field weed (cp. Fig. 14) flowered abundantly. From the table it is seen that out of 63 individuals belonging to 9 different samples of *E. cicutarium* subsp. *dunense* from the British dunes only 4 had started flowering on 5. August, while 59 were still vegetative rosettes. The 4 flowering individuals still had large rosettes. One and a half month later, on 25. September, 19 out of 56 individuals alive

at that time were still vegetative, while 35 were flowering, but all the 35 with rosettes, only the two plants of No. 4452 from Wales had lost their rosette leaves. Perhaps this deviating strain had

Fig. 15. *E. danicum* No. 4240 grown in the experimental field, collected Sept. 1955.

suffered hybridization with *E. cicutarium* subsp. *arvale*. The 3 samples of *E. cicutarium* subsp. *arvale* all lost their rosette leaves during the autumn and flowered abundantly. These facts suggest that subsp. *dunense* in nature is a hibernating annual.

In the rather severe winter 1955/56 all strains died, whether they had flowered or not.

Cultivations carried out in previous years are all in close accordance with those of ANDREAS, thus in the mild winter of 1950/51 some few individuals of the strains 1202 from Holland and 2212 from Denmark, N. Zealand, hibernated.

It is evident that the general conditions given in the expe-

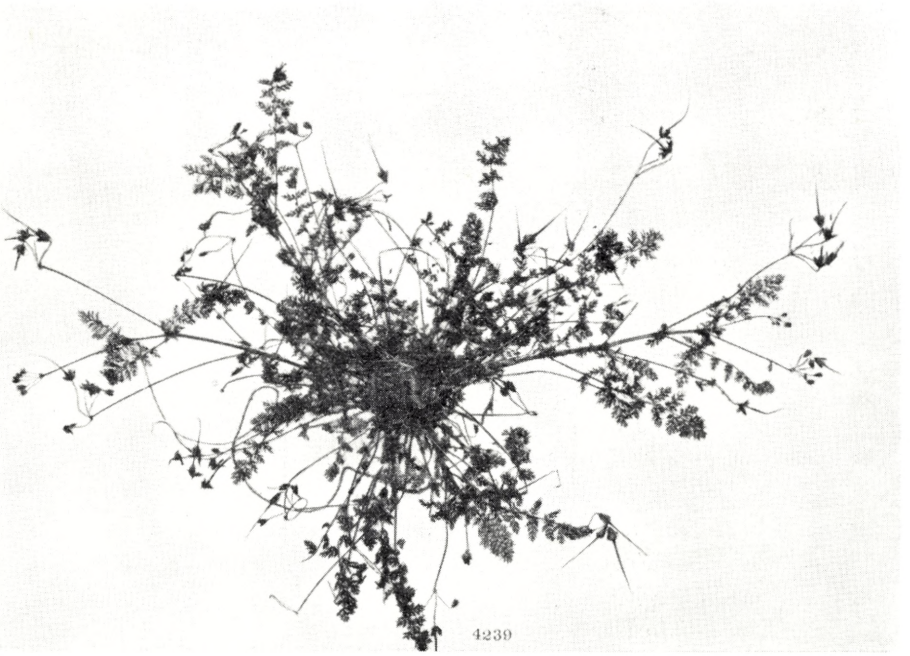


Fig. 16. *E. danicum* No. 4240 the type specimen grown in the experimental field, collected Sept. 1955.

rimental field have promoted the development of the vegetative part of the plants. These large rosettes are much more sensitive to the winter than the small rosettes developed in nature.

E. danicum K. Lars. behaved nearly as subsp. *arvale* (cp. Table IV). It developed a short-lived rosette and got very long branches which in the autumn, as described above, developed new rosettes in the leaf axils.

E. glutinosum Dum. In the vegetative parts this species deviated from the other dune *Erodiums* in so far as the rosette leaves were ascending, while in *E. cicutarium* (L.) L'Her. they were always decumbent. The batch consisted of two plants only. In

the last part of September they ceased flowering and were without rosette leaves, but had many vegetative shoots.

E. maritimum (Burm. fil.) L'Her. The seeds germinated in the last part of April. The plant flowered luxuriantly all the summer from the last part of June until the autumn, when the rosettes had reached a diameter at about 25 cm. In August new seedlings originated from seeds of the fruiting plants. They germinated in a large number in the experimental field; no doubt the species is able to produce two generations a year in climates which allow the seedlings to hibernate.

E. hirtum. The species remained vegetative during the first summer. The plants developed glabrous rosettes and a root system bearing characteristic globular tubers. These vegetative plants all died during the winter, even if they were hibernated in greenhouse at 7° C.

V. Observations in Nature.

According to JOHANSEN (1899) *Erodium cicutarium* (L.) L'Her. as a rule is a hibernating annual on Gotland. FRIES (1866) mentions that in dry places in Scania (S. Sweden) an early spring type is to be found which has hibernated with a very short stem ("subacaule"). FRIES regards this type (designated as a species by JORDAN) as a biennial form of the common annual field weed. The biennial form may be identical with var. *crassicaule* in SYLVÉN (1906, p. 179, Fig. 2 c, d, and Fig. 17 in the present paper), a variety to be found to be the commonest type on the limestone pavement ("alvar") on Öland. It seems exclusively to be a hibernating annual. Also summer annuals are reported from Scandinavia by several authors.

In Denmark it is obvious that on dry sandy hills in Northern Zealand *E. cicutarium* (L.) L'Her. behaves as a hibernating annual together with a number of other hapaxanthic species (e. g. *Trifolium arvense, Jasione montana, Cerastium semidecandum*, etc.). Small rosettes were observed in the middle of December 1955; at that time rosette diameters of 5—8 cm were measured on the slopes at the southern end of the lake Esrum Sø. The same locality was visited again in the middle of April 1956. The rosettes had all hibernated in contrast to those of the experimental field. They had not increased in diameter, but small buds were

observed among the rosette leaves. Some plants transferred to room temperature (Fig. 17) developed flowers within ten days. These specimens could be referred to the same spring type (var. *crassicaule*) as mentioned by SYLVÉN and FRIES.

Some of the "spring-type plants" were transplanted to the

Fig. 17. Hibernated rosette of *E. cicutarium* collected in the middle of April 1955 (cp. the text on p. 22).

Botanical Gardens. They developed to normal subsp. *arvale* and showed the chromosome number $2n = 40$. Thus the statement of LÖVE & LÖVE (mentioned above, p. 6) could not be confirmed on Danish material.

It is the author's impression that *E. cicutarium* (L.) L'Her. is most frequently a hibernating annual on dry slopes and in dunes (both subsp. *arvale* and subsp. *dunense*), whereas the field weed most frequently is a summer-annual.

Another purpose of the present investigations has been to establish whether the northern limit of *E. glutinosum* does reach Scandinavia. A study of the collections from Denmark in Herb. C

has revealed some small glutinous plants which have not developed mature fruits. In morphological appearance they were close to the description of the hybrid *E. cicutarium* × *glutinosum* (*E. anaristatum* Andreas). *E. glutinosum*, however, was not represented in the Danish collection. On the other hand, few sheets from the West Jutland dune areas were available. On travels in 1951 along the Jutland west coast from the South to the North the dune types of *Erodium* were studied; only subsp. *dunense* (Figs. 9—10) seems to be present there. The sterile forms mentioned above may have developed through poor nutrition or other unfavourable environmental factors.

VI. Summary.

1. The chromosome number of the following species of *Erodium* have been counted in material from natural habitats (see Table II): *E. hirtum* (Forsk.) Willd. subsp. *maroccanum* Maire, $2n = 20$; *E. maritimum* (Brum. f.) L'Her., $2n = 20$; *E. glutinosum* Dumort., $2n = 20$; *E. cicutarium* (L.) L'Her., $2n = 40$ (both subsp. *dunense* Andreas and subsp. *arvale* Andreas); *E. danicum* K. Lars., $2n = 60$.
2. A hexaploid cytotype of *E. cicutarium* coll. found in five localities in Denmark is established as a new species, *E. danicum* sp. nov. It is suggested to be an amphidiploid between *E. glutinosum* and *E. cicutarium* subsp. *arvale*.
3. Experimental cultivations with the two subspecies of *E. cicutarium* and *E. danicum* were carried out and summarized in Table IV. When grown as summer annuals plants of subsp. *dunense* very often do not reach flowering, while subsp. *arvale* and *E. danicum* always developed flowers.
4. Observations on *E. cicutarium* in nature has shown that summer-annuals as well as winter-annuals occur in Denmark. They were both found to be tetraploid. Thus the statement of diploid spring forms from Sweden could not be confirmed.
5. A search for *E. glutinosum* in the dune areas along the Danish North Sea coast gave no results. It is possible that it has its northern limit close south of the Danish frontier and that only the more robust *E. danicum* has been able to migrate further North.

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ON THE SCALES OF SOME SYNURA SPECIES

II

BY

JOHS. BOYE PETERSEN AND J. BENTH HANSEN

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Synopsis.

The scales of some Synura-species viz. *S. sphagnicola* Korsh., *S. Conradii* Kuff., *S. lapponica* Skuja and two new forms of *S. Petersenii* Korsh. (f. *Kufferathii* and f. *macracantha*) are examined by electron microscopy. It is established that *S. Conradii* is identical with *S. echinulata* Korsh. Finally some general remarks on the genus Synura.

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After the publication of our first paper on Synura scales examined by electron microscopy (Boye Petersen & J. Benth Hansen 1956), there appeared a paper on the same subject by Fott & Ludvík (1957), dealing with the scales of the same species we had discussed, as well as with those of *S. sphagnicola* Korsh. Our descriptions of the scales are in perfect agreement, apart from a few details, but concerning *S. sphagnicola* we have something to add, namely figures of the basal scales, which Fott & Ludvík only mention in passing. We have, besides, received material from Dr. H. Kufferath and from Professor Skuja, for which we are very grateful.

1. *Synura sphagnicola* (Korsh.) Korsh.

The species was established by Korshikov (1927) under the name of *Skadovskiella*. Owing especially to its peculiar scales and the position of the chromatophores in the cell, he considered it right to refer this species to a new genus. In the known Synura species the chromatophores are parietal, but in *S. sphagnicola* he found them placed centrally in the cell, separated from the outer side by two leucosin vesicles. The scales, which he saw for the first time in a Synura on this occasion, he interpreted as an oval ring provided with a handle, like a tennis racket. Korshikov does not give the dimensions of the scales. Later he changed his view of the species (1929), referring it to the genus Synura, because he realised that he was here concerned with real scales (not rings), and that the position of the chromatophores was hardly any good generic character always to be found in this species.

This species was found in Lille Skidendam in Teglstrup Hegn (leg. Berit Asmund), and in Bøllemose near Skodsborg (leg. J. Kristiansen). Both these localities are pronounced Sphagnum bogs with acid water. In Bøllemose ph 3.7 has been

measured (Boye Petersen 1943, p. 34), and in Lille Skidendam ph 3.9. The specimens found here agreed exactly with Korshikov's description of *Skadovskiella*, even the chromatophores did not seem closely appressed to the outer surface of the cell, as is usual in the Synura species. Nygaard (1949, p. 138) gives some localities for the species, but the determinations have not been verified by electron microscopy. While the other Synura species often occur in such large amounts that they give the water a brownish colour, we have only seen comparatively few specimens of *S. sphagnicola*.

After drying on cover slips the scales showed quite clearly the appearance described by Korshikov, so we must consider the determination as certain as it can be without the original material for comparison, material which we tried in vain to procure (Korshikov † 1943).

Specimens were dried on formvar film and after shadow-casting with palladium electron micrographs were taken. The scales then proved to resemble those of *S. spinosa* in many respects, but they were totally different from the latter by the fact that, though the surface of the scale was uniform, with small round holes like the lower part of the surface of the spinosa scale, the reticularly joined ribs on the upper surface of that scale were entirely absent. The basal scales proved to be ovoid and of the same type as in *S. spinosa*. Under the light microscope it is not very easy to distinguish the scales of these two species from each other. Korshikov (1927) draws the scales in such a way that the thorn of the scale must be presumed to be on the same plane as its plate. In *S. sphagnicola* this is no more the case than in the rest of the Synura species, and the thorn is also attached a short distance from the front edge of the scale.

Description of the scales of
Synura sphagnicola (Korsh.) Korsh. Korshikov 1929, p. 287.

Syn.: *Skadovskiella sphagnicola* Korshikov 1927, p. 450.
Pl. I, figs. a—c.

Apical scales oval, length 2.7—3.3 μ, breadth 1.9—2.5 μ, below with an upturned edge 0.3 μ broad, reaching about $^2/_3$ up the

scale. Here it seems to turn so as to form an edge at right angles to the surface of the scale. The whole of the surface is uniform with round holes about 0.06 μ in diameter, evenly distributed, about 8 in 1 μ. The spine is attached a little way inside the edge, ending in 2—3 short points, length 2.2—2.7 μ, breadth 0.2—0.3 μ. Basal scales ovoid, pointed at top (length 3.3 μ, breadth 2.1 μ), all the way round with a inflexed edge (breadth 0.3 μ) which at the top covers a space of about $^1/_3$ down the scale from the tip. The surface uniform with round holes about 0.06 μ in diameter, about 7 in 1 μ, without spine. In material from Abisko (sent by Professor SKUJA) there were also scales with an oblong, nearly rectangular outline (Pl. I c), length 3 μ, breadth 1 μ. We have not been able to see with certainty where these scales were placed on the cell, for we have only had loose scales for examination. It is possible that they have been situated among the normal scales provided with spines. The same material also contained the usual basal scales.

FOTT & LUDVÍK (1957) also mention the scales of *S. sphagnicola* and in the main their description agrees with our observations. In addition to the ordinary scales with spines we have also depicted basal scales without spines.

The small holes in the surface of the scale are described as hexagonal by FOTT & LUDVÍK. In our figures they are quite circular, and this, we think, is the case too in the figures of the two authors. Probably we have here an optical delusion. FOTT & LUDVÍK mention *Syncrypta volvox* Ehrb. as a synonym of *S. sphagnicola* (l. c.). As to this it must be urged that though Syncrypta is most certainly identical with Synura, it would seem that several, perhaps all, Synura species may enter into a Syncrypta stage. Thus we have had occasion to see such stages in *S. Petersenii* (see also CONRAD 1920, p. 177).

2. On the Validity of the Name *Synura Conradii* in CONRAD 1946, p. 5.

Pl. II.

Owing to the great kindness of Dr. KUFFERATH and the Musée royale d'histoire naturelle de Belgique, CONRAD's original preparations containing this species have been sent to us, as well

as CONRAD's and KUFFERATH's drawings and notes dealing with the genus Synura. The material for the preparations is derived from Mare des Clabots by Rouge Cloître near Brussels and was collected by W. CONRAD in February 1943. In most of the preparations there were numerous Synura scales, for the most part evenly distributed, while whole cells were rare. This, as described by CONRAD (1946, p. 3), is due to the way in which the samples were collected and examined. Precisely for the purpose of making the scales clearly visible it was especially the sediment in the plankton samples which was investigated, the cells there being separated, and the scales more or less detached from them. By means of the light microscope we have found perfect agreement between the drawings and the finds on the slides. We then soaked some of the preparations in xylene and removed the cover slip, after which part of the material which had dried on the slide or cover slip was removed with collodion and transferred to formvar film for use in electron microscopy. We were able to do so without removing all the material, so that the essential part of it could be left and the preparation restored to its original appearance. In this way the following preparations were examined: Nos. I, VII, XIII, and No. XIV. The preparations were mounted as follows:

No. I. Gentian violet, Canada balsam. No. VII. Gentian violet, euparal. No. XIII. Cresyl blue, without cover slip. No. XIV. Safranin, without cover slip.

The scales of the Synura species stain only faintly with the ordinary stains, presumably because they consist chiefly of silicic acid. When immersed in a medium with about the same refractive index as silicic acid, they will be very difficult to see clearly. In the preparations without cover slips the scales were distinct, and were sharply defined with the high dry system, whereas with oil immersion the scales will again be in a medium with about the same refractive index as the silicic acid, and the picture loses clearness of contrast. It is understandable, therefore, that CONRAD and KUFFERATH were not able to determine the species with certainty. The best method, which has previously been adopted by KORSHIKOV, is to dry the material on a cover slip and then fasten this on to a slide with a little canada balsam at the corners and with the material turned downwards. Such a pre-

paration can be examined with an oil immersion objective, and distinct pictures of the scales can be obtained. Further, the method has the advantage that the material can be preserved unchanged for an indefinite time.

In the e. m. examination we obtained the best results from the preparations without cover slips. In the preparations mounted in euparal or canada balsam some obscurity still remained in the shape of a fine veil, even though they were carefully treated with all the usual dissolving agents.

According to KUFFERATH's notes the preparations should contain the following species: *Synura Conradii* (in abundance), *S. uvella*, *S. Petersenii*, and *S. spinosa*. The three latter species we have, indeed, found. Of *S. Petersenii* a deviating form was seen, to which we shall revert presently. *S. spinosa* was found as f. *spinosa*.

It turned out, however, that by far the commonest scales in the preparations were from *S. echinulata* (Pl. II), which is not mentioned at all, and since none of the other scales present could be mistaken for *S. Conradii*, we must conclude that *Synura Conradii* Kuff. is identical with *S. echinulata* Korsh.

3. *Synura lapponica* Skuja.
Pls. III, IV.

SKUJA (1956, p. 275, Pl. 57, figs. 10—14; Pl. 58, figs. 1—2) has described a new species, *Synura lapponica*. Of this species, which in all other respects resembles the rest of the Synura species, he depicts the scales, which differ in appearance from those of all others. They are scutiform without any apical thorn, oval in shape, and have a small boss in the middle. Professor SKUJA has kindly placed at our disposal material of this species, taken from a small pond at Abisko and dried on slides. The sample contained a number of specimens with cysts. On these specimens it was impossible for us to find the scales, but scattered throughout the preparation we found single scales or small groups of such, which were transferred to formvar and photographed. These scales corresponded closely in shape and size to SKUJA's description, but it was difficult to point out with certainty any particular scale as apical or basal. Their shape differed some-

what, some were elliptical and large, presumably apical scales; others were smaller, almost circular, presumably basal scales.

A scale may be described as follows:

Shape elliptical, length 7.5 μ—8.9 μ, breadth 3.4—4.8 μ, with an upturned edge (ca. 0.2 μ broad) all the way round. The surface smooth with small holes (ca. 0.03 μ in diam.), about 16 in 1 μ. In the middle there is an annular elevation within which the surface continues with holes. The smallest scales have the same structure, length 4.9 μ, breadth 4.1 μ.

In principle these scales differ very much from those of the species previously known. Consequently this species cannot be referred to any of the previously mentioned sections of the genus (Boye Petersen & J. B. Hansen 1956, p. 6), and probably it may even be best to interpret *S. lapponica* as belonging to a separate genus. That, however, we will not do for the present, since there might, amongst other things, be a possibility that it is identical with genera already described (*Synuropsis*?) in which no scale-covering is known, though it may nevertheless be present.

4. *Synura Petersenii* Korsh. formae.

Of *Synura Petersenii* we have seen a large material and it turns out that the scales are of somewhat variable structure. As the type we must establish the form whose scales were originally described by Korshikov (1929), I. Manton (1955), and Boye Petersen & Hansen (1956). It must be named *Synura Petersenii* Korsh. f. *Petersenii* nomen novum.

In the material from Kufferath there were scales differing from the type (1) in that the middle cavity was short and (2) in that the ribs were connected by transverse folds so that a network of ribs was formed at the lower end of the scale. This form might be called *S. Petersenii* f. *Kufferathii* n. f. In the material from Abisko sent by Professor Skuja there were scales remarkable for (1) a very large and strong spine and (2) a very stout network of connected ribs. The dimensions of the scales were: length 4.3 μ, breadth 2.2 μ; spine: length 1.5 μ, breadth 0.5 μ, that is to say, of the same size as in the type but with a stronger spine than the latter. This form may be called *S. Petersenii* f. *macracantha* n. f. It is possible that *Synura intermedia*

(Bioret) Kufferath, of which we have in vain tried to obtain authentic material (Conrad 1946, p. 5, fig. 23) is a form of Petersenii with a still stronger spine.

5. General Remarks on the Genus Synura.

In the present paper we have established some new forms of *S. Petersenii*, and we have previously described five forms of *S. spinosa* (Boye Petersen & J. B. Hansen 1956, p. 20—21). These forms show characteristic differences and under the electron microscope they may easily be distinguished from one another. At present, however, our knowledge of the range of variation of the various species is very imperfect. It might be supposed, for instance, that within the same species the appearance of the scales might vary with the seasons. We have considered it sufficient, therefore, to call these varieties forms, but it is possible that they actually represent well separated species.

Successively, the scales of a number of species of the genus Synura have been examined, partly by light microscopy, partly by electron microscopy. The scales are so small and their structure is so fine that but little satisfactory pictures of it can be obtained by light microscopy. In most cases, however, if the best optical instruments that can be had are employed, it will be possible to distinguish the species from one another under the light microscope, particularly if a distinct impression of their structure has first been gained from e. m. pictures. All species of the genus which have been established without taking into account the structure of the scales must be considered doubtful and are therefore entered in the list below as species inquirendae. In the same list some species have also been entered which are referred to affiliated genera, though it may be conjectured that they actually belong to the genus Synura, where the scale-covering hitherto has not been observed. Unfortunately it has proved impossible to procure authentic material of most of these species, despite the fact that merely a sample containing the species dried on a slide would be sufficient to determine the position of the species.

Before concluding this paper we must refer the reader to Harris & Bradley (1956, I and II), who have given very fine pictures of Synura scales produced by the so-called carbon

replica process. These pictures emphasise certain details which are less distinct in ours. On the other hand, other details are not seen in them.

Acknowledgements.

It is our pleasant duty to thank all who have helped us with material for this paper, namely Lektor BERIT ASMUND, Professor H. SKUJA, the late Dr. H. KUFFERATH, and the Musée royale d'histoire naturelle de Belgique. Further we wish to thank Professor J. KOCH for permission to use the electron microscope of the Biophysical Laboratory of the University of Copenhagen, as well as Mr. F. CARLSEN M. Sc. for his never-failing aid. The authors express their grateful acknowledgement to the Trustees of the Carlsberg Foundation and of the Danish State Research Foundation for support which has rendered possible the completion of this work. The paper has been translated into English by Miss ANNIE I. FAUSBØLL M. A.

Diagnoses formarum novarum.

S. Petersenii f. *Kufferathii* n. f. a forma Petersenii differt cavitate centrali curta, costis marginalibus in parte inferiore reticulatim connectis.

S. Petersenii f. *macracantha* n. f. a forma Petersenii differt spina maxima costis marginalibus usque ad apicem reticulatim connectis.

Species et varietates delendæ.

Synura caroliniana Whitford 1943, p. 158 = *S. Petersenii* Korsh.
– *Conradii* Kufferath in Conrad 1946, p. 5 = *S. echinulata* Korsh.
– *uvella* var. *punctata* Awerinzev 1899, p. 264 = *S. sphagnicola* Korsh. (fide Fott and Ludvík 1957).
– *uvella* f. *turfacea* Steinecke 1916, p. 32 = *S. sphagnicola* Korsh. (fide Fott and Ludvík).

Species inquirendæ.

Synura Adamsii G. M. Smith 1924.
– – f. *malabarica* Philipose 1953, p. 239.
– *australiensis* Playfair 1915, p. 315.
– *Bioretii* Huber-Pestalozzi 1941, p. 141.
– *granulosa* Playfair 1915, p. 314.
– *intermedia* (Bioret) Kufferath in Conrad 1946, p. 5.
– *Lohammeri* Skuja 1956, p. 274.
– *reticulata* Lemmermann 1903, p. 119.
– *splendida* Korshikov 1942, p. 27.
– *uvella* var. *lævis* Kisselew 1931, p. 243.
– – – *punctata* Kisselew 1931, p. 243.
– – – *longipes* Virieux 1916.
– *verrucosa* Pascher 1913, p. 51.
Chlorodesmus hispidus Philipps 1884.
Pseudosynura urogleniformis Kisselew 1931, p. 243.
Synuropsis danubiensis Schiller 1929, p. 443.
– *globosa* Schiller 1929, p. 445.
Actinoglena Klebsiana Zacharias 1897, p. 5 = *Synura Klebsiana* Lemmermann 1899, p. 110.

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Plates.

PLATE I.

Synura sphagnicola, a. apical scale seen from the inner side. Shaded with palladium, negative print. ×15.000. Ll. Skidendam 9/8 1956. b. Apical and basal scale seen from the outer side. Shaded with palladium, negative print. ×15.000. Ll. Skidendam 9/8 1956. c. Positive print. ×15.000. Abisko.

PLATE II.

Synura echinulata (= *S. Conradii* Kuff.). From Conrad's slide nr. VII. Positive print. ×15.000.

PLATE III.

Synura lapponica, a. scale shaded with palladium, negative print. ×15.000. b. Central part of a scale, positive print. ×32.000.

PLATE IV.

Three different scales of *Synura lapponica*, shaded with palladium, negative print. ×15.000.

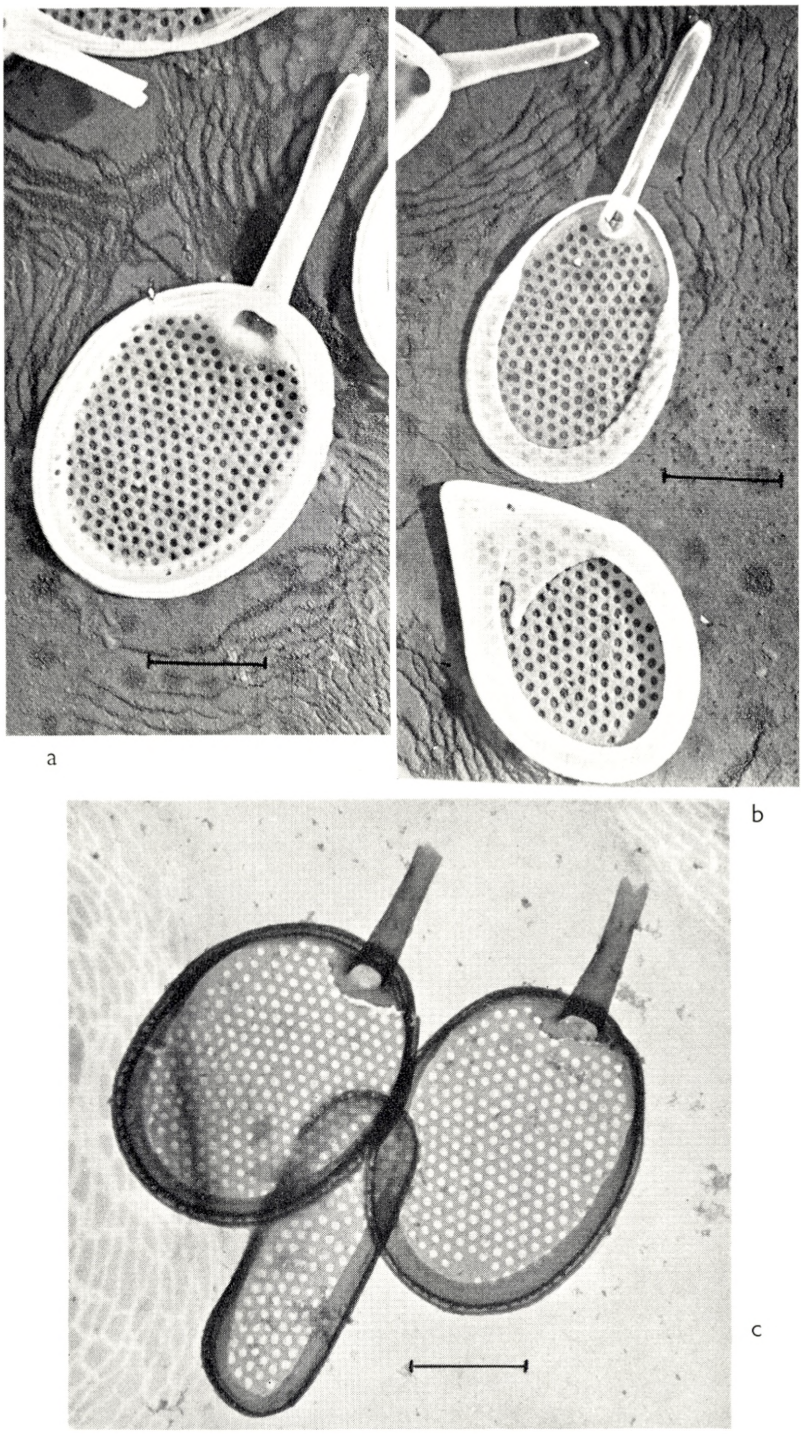
PLATE V.

Synura Petersenii f. *Kufferathii*. From Conrad's slide nr. XIII. Shaded with palladium, negative print. ×20.000.

PLATE VI.

Synura Petersenii f. *macracantha*. Shaded with palladium, negative print. ×20.000. Abisko.

The unit of measurement represents 1 μ.



a

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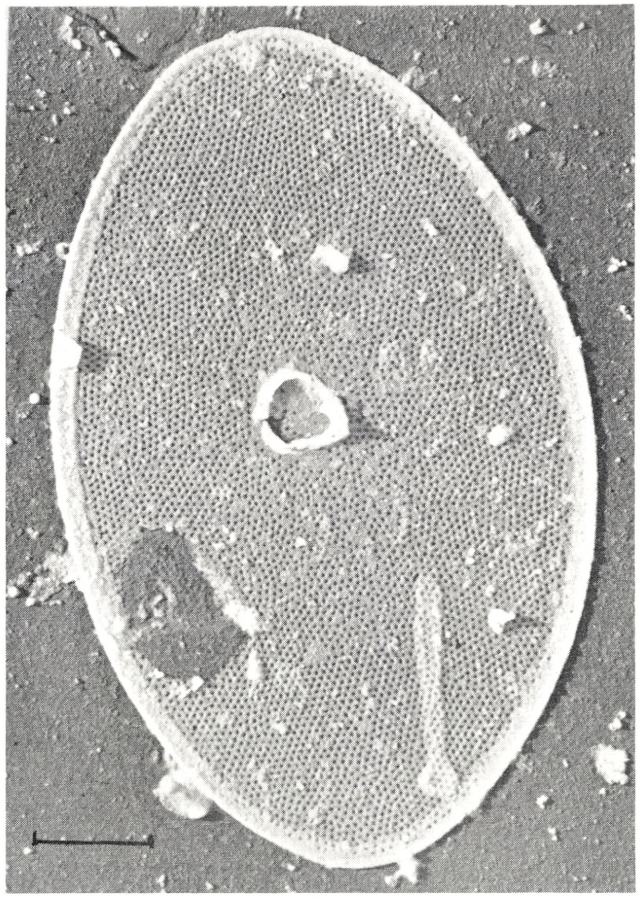
PLATE II.



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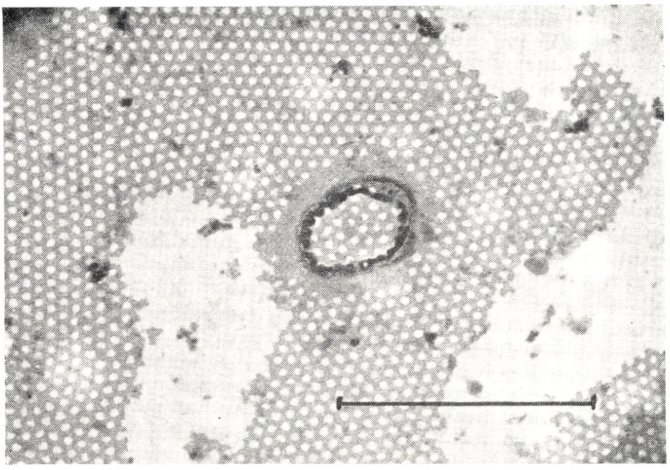

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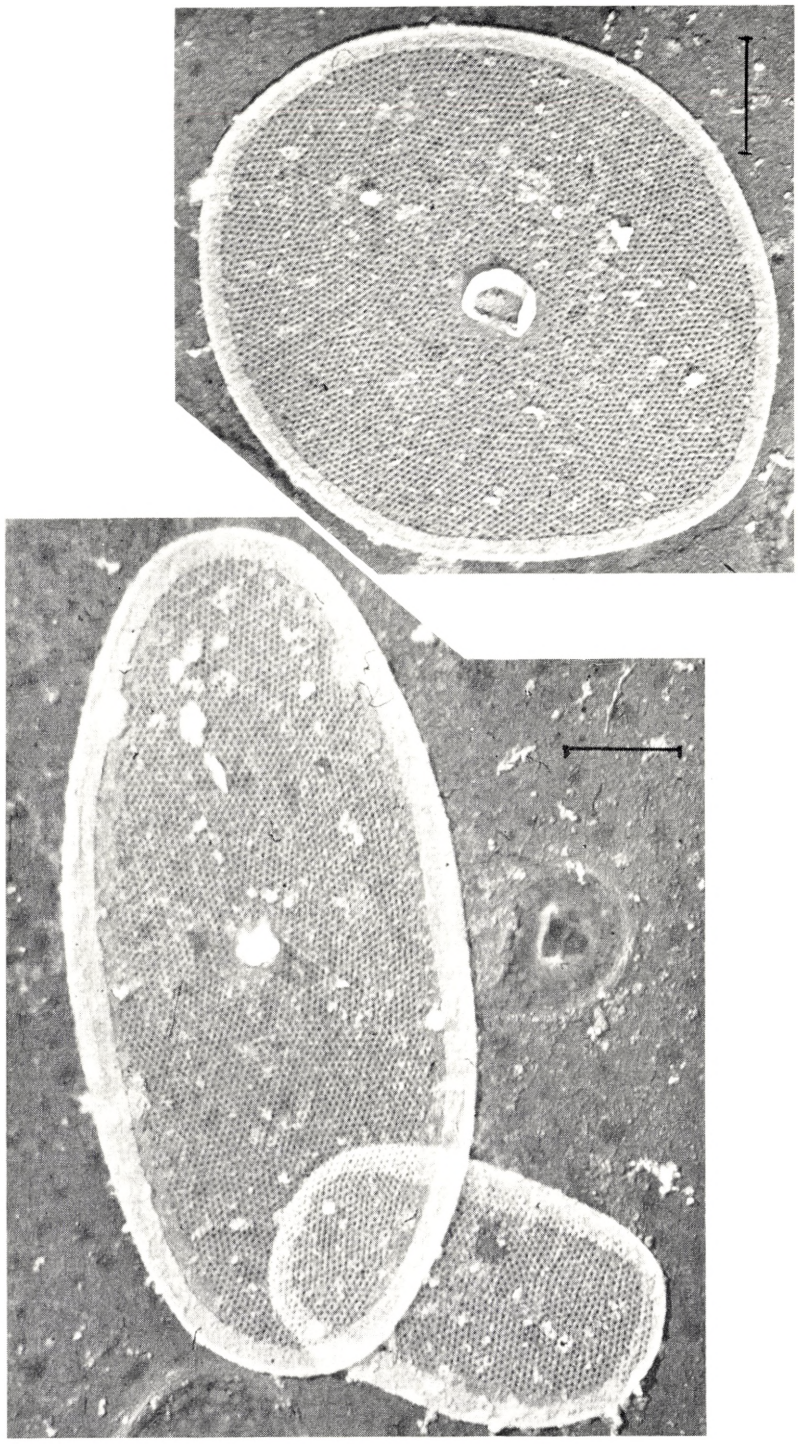
PLATE IV.



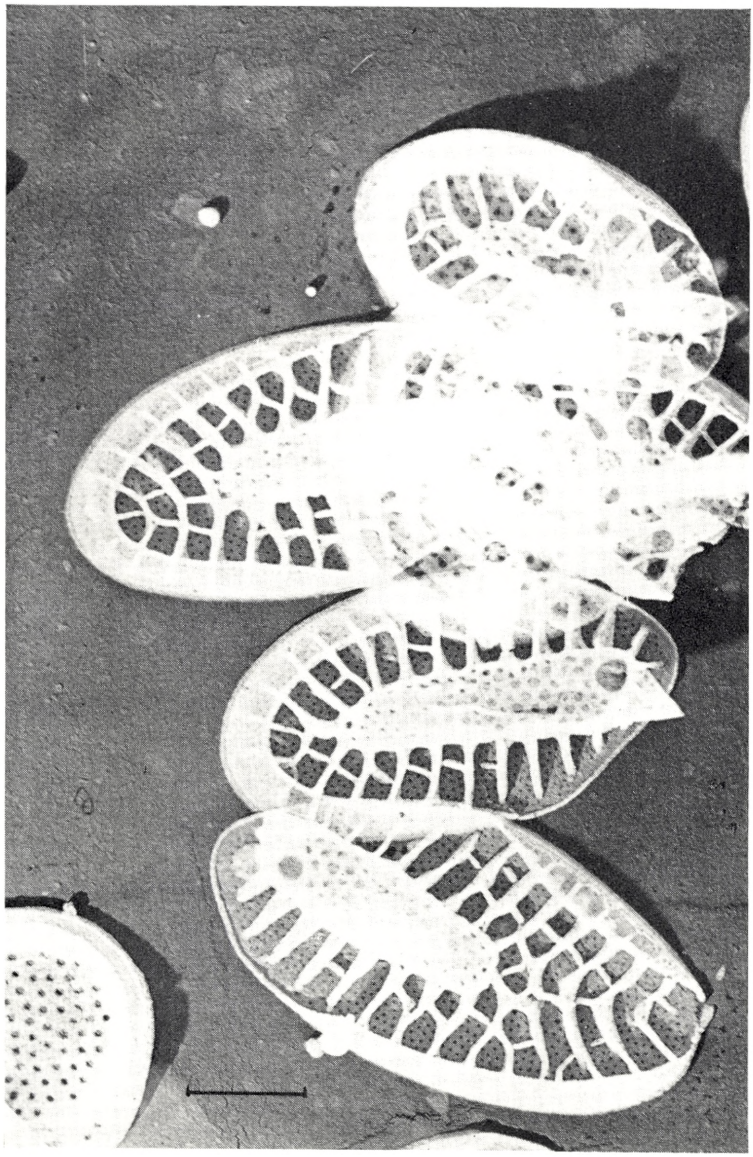
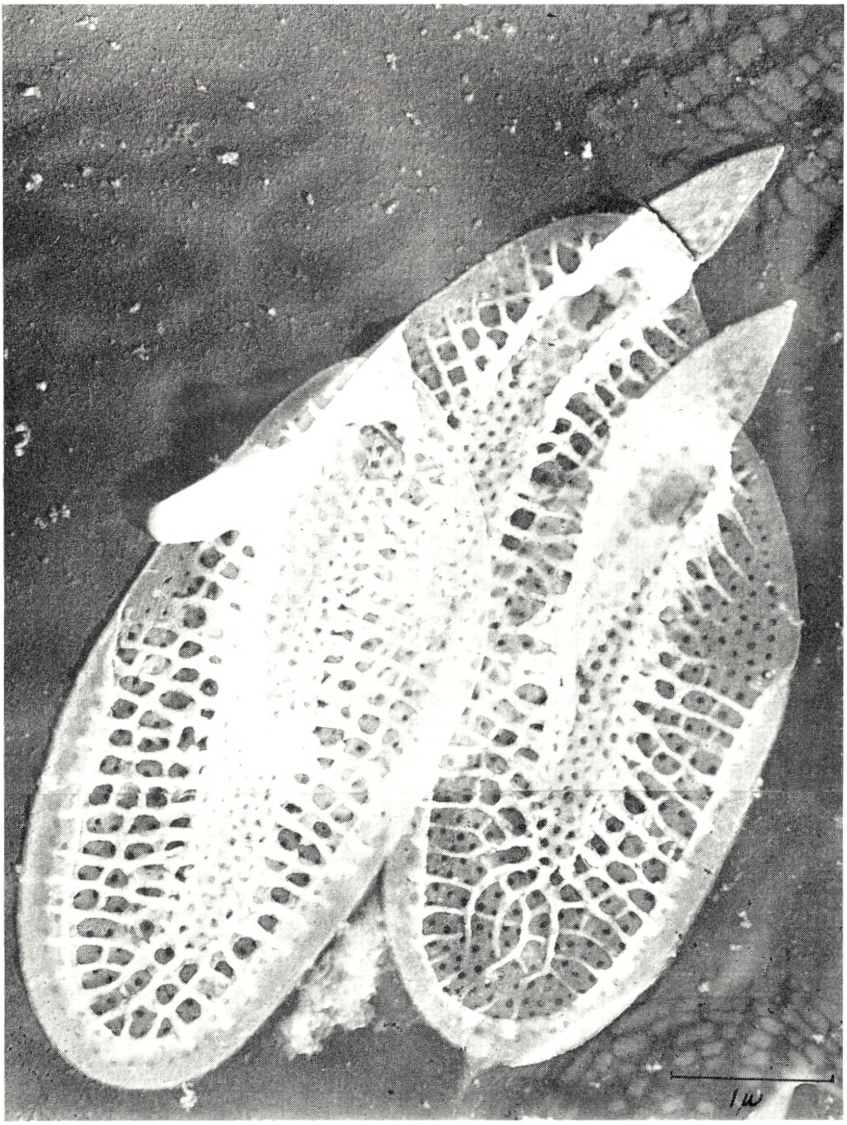


PLATE VI.



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UNTERSUCHUNGEN ÜBER DETERMINATION UND DIFFERENZIERUNG

5. ÜBER DIE WIRKUNGSWEISE DES WUCHSSTOFFES IN DEM EPIKOTYL VON *PHASEOLUS*

(DIE BRÜCKENTHEORIE DER WUCHSSTOFFWIRKUNG)

VON

P. BOYSEN JENSEN

With an English Summary

København 1958
i kommission hos Ejnar Munksgaard

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CONSTRUCTION AND PROPERTIES OF COMMA-FREE CODES

BY

S. W. GOLOMB, L. R. WELCH, AND M. DELBRÜCK

København 1958
i kommission hos Ejnar Munksgaard

CONTENTS

Synopsis.

The sequence of bases in deoxyribonucleic acids is assumed to represent a coded message, embodying information concerning the sequence of amino acids in proteins. Crick *et al.* [7] suggested that the code might be a "comma-free triplet" code. This means that each amino acid is coded by a triplet of bases, and that the triplets are chosen such that no overlap between any pair of triplets codes for an amino acid. In such a code the triplets do not have to be separated from each other by some kind of comma; they can be run together without causing ambiguities in the message.

This paper concerns the following aspects of comma-free codes:

1) Procedures for the construction of all comma-free triplet codes involving the maximum number (20) of triplets. It is shown that there are five classes of such codes and a total of 408 codes.

2) It is shown that no message written with any of these codes ever contains a fourfold repeat of any base, and that in some of the codes certain threefold repeats are excluded.

3) Certain misprints in the coded message will produce nonsense (the resulting triplet does not code for any amino acid), other misprints will produce missense (the resulting triplet codes for a different amino acid). The codes were studied with respect to missense/nonsense ratio produced by various classes of misprints.

4) DNA has a directional symmetry. The basic structure is such that the message could be read in either direction. The question is posed whether codes could be devised such that if they are read in the wrong direction they give nonsense everywhere, i. e., no triplet or overlap between triplets read in reverse corresponds to any amino acid. Such codes are termed transposable codes. It turns out that a transposable triplet code can code for at most 10 amino acids, which is too few. Therefore quadruplet codes were taken into consideration. These are mathematically more difficult to handle and only a few fragmentary results have been obtained so far.

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Part I.

Origin of the Problems, Summary and Discussion of Results.

By M. Delbrück.

The discovery that genetic information in many organisms is transmitted from parent to offspring through desoxyribonucleic acid (*DNA*) and the discovery of the structure of *DNA* by Watson and Crick [1] have raised the problem as to the nature of the code used to carry this information and as to the mechanism by which the code is read. It is believed that one of the intermediate steps of the translation consists in the synthesis of specific proteins and that the essential element of this specificity consists in the sequence of the amino acids in the proteins. Both the *DNA* and the protein are linear polymers. The *DNA* molecule is a duplex of two chains containing principally four bases (adenine = *A*, cytosine = *C*, guanine = *G*, and thymine = *T*). In addition, there are one or more bases occurring in very small proportion which may or may not have any particular significance. The two chains have base sequences which are complementary, *A* always opposite *T*, and *C* always opposite *G*. The duplex as well as the single chain may therefore be looked upon as a message written in a code involving four symbols. In the case of the single chain, the symbols are *A*, *C*, *G*, and *T*; in the case of the duplex, the symbols are the base pairs *A*–*T*, *T*–*A*, *C*–*G*, and *G*–*C*. The proteins are polypeptide chains of some 20 amino acids and can thus be looked upon as messages written in a code containing some 20 symbols. The problem is thus reduced to one of coding the information contained in a message employing some 20 symbols in a code employing only four symbols and to finding the mechanism for its translation.

The *DNA* as a message container confronts us at once with a peculiar duality feature: it contains *two* complementary messages which are chemically quite different. This is so whether the single chain or the duplex as a whole is the message container. If a single chain is the container, then the complementary chain contains a message which differs from the first one by two operations: (1) reading backward, (2) substitution of the complementary symbol X' for each of the symbols X (A for T, T for A, G for C, and C for G). If the duplex as a whole is the message container, with base pairs as symbols, then we are still dealing with two complementary messages. This is so because the duplex as a whole is symmetric, it can be read in either direction, and the two messages so obtained differ exactly by the same operations as those on the two chains: reversal of direction and substitution of the complementary symbol (base pair $A-T$ for $T-A$, etc.).

By the time the message is translated into protein this duality is apparently gone, as there is no evidence that one piece of genetic material is regularly responsible for two different proteins. Along the path from *DNA* to protein one of the messages is therefore eliminated. The last chapter of this paper arose from a specific suggestion as to the nature of this elimination. To introduce this notion, as well as those which prompted the other mathematical questions and answers dealt with in this paper, it will be necessary to insert a few comments on the biochemical aspects of the problem.

It seems fairly certain that the centers of protein synthesis in the cell are the microsomes, particles which contain no *DNA* but which do contain ribonucleic acid (*RNA*), a linear polynucleotide also containing four bases. It is a likely conjecture that the *RNA* represents an intermediate translation of the code. If so, it would be of the greatest interest to know whether at this point of the translation process the duality of the message has already been eliminated. The fact that the base ratios of *RNA* in some cases deviate from those imposed in *DNA* by the complementarity feature would seem to speak in favour of the idea that the duality has been removed, but neither this fact, nor, if it is a fact, the method by which it is accomplished are clear.

Regarding the actual synthesis of proteins it is now believed [2, 3, 4] that the amino acids are first activated, in two steps: in

the first step a complex is formed between amino acid and adenosine monophosphate (*AMP*); while in a second step the amino acid is transferred from *AMP* to a soluble *RNA* fraction. More precisely, there is a specific enzyme for each amino acid which couples only this amino acid to *AMP*. The amino acid is then transferred to a specific site on the soluble *RNA*. At first sight it would seem surprising that for each amino acid there should be a specific enzyme to couple it to *AMP*, since no confusion, i.e., no false synthesis, would occur if this step were unspecific. It is conceivable, however, that in vivo not soluble *RNA* molecules but tri- or tetra-nucleotides play the role of intermediate amino-acid carriers, supplying a specific adaptor to each, this adaptor serving the purpose of fitting it to the code letters in the message. If the same enzyme which couples the amino acid to *AMP* were responsible for the transfer of the amino acid to the specific adaptor then the specificity of the enzyme would make sense. Obviously, the same enzyme could not be charged with the duty of coupling each amino acid to its specific adaptor.

Four years ago Gamow [5] published in these proceedings an important paper in which a first attempt was made to "break the code". The main characteristics of Gamow's attempt were the following: first, it assumed a *direct* translation from *DNA* into protein; second, it assumed an *overlapping* code, the piece of *DNA* determining one amino acid (a diamond shaped structure extending over three base pairs) and the piece of *DNA* determining the next neighboring amino acid overlapping by two thirds of their length; third, it assumed a *degenerate* code, in that several different triplets of base pairs coded for the same amino acid. The reason why it was assumed that a triplet of three pairs code for one amino acid was simple. Two base pairs give only 16 possibilities, which is not enough to code for some 20 amino acids. Three base pairs give 64 possibilities which is more than necessary and therefore permits degeneracy. The overlap feature was introduced for geometrical reasons: with this amount of overlap the spacing from one amino acid to the next would correspond roughly to the spacing from one base pair to the next and this seemed reasonable on structural grounds.

With the increase in our knowledge of amino acid sequences in proteins Gamow's particular scheme, and several others, have

been demonstrated to be untenable, and in fact BRENNER has given [6] an elegant proof of the impossibility of all overlapping triplet codes. In 1957 CRICK, GRIFFITH and ORGEL [7] introduced a new idea into the problem. If it is true that groups of base pairs, say triplets, code for one amino acid, and that these triplets are not overlapping, and if the message is formed simply by tacking these triplets end to end, how do we know where one triplet ends and the next begins? Either the message would have to be read strictly in sequence starting at one end, or the triplets might be chosen such that no overlap makes "sense". The triplets which code for amino acids might form a "dictionary" of "words" such that no overlapping triplet in a message written from these words is a word in this dictionary. This is the idea of the comma-free code, and CRICK *et al.* proved that in the case of four symbols and words of length three the maximum size of such a dictionary is 20. They also constructed some of the dictionaries of this size. In such a code, then, the freedom resulting from the fact that there are 64 possible triplets and only 20 amino acids to code for is used to select a comma-free dictionary. GOLOMB, GORDON and WELCH [8] addressed themselves to a mathematically interesting generalization of this problem: what is the maximum size of a comma-free dictionary in the case of an arbitrary number of symbols and an arbitrary length of the words? They were able to obtain a partial solution of this problem, and to develop methods which are useful also for the mathematical developments presented in this paper.

These developments were motivated directly by certain aspects of the *DNA*-protein problem. It is the purpose of this Part I to explain the biological interest of these questions and to summarize and discuss the results. It is hoped that they will stimulate new experimental approaches and point the direction which further mathematical analysis might take, to be of the greatest interest to the biologist.

The overlapping codes considered by previous authors implied no restriction on base neighbours, but did imply certain restrictions, statistical or absolute, regarding amino acid neighbors. It had been hoped that these restrictions would afford clues for breaking the code without any actual knowledge of base sequences in the *DNA*. As it turned out, these restrictions only

served to *eliminate* the overlapping codes. The comma-free codes present the opposite situation. They are non-overlapping codes, and as such they contain no restrictions, statistical or absolute, regarding amino acid neighbors, but they do imply restrictions on base sequences. If this is the type of code actually used, then the study of amino acid sequences by itself will be useless for breaking the code. However, information on the neighbor relations among the *bases* may be indicative as to the specific code used, and it becomes of interest to examine these codes closely to see what they imply, statistically or absolute, with respect to base neighbor relations.

A prerequisite for such a study is a method for constructing all possible comma-free codes. This is accomplished for the three letter codes in the first three chapters of Part II of this paper. The key to this construction is the important Theorem 2 which states a surprisingly simple condition which is both necessary and sufficient for a collection of 20 triplets to constitute a comma-free dictionary. With the help of this theorem it can be shown that there are five types of maximal comma-free dictionaries using triplets. Of each type there are a large number of different dictionaries, dictionaries of the same type differing from each other by permutations of letters and by reversals of sections of the dictionary. Chapter 3 is devoted to a study of these permutations and reversals.

Being in possession of these dictionaries we would like to enquire into properties of these dictionaries which might find expression in an experimentally verifiable manner and might thus serve the diagnostic purpose of differentiating between various codes.

The most direct route for breaking the code would of course be available if the conjecture of a specific oligonucleotide adaptor for each amino acid were found to be valid, and if these adaptors should stand in a complementarity or identity relation to the words of the coded message. It would then only be necessary to isolate and characterize the amino acid-adaptor complexes.

It is more likely that a less direct approach will be needed, involving properties of the *DNA* by itself or of the *RNA* by itself, and here a promising attack would lie in the examination of oligonucleotide fragments prepared by hydrolytic procedures with known specificities. Here it is important, before comparing ex-

perimental data with the mathematical properties of the codes, to take proper account of the duality feature.

We will illustrate this by examining three rules, proved in Part II of this paper, concerning forbidden symbol combinations.

1) In stochastic messages written from some of the triplet codes one and only one of the triplets of type XXX does not occur.

This is not a property which is verifiable by fragment analysis if the material studied still has the duality feature. Indeed, since the triplet $X'X'X'$ (the complement of XXX) will occur in the *message*, the triplet XXX will occur in the complementary message. If one of the single chains is the message, and does not contain XXX, then its complement will contain it, and its absence from the message proper will not be apparent from a hydrolyzate which does not distinguish the breakdown products of the real message from those of the complement. Similarly, if the duplex, read in one direction, is the real message, and this excludes of the triplets only the triplet XXX (X representing, say, the base pair $A-T$), then one chain excludes the triplet AAA and the other TTT, but each chain will contain the triplet which the other excludes, and the exclusion will not be observable by fragment analysis.

In fact, what is observable is not any rule as expressed in terms of the symbols of the message, but only such a rule after "mixing" it with the complementary rule. Thus, the rule "the triplet XXX does not occur and the triplet $X'X'X'$ does occur" is to be mixed with the complementary rule "triplet XXX does occur and triplet $X'X'X'$ does not occur." The mixed rule then says that both triplets occur.

2) No quadruplet of type $XXXX$ can occur in any message written from any maximal comma-free triplet code.

This rule is not affected by mixing with the complementary rule, since in this case the original and the complementary rule are identical.

3) In two of the five types of triplet code *two* triplets are excluded from the messages. If the two excluded triplets are complementary, then the rule is not changed by mixing, while if they are not complementary, then there are no exclusions after mixing. Whether the two triplets are complementary or not depends on the identification of the symbols with the bases or base pairs.

Up to this point we have been talking about absolute rules: complete exclusions of certain symbol combinations. We now turn to statistical rules. Here the first rule (theorem 1) is that in each maximal comma-free dictionary every letter occurs equally frequently and in the same number of words. Any deviation from equality between the four symbols therefore indicates an unequal frequency of the words, as is indeed obvious from the inequalities between the frequencies of the different amino acids. This inequality is of course insufficient to characterize the type or the specific dictionary. It follows further that it would be very difficult to make predictions concerning the frequencies of permitted digrams and longer combinations. For digrams, for instance, it is easy to calculate the frequencies with which they occur in the different dictionaries, and wide differences will be found between different dictionaries. However, the observable digrams include the overlaps, and their frequencies depend critically on the relative word frequencies which are not known in terms of the words of the code, even though they may be ascertainable, in some cases, in terms of the amino acids. For these reasons it does not seem worth while at this stage to go into a detailed analysis of the statistical relations.

The comma-free codes differ in another important respect from those previously considered: every misprint of necessity alters the message. This is in contrast to degenerate codes, where a large class of misprints produces no alteration at all in the message. The non-degenerate codes are therefore more vulnerable. The errors which occur, may, moreover, be divided into two classes: those which change a word of the dictionary into another word of the dictionary, and therefore one amino acid into another amino acid, one polypeptide into an altered one; and those which change a word of the dictionary into a combination of letters which is not in the dictionary, and therefore one amino acid into no amino acid, one polypeptide of length n into two polypeptides of total length $n-1$. Let us call these two classes of misprints the mis-sense class and the non-sense class. Presumably the non-sense class represents on the average a more severe functional change. It seems likely that the mis-sense to non-sense ratio is an important characteristic of each code and might have played a role in its natural selection. Moreover, it may be that

among the individual symbol errors those that interchange similar ones (the purines among themselves and the pyrimidines among themselves) are much more probable than the others.

The mis-sense to non-sense ratios are shown in Tables III and IV for the five types of dictionaries and for various restricted classes of misprints. There exist only slight differences between the five types of dictionaries when the types of misprints are unrestricted. In a maximal comma-free dictionary using four symbols and three-letter words there are sixty letters (20 words with three letters each) and each of them can be misprinted in three different ways, giving a total of 180 different misprints. The class of non-sense misprints ranges in size from 80 (in type I) to 92 (in type V). The differences become more pronounced when restricted classes of errors, of the types alluded to above, are considered. In this case, each letter can be misprinted in only one way (each pyrimidine by the other pyrimidine and each purine by the other purine). The total number of possible misprints is now sixty, and the class of non-sense misprints ranges in size from 16 (for type I) to 32 (for type V).

We turn now to the problem of how the duality of the message in the *DNA* is handled in the translation process. Here we put to ourselves the question whether the dictionary might not be so constructed that it automatically eliminates the duality. This would be accomplished if one of the two complementary sequences of letters contained nowhere letter combinations which occur in the dictionary. This question is examined in the last section of Part II of this paper. It turns out that it is indeed possible to construct dictionaries such that the complement to any message composed of words of the dictionary contains nowhere, neither as the complements of words nor as complements of the overlaps, a word of the dictionary. Such dictionaries are called *transposable* dictionaries and the very strong constraint which they fit does not reduce their size inordinately. A general theorem (theorem 15) about transposable dictionaries is given, establishing an upper bound as to their sizes. In the case of four symbols and three letter words, this maximum size is 10, as compared to 20 in the absence of the constraint. Several such dictionaries are given. Ten words is too few to code for all the amino acids and the simplest way to increase the number of words is by increasing the length of the words to four letters. In this case the upper bound turns

out to be 27 and an actual transposable dictionary of size 26 has been constructed. [Added in proof: numerous examples of 27 word dictionaires are now known.]

We wish to emphasize that we consider the postulate of comma-freedom and the postulate of transposability to be almost on the same footing. Indeed the principal virtue of comma-freedom is that any message can be read unambiguously starting at any point, with the proviso, however, that one must know in advance *in which direction to proceed.* Since the equivalence of the two opposite directions in a structural sense seems to be one of the more firmly established features regarding the *DNA* molecule the advance knowledge as to the direction in which to read cannot come from the basic structure. Comma-freedom would therefore seem to be a worthless virtue unless it is coupled with transposability.

Transposable comma-free dictionaries, in contrast to ordinary comma-free dictionaries, are strongly asymmetric with respect to symbol frequencies and symbol combinations. This is due to the fact that in the ordinary comma-free dictionaries every complete equivalence class[1] is represented, whereas in the transposable ones at least one half of these classes are not represented. This may be illustrated by the 26 word dictionary using 4-letter words exhibited in the last chapter. In this dictionary the pairs A and B, and C and D are complementary. The four symbols occur in the dictionary with the frequencies 21, 31, 41, 11. The complementary pairs A, B and C, D occur equally frequently in the dictionary. This is a general rule for transposable dictionaires in which each non-symmetric equivalence class[1] is represented. In the example given each class, with one exception, is, in fact, represented. This exception is the class $ADBC$. Its omission does not lead to a violation of the equality rule for complementary pairs, since it contains an equal number of each pair.

In terms of the bases this rule implies that in the dictionary the pair of bases Adenine-Thymine occurs as frequently as the pair of bases Guanine-Cytosine. For *DNA* molecules this equality in general does not hold. The inequality would have to be due to the unequal frequencies of the words (amino acids) of the dictionary in the coded message (*DNA* molecule).

A strong asymmetry may be inherent in the dictionary with

[1] for definition of this term see Part II, chapter 1.

respect to *purines* vs. *pyrimidines*. This we will illustrate again with the 26 word dictionary. Let us make the identification:

A = adenine
B = thymine
C = cytosine
D = guanine

Thus A and D are the purines (collectively designated as Pu), and B and C are the pyrimidines (collectively designated as Py). The dictionary is thus seen to contain 32 Pu and 72 Py. The symbol frequencies in the message will of course depend on the word frequencies, and this may affect the letter frequencies appreciably. However, even for equal word frequency, there would be on one of the chains a large excess of Py. Let us call this the Py chain. This would be compensated by a corresponding excess of Pu on the other chain, the Pu chain, and would not be observable as an asymmetry in the total hydrolysate. The asymmetry would be observable, however, in an experiment of the type described by MESELSON and STAHL [9], where N^{15} labelled *DNA* is permitted to duplicate once in the presence of N^{14}, producing "hybrid" *DNA* molecules, half labeled with N^{15}. If the "halves" in these experiments are indeed single polynucleotide chains (of which there is still doubt), then we should obtain two classes of hybrids, those whose Py chain is N^{15} labelled and Pu chain N^{14} labelled, and vice versa, and these two types of hybrids would differ sufficiently in density (by 0.2 %) to be resolvable by the equilibrium density gradient technique.

The unequal distribution of the purines and pyrimidines over the two chains will lead also to a phenomenon recently reported by SHAPIRO and CHARGAFF [10]. These authors found that the pyrimidines are bunched, i.e., that runs of pyrimidines of length one and two, flanked by purines, occurred less frequently than would be expected for random sequences in which purines and pyrimidines occur equally frequently. Messages constructed from a given dictionary are certainly not to be equated statistically with random letter sequences. The statistical proportion of the symbol relationships in such messages cannot be assessed in detail without a knowledge of the statistics of words. It is clear, however, that a strongly unequal distribution of the pyrimidines

over the two chains will introduce a bias in the direction of bunching.

It is perhaps unlikely that the words in the dictionary are of equal length. Indeed if economy with respect to total length of the coded message were the overriding principle in the choice of the code, nature would certainly have selected short words for frequently occurring amino acids and vice versa. We know at present too little about other aspects, particularly those of steric convenience, that might have more decisive influences affecting the selective advantages of various possible codes. The arguments and mathematical results presented in this paper can do no more than to help clarify ideas and direct attention to the types of data on base sequences that would be most helpful.

Part II.

Mathematical Developments.

By S. W. Golomb and L. R. Welch.

1. Definitions and General Theorems.

Let n be a fixed positive integer, and consider an alphabet consisting of the numbers 1, 2 . . . n. With this alphabet form all possible k-letter words $(a_1 a_2 \ldots a_k)$, where k is also fixed. There are evidently n^k such words in all.

Def. A set L of k-letter words is called a *comma-free dictionary* if whenever $(a_1 a_2 \ldots a_k)$ and $(b_1 b_2 \ldots b_k)$ are in L, the "overlaps" $(a_2 a_3 \ldots a_k b_1)$, $(a_3 \ldots a_k b_1 b_2)$, ... , $(a_k b_1 \ldots b_{k-1})$ are not in L.

Let $W_k(n)$ denote the greatest number of words that such a dictionary can contain. In [8] the upper bound

$$W_k(n) \leq \frac{1}{k} \sum_{d/k} \mu(d)\, n^{k/d} \tag{1}$$

was obtained, where $\mu(d)$ is the Möbius function, and the summation is extended over all divisors d of k. It was further shown that the upper bound (1) is actually attained by some dictionary for all values of n when $k = 1, 3, 5, 7, 9, 11, 13, 15$, and this is *conjectured* to extend to all *odd* k. On the other hand, if k is *even*, the upper bound (1) is *not* attained by $W_k(n)$ for any $n > 3^{k/2}$. Specifically, for $k = 2$ it was demonstrated that $W_2(n) = \left[\frac{n^2}{3}\right]$, whereas (1) merely asserts that $W_2(n) \leq \frac{n^2 - n}{2}$. The treatment of the case $k = 2$ actually contained the method for finding

all possible comma-free dictionaries containing the maximum number of words, $W_2(n) = \left[\frac{n^2}{3}\right]$.

The primary objective here is to provide a constructive method for finding all maximum comma-free dictionaries for $k = 3$. Here the size of such a dictionary is $W_3(n) = \frac{n^3 - n}{3}$.

Before confining attention to the case $k = 3$, certain observations are appropriate concerning all dictionaries for which the upper bound (1) is attained.

Def. Two k-letter words are in the same *equivalence class* if they differ only by a cyclic permutation of the letters. (Thus *ABC*, *BCA*, *CAB* are all in the same equivalence class.) An equivalence class is *complete* if it contains k distinct members. (The example *ABC*, *BCA*, *CAB* is complete. The equivalence class of *AAA* is *degenerate*, containing only one member.)

Degenerate equivalence classes are never represented, and complete equivalence classes are represented at most once, in any comma-free dictionary, as shown in [7] and [8].

Theorem 0. The upper bound (1) is attained by a comma-free dictionary if and only if every complete equivalence class has *exactly* one representative in the dictionary.

Theorem 1. In any comma-free dictionary for which $W_k(n)$, the size of the dictionary, attains the upper bound (1), all n letters of the alphabet occur equally often among the $kW_k(n)$ letters in the dictionary. Also, each letter occurs in the same number of different words.

Proof. The dictionary contains one representative from each complete equivalence class. *Which* representative is inconsequential insofar as the letters comprising the individual words and the entire dictionary are concerned. Since any permutation of the alphabet leaves the class decomposition of the k-letter words invariant, permutation of the alphabet does not affect the distribution of letters in the dictionary, nor the frequency with which letters appear in distinct words. Therefore, these distributions and frequencies must be the same for all n letters of the alphabet.

Corollary. If all words containing a given letter are dropped from a maximum comma-free dictionary L attaining the upper bound (1) for $W_k(n)$, the remaining words form a maximal

comma-free dictionary L' for the $(n-1)$-letter alphabet. The number of different words of L in which a given letter appears is thus $W_k(n) - W_k(n-1)$.

Proof. The deletion of certain words from a comma-free dictionary cannot destroy the comma-free property. Thus expurgation of all words of L containing the letter X leaves a comma-free dictionary L' involving only an $(n-1)$-letter alphabet. Moreover, every complete equivalence class for these $n-1$ letters had a unique representative in L, which has not been dropped during the expurgation process. Thus L' is maximal by Theorem 0.

2. Classes of Dictionaries for $k = 3$.

The fundamental result concerning the structure of maximal comma-free dictionaries for $k = 3$ is expressed in the following theorem. Unless otherwise specified it will henceforth be assumed that $k = 3$.

Def. The first two letters of a word are an *initial digram*; the last two letters are a *final digram*.

Theorem 2. For $n > 2$, the necessary and sufficient condition that a collection of $(n^3 - n)/3$ words constitute a maximal comma-free dictionary is that no initial digram ever occurs as a final digram.

Proof. The condition is *sufficient* for maximal comma-freedom, since $(n^3 - n)/3$ is the correct dictionary size, and violation of comma-freedom from $(ABC)(DEF)$ could only occur if either BCD or CDE were in the dictionary along with ABC and DEF. But BCD begins with the final digram of ABC, and CDE ends with the initial digram of DEF.

To show *necessity*, suppose that ABC and BCD were both in the maximum comma-free dictionary L. To prevent conflicts, A must never end a word, nor D begin a word, and in particular, $A \neq D$. Therefore, the equivalence class $\{ AAX, AXA, XAA \}$ must be represented by AAX, and the class $\{ YDD, DYD, DDY \}$ must be represented by YDD, for all $X \neq A$ and $Y \neq D$. For any Z distinct from both A and D, consider the class $K = \{ ADZ, DZA, ZAD \}$. (Here the hypothesis $n > 2$ is required.) Observe that ADZ is contained in the overlap of $(AAX)(YDD)$ when $X = D$, $Y = Z$; while ZAD is in the overlap of $(AAX)(YDD)$ when $X = Z$,

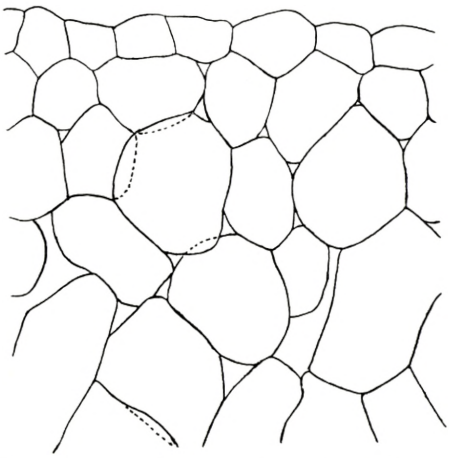

Abb. 4. Horizontale Lage. IES-Paste an der Oberseite. Oberseite.
Vers. 8 in Tab. 3. Zeichenapp. 215/1

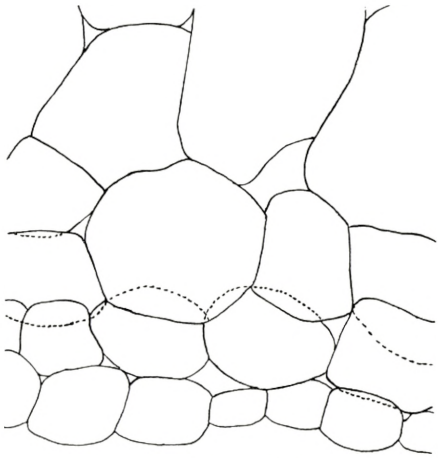

Abb. 5. Auslöschung der Verdickungen. Umkehrungsversuche. Unterseite.
Vers. 5 in Tab. 4. Zeichenapp. 215/1

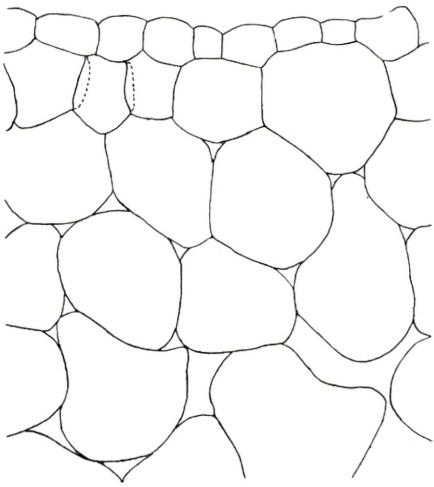

Abb. 6. Auslöschung der Verdickungen. IES-Paste an der Oberseite. Oberseite.
Vers. 4 in Tab. 5 Zeichenapp. 215/1

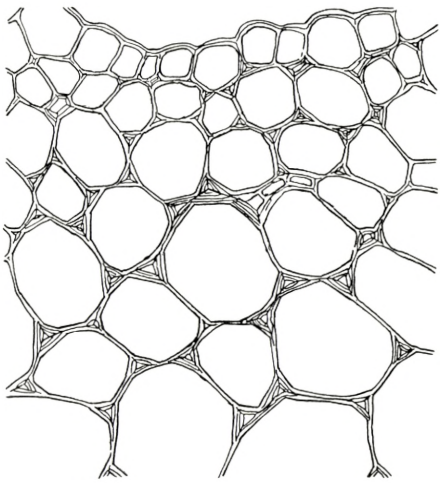

Abb. 7. Dekapitierung.
Vers. 11 in Tab. 6. Zeichenapp. 215/1

Tabelle 3. Horizontallage mit IES-Paste an der Oberseite, 24 Stunden.

1, 2. 4, 5) Spitze nach unten, 3) Spitze nach oben, 6, 7, 8, 9) Spitze nach der Seite. 1) 1 g Lanolin + 20 mg IES, 2, 3, 4, 5, 6, 9) 1 g Lanolin + 5 mg IES, 7, 8) 1 g Lanolin + 2 mg IES.

Nr.	Epikotyl länge, cm	Zuwachs der Zonen in 24 Stunden, cm								Gesamtzuwachs, cm	Diameter vertik., mm	Rindendicke, mm		Zellwanddicke, μ	
		I	II	III	IV	V	VI	VII	VIII			Ob.	Unt.	Ob.	Unt.
1	3,8	0	0,3	0,3	0,2	0,2	0,1	0	..	1,1	4,8	0,64	0,41	0,77	0,96
2	4,5	0,2	0,2	0,2	0,2	0,1	0,1	0,1	0	1,1	5,3	0,75	0,47	1,05	1,65
3	4,9	0,2	0,6	0,3	0,2	0,2	0,1	0,1	0,1	1,8	4,1	0,40	0,46	0,75	1,65
4	4,0	0,3	0,5	0,5	0,3	0,2	0,1	0,1	..	2,0	4,9	0,50	0,45	0,95	1,11
5	6,4	0,3	0,4	0,5	0,3	0,1	0,1	0,1	0	1,8	5,0	0,55	0,47	0,87	0,68
6	6,9	0,1	0,4	0,5	0,5	0,4	0,4	0,3	0,3	2,9	4,9	0,47	0,52	0,60	1,22
7	4,8	0,1	0,2	0,4	0,2	0,1	0	0	0	1,0	4,4	0,61	0,54	0,50	0,50
8	4,5	0,2	0,2	0,4	0,3	0,2	0,1	0,1	0	1,5	4,4	0,62	0,51	0,51	0,53
9	7,0	0	0,5	0,7	0,3	0,3	0,2	0,2	0,2	2,4	5,4	0,51	0,50	0,69	1,29
Durchschnitt		0,2	0,4	0,4	0,3	0,2	0,1	0,1	0,1	1,7	4,8	0,56	0,48	0,74 ± 0,06	1,07

zu gross ist. Dagegen ist es sicher — und das ist das Entscheidende — dass auf der Oberseite keine Verdickungen vorhanden sind. Die durchschnittliche Zellwanddicke auf dieser Seite beträgt 0,74 μ (Abb. 4). Es geht daraus hervor, dass man durch Zufuhr von Wuchsstoff an die Oberseite von Epikotylen in horizontaler Lage die Entstehung der Verdickungen, die sonst normalerweise gebildet werden, verhindern kann, und man wird daher schliessen können, dass die oben genannte Auffassung, dass diese Verdickungen durch Verminderung des Wuchsstoffgehaltes hervorgerufen werden, richtig ist.

c. Auslöschung der durch horizontale Zwangslage erzeugten Verdickungen an der Oberseite der Epikotyle.

1. *Umkehrungsversuche* (Tab. 4). Epikotyle wurden 24 Stunden in horizontaler Zwangslage in Glasröhren gelegt. In den zahlreichen Versuchen, die in Tab. 2 wiedergegeben sind, sind die Zellen an der Oberseite dann ausnahmslos verdickt. Man muss

Tabelle 4. Horizontale Zwangslage 24 Stunden. Umkehrung. Horizontale Zwangslage 24 Stunden.

1—3) Spitze nach unten (oben), 4—9) Spitze nach der Seite.

Nr.	Epikotyllänge, cm	Tage	Zuwachs der Zonen in 24 Stunden, cm								Gesamtzuwachs, cm	Diameter vertik. mm	Rindendicke, mm		Zellwanddicke, μ	
			I	II	III	IV	V	VI	VII	VIII			Ob.	Unt.	Ob.	Unt.
1	4,4	1.T	0,2	0,2	0,1	0,1	0	0	0	0	0,6					
		2.T	0,2	0,2	0,1	0,1	0	0	0	0	0,6	5,2	0,57	0,50	0,99	0,69
2	6,4	1.T	0,2	0,2	0,2	0,1	0	0	0	0	0,7					
		2.T	0,7	0,3	0,1	0,1	0	0	0	0	1,2	6,2	0,48	0,55	1,75	0,75
3	4,4	1.T	0,1	0,2	0,2	0,1	0,1	0	0	0	0,7					
		2.T	0,6	0,5	0,1	0,1	0	0	0	0	1,3	5,3	0,55	0,60	0,75	0,77
4	6,4	1.T	0,3	0,4	0,3	0	0	0	0	0	1,0					
		2.T	0,7	0,5	0,2	0,2	0,2	0,2	0	0	2,0	5,8	0,69	0,66	1,95	0,96
5	6,6	1.T	0,3	0,5	0,2	0,1	0,1	0	0	0	1,2					
		2.T	0,6	0,1	0,1	0,1	0,1	0,1	0	0	1,1	5,8	0,62	0,80	0,84	0,66
6	4,4	1.T	0,4	0,1	0,1	0	0	0	0	0	0,6					
		2.T	0,6	0,4	0,1	0,1	0	0,1	0	0	1,3	5,0	0,62	0,42	0,72	0,53
7	4,3	1.T	0,3	0,2	0	0	0	0	0	0	0,5					
		2.T	0,6	0,4	0,3	0,1	0,1	0	0	0	1,5	4,3	0,51	0,55	0,66	0,59
8	3,7	1.T	0,3	0,3	0,1	0,1	0,1	0	0,1	..	1,0					
		2.T	1,0	0,4	0	0	0	0	0	..	1,4	4,7	0,52	0,52	0,72	0,74
9	3,8	1.T	0,1	0,4	0,3	0,1	0,1	0,1	0	..	1,1					
		2.T	1,0	0,8	0,1	0,1	0	0	0	..	2,0	5,7	0,50	0,53	0,72	0,87
Durchschnitt			0,2	0,3	0,2	0,1	0	0	0	0	0,8					
			0,7	0,4	0,1	0,1	0	0	0	0	1,4	5,3	0,56	0,57	1,01	0,73 ± 0,04

daher folgern, dass auch in dieser Versuchsreihe die Zellwände der Oberseite nach 24 Stunden stark verdickt waren, obwohl man nicht imstande ist, dieses direkt zu beobachten. Die Epikotyle wurden nun umgekehrt. In den folgenden 24 Stunden lagen sie in horizontaler Zwangslage mit der früheren Oberseite nach unten. Man muss annehmen, dass diese Seite nun die grösste Wuchsstoffmenge erhält.

Der Zuwachs am zweiten Tage war grösser als am ersten Tage, nämlich 1,4 cm; er bleibt somit weit hinter dem normalen zurück. Bei der Beurteilung dieses Ergebnisses muss man jedoch erinnern, dass die Zuwachsmöglichkeiten der oberen Zonen in den ersten 24 Stunden teilweise erschöpft sind.

An der Unterseite (der früheren Oberseite) waren die Verdickungen, die während des ersten Tages gebildet worden sein müssen, verschwunden. Die durchschnittliche Zellwanddicke war 0,73 μ, ein wenig kleiner als diejenige der Kontrollpflanzen (Abb. 5). Gleichzeitig war die Rindendicke bis auf 0,57 mm gewachsen. Es hat somit auf dieser Seite ein starkes Transversalwachstum stattgefunden.

An der Oberseite (der früheren Unterseite) waren, wie es zu erwarten war, bisweilen schwache Verdickungen entstanden. Die durchschnittliche Zellwanddicke war jedoch nur 1,01 μ.

2. *Wuchsstoffzufuhr* (Tab. 5). Epikotyle, die 24 Stunden in horizontaler Zwangslage verbracht hatten, wurden aus den Glasröhren herausgenommen. Es entstand bald eine starke negative Krümmung, wobei die Oberseite (deren Zellwände verdickt sein müssen) stark konkav wurde, so dass der obere Teil der Epikotyle ungefähr senkrecht wurde. Es wurde nun diese Seite mit einer Wuchsstoffpaste (1 g Lanolin + 20 oder 5 mg IES) belegt. Nach 24 Stunden wurden Schnitte aus der dritten Zone hergestellt.

Es geht aus Tabelle 5 hervor, das der Zuwachs 1,3 cm betrug. Wenn sie nicht grösser ist, ist die Ursache, einmal, dass die Wachstumsmöglichkeit der oberen Zonen in den ersten 24 Stunden, wie oben bemerkt, teilweise erschöpft ist, und ferner, dass neben dem longitudinalen auch ein starkes transversales Wachstum stattfindet. Die Rindendicke an der Oberseite war von 0,36 mm (Tab. 2) auf 0,62 mm gewachsen. An der Unterseite war sie nur wenig verändert.

Die Zellwanddicke auf der Oberseite war von 2,71 μ (Tab. 2) auf 0,72 μ gesunken (Abb. 6). Es sind somit die Zellwandverdickungen an der Oberseite durch die Wuchsstoffbehandlung verschwunden. An der Unterseite ist die Zellwanddicke ungefähr unverändert.

Man wird aus den in Tab. 4 und 5 dargestellten Versuchen schliessen müssen, dass die durch horizontale Zwangslage erzeugten Verdickungen an der Oberseite der Epikotyle zum Verschwinden gebracht werden können entweder als Folge der durch eine Umkehrung hervorgerufenen Steigerung des Wuchsstoffgehaltes oder durch Zufuhr von Wuchsstoff.

TABELLE 5. Horizontale Zwangslage 24 Stunden; IES-Paste an der Oberseite 24 Stunden.

1) Spitze nach unten, 3—10) Spitze nach der Seite. 1) 1 g Lanolin + 20 mg IES, 2—10) 1 g Lanolin + 5 mg IES.

Nr.	Epikotyllänge, cm	Tage	Zuwachs der Zonen in 24 Stunden, cm								Gesamtzuwachs, cm	Diameter vertik. mm	Rindendicke, mm		Zellwanddicke, μ	
			I	II	III	IV	V	VI	VII	VIII			Ob.	Unt.	Ob.	Unt.
1	3,2	1.T	0,3	0,3	0,2	0,2	0,2	0	..	..	1,2					
		2.T	0,7	0,4	0,1	0	0	0	..	..	1,2	6,5	0,70	0,51	0,77	1,04
2	7,4	1.T	0,3	0,4	0,3	0,2	0,1	0,1	0,1	0	1,5					
		2.T	0,4	0,8	0,2	0,2	0,1	0	0	0	1,7	6,7	0,58	0,70	1,34	1,50
3	4,7	1.T	0,3	0,2	0,2	0,1	0,1	0,1	0,1	0	1,1					
		2.T	0,1	0,1	0,1	0	0	0	0	0	0,3	5,2	0,55	0,60	0,60	0,90
4	2,4	1.T	0,6	0,5	0,4	0,3	..	..	..	..	1,8					
		2.T	0,3	0,4	0,1	0	..	..	..	..	0,8	5,0	0,72	0,67	0,74	0,75
5	8,3	1.T	0,4	0,5	0,3	0,2	0,1	0,1	0,1	0,1	1,8					
		2.T	0,3	0,1	0,2	0,2	0,2	0	0	0	1,0	6,3	0,72	0,74	0,65	0,69
6	3,8	1.T	0,3	0,3	0,2	0,1	0,1	0	0	..	1,0					
		2.T	0,7	0,6	0,3	0,1	0	0	0	..	1,7	4,4	0,55	0,52	0,53	0,54
7	4,7	1.T	0,2	0,1	0,1	0,1	0,1	0,1	0	0	0,7					
		2.T	0,2	0,4	0,1	0,1	0,1	0	0	0	0,9	4,5	0,55	0,52	0,51	0,74
8	3,7	1.T	0,2	0,3	0,2	0,1	0,1	0	0	..	0,9					
		2.T	0,2	0,6	0,2	0,1	0,1	0	0	..	1,2	4,7	0,75	0,60	0,80	0,72
9	7,2	1.T	0,2	0,2	0,2	0,2	0,2	0,1	0	0	1,1					
		2.T	0,6	0,7	0,4	0,3	0,2	0,1	0	0	2,3	4,8	0,56	0,55	0,57	0,66
10	3,7	1.T	0,3	0,2	0,2	0,1	0	0	..	..	0,8					
		2.T	1,1	0,7	0,3	0,2	0	0	..	..	2,3	4,6	0,50	0,55	0,66	0,80
Durchschnitt			0,3	0,3	0,2	0,2	0,1	0,1	0	0	1,2					
			0,5	0,5	0,2	0,1	0,1	0	0	0	1,3	5,3	0,62	0,60	0,72 ± 0,08	0,83

Was aus den Verdickungen wird, war nicht möglich zu ermitteln. Sie können entweder aufgelöst werden, oder es kann bei dem Flächenwachstum eine plastische Dehnung der Zellwände eingetreten sein, wobei dieselben dünner wurden. Die letztere Möglichkeit ist wohl die wahrscheinlichste.

Tabelle 6. Dekapitierung.

1—11) 24 Stunden, 12) 72 Stunden.

Nr.	Epikotyllänge, cm	Zuwachs der Zonen in 24 Stunden, cm						Gesamtzuwachs, cm	Diameter mm		Rindendicke, mm		Zellwanddicke, μ
		III	IV	V	VI	VII	VIII		gr.	kl.	gr.	kl.	
1	5,2	0	0	0,1	0	0	0,1	0,1	3,6	2,8	0,35	0,27	1,26
2	7,5	0	0	0,1	0	0	0	0,1	4,2	3,1	0,35	0,37	1,14
3	8,0	0	0	0	0	0	0	0	4,4	3,1	0,37	0,28	1,56
4	8,2	0	0	0	0	0	0	0	4,3	3,3	0,30	0,33	1,90
5	5,8	0	0,1	0	0	0	0	0,1	4,4	3,2	0,43	0,38	2,20
6	7,4	0	0,1	0	0,1	0	0	0,2	3,6	3,1	0,37	0,31	1,20
7	4,3	0	0,1	0	0,1	0	0	0,2	5,2	3,2	0,32	0,35	1,80
8	6,5	0	0,1	0,1	0	0	0	0,2	4,4	3,1	0,38	0,36	1,53
9	3,4	0	0,1	0,1	0,1	..	..	0,3	4,7	3,1	0,34	0,37	1,53
10	4,5	0	0	0	0	0	0	0	5,4	3,3	0,37	0,43	1,80
11	4,9	0	0	0	0,1	0	0	0,1	4,1	3,1	0,41	0,34	3,15
Durchschnitt		0	0	0	0	0	0	0,1	4,4	3,1	0,36	0,34	1,73 ± 0,17
12	5,0	72 Stunden						0	4,3	3,3	0,40	0,36	1,44

5. Versuche mit dekapitierten Epikotylen.

Wenn man Agarwürfelchen mit wechselndem Wuchsstoffgehalt einseitig auf dekapitierte *Avena*koleoptilen aufsetzt, erhält man bei der Messung der entstandenen Krümmungen eine Kurve, die die Wirkung der Wuchsstoffkonzentration auf die Wachstumsgeschwindigkeit der Koleoptile wiedergibt. Man würde nun vielleicht erwarten können, dass es möglich sein sollte, eine ähnliche Kurve für die Wirkung des Wuchsstoffes auf die Wachstumsgeschwindigkeit und die Zellwanddicke des *Phaseolus*epikotyls zu bestimmen. Dieses ist zwar nicht möglich; aber man kann doch die Wirkungsweise des Wuchsstoffes untersuchen.

a. Hervorrufung von Verdickungen (Tab. 6).

Die Dekapitierungen wurden in folgender Weise ausgeführt. Es wurden wie oben vom Knie herab 8 Zonen, je 0,5 cm, abgesetzt. Von diesen wurde die zweite wieder in zwei Teile geteilt. Die Epikotyle wurden dann dreimal mit Zwischenräumen von

$1^1/_2$ Stunde bzw. 0,5, 0,75 und 1 cm unter dem Knie dekapitiert. Bei jeder Dekapitierung wurde die Schnittfläche zweimal mit Nährlösung ($^1/_2$ Ib + II) abgewaschen und nachher mit einem feuchten Wattebausch bedeckt. Nach der dritten Dekapitierung wurde auf die Schnittfläche reines Lanolin gelegt.

Während der Dekapitierungen standen die Pflanzen unter einer Metallstülpe, so dass die Luft mit Wasserdampf gesättigt sein dürfte, während der Versuche standen sie unter einer Pappstülpe.

Wie zu erwarten war, sank der Zuwachs in den dekapitierten Pflanzen sehr stark herab, bis 0,1 cm. Die Rindendicke war ungefähr dieselbe wie in den Kontrollpflanzen. Die durchschnittliche Zellwanddicke in der Rinde betrug 1,73 μ, es ist somit eine nicht starke, aber doch deutliche Verdickung der Zellwände entstanden (Abb. 7).

b. Verhinderung der Entstehung der Zellwandverdickungen in dekapitierten Epikotylen (Tab. 7).

Nach der dritten Dekapitierung wurde eine Wuchsstoffpaste (1 g Lanolin + 20 oder 6 mg IES) teils auf die Schnittfläche und teils als zwei Streifen auf die oberen 4 cm der flachen Seiten der Epikotyle gelegt.

Der Zuwachs betrug 1,0 cm. Obwohl somit eine starke Vergrösserung desselben als Folge der Wuchsstoffzufuhr entstanden ist, steht er doch hinter demjenigen der Kontrollpflanzen sehr zurück. Man muss bei der Beurteilung dieses Ergebnisses jedoch berücksichtigen, dass die beiden ersten Zonen, die normalerweise stark wachsen, bei der Dekapitierung entfernt worden sind, und ferner, dass, wie unten bemerkt, auch ein Transversalwachstum stattfindet. Drittens kommt noch ein Faktor hinzu, der unten besprochen werden soll.

Die Rindendicke ist auf den flachen Seiten 0,54 mm, sie ist somit viel grösser als in der entsprechenden Versuchsreihe ohne IES. Man muss hieraus schliessen, dass die Wuchsstoffzufuhr sowohl ein Longitudinal- als ein Transversalwachstum hervorruft.

Die Zellwanddicke betrug auf den flachen Seiten 0,88 μ. Auch bei den dekapitierten Pflanzen ist man somit imstande, die Entstehung der Verdickungen der Zellwände, die durch die Dekapitierung normalerweise erzeugt werden, durch Wuchsstoffzufuhr zu verhindern.

TABELLE 7. Dekapitierung, IES-Paste.

1) 1 g Lanolin + 6 mg IES, 2—9) 1 g Lanolin + 20 mg IES.

Nr.	Epikotyllänge, cm	Zuwachs der Zonen in 24 Stunden, cm						Gesamtzuwachs, cm	Grösste Achse			Kleinste Achse mit IES-Paste		
		III	IV	V	VI	VII	VIII		Diameter, mm	Rindendicke, mm	Zellwanddicke, μ	Diameter, cm	Rindendicke, mm	Zellwanddicke, μ
1	6,2	0,1	0,3	0,2	0,2	0,3	0,1	1,2	4,2	0,51	0,99	3,2	0,38	1,04
2	8,7	0,1	0,4	0,3	0,2	0,2	0,1	1,3	4,9	0,58	1,18	3,8	0,55	0,96
3	6,3	0,1	0,2	0,2	0,1	0,1	0	0,7	4,1	0,43	1,13	3,0	0,50	1,07
4	9,6	0,2	0,5	0,4	0,3	0,2	0,1	1,7	4,2	0,58	0,81	4,1	0,48	0,87
5	5,4	0	0,3	0,3	0,2	0,1	0,1	1,0	5,7	0,54	0,69	3,9	0,55	0,80
6	4,4	0,2	0,3	0,2	0,1	0,1	0	0,9	4,2	0,70	0,67	4,0	0,58	0,90
7	5,0	0,1	0,2	0,2	0,1	0	0	0,6	5,7	0,70	0,83	4,0	0,73	0,95
8	6,3	0,1	0,2	0,2	0,2	0,1	0,1	0,9	4,9	0,46	1,85	4,2	0,54	0,62
9	6,3	0	0,3	0,2	0,2	0,2	0,1	1,0	4,5	0,51	1,28	4,0	0,52	0,72
Durchschnitt		0,1	0,3	0,2	0,2	0,1	0,1	1,0	4,7	0,56	1,05	3,8	0,54	0,88 ± 0,05

c. Auslöschung der durch Dekapitierung erzeugten Verdickungen (Tab. 8).

Die Epikotyle wurden in der unter a beschriebenen Weise dekapitiert und 24 Stunden unter einer Pappstülpe gelassen. Es wurde dann die Lanolinpaste an der Schnittfläche entfernt, und es wurde eine Wuchsstoffpaste (1 g Lanolin + 20 oder 5 mg IES) an die Schnittfläche und als zwei Streifen an die flachen Seiten der Epikotyle gelegt.

Die Epikotyle, die in den ersten 24 Stunden nur 0,1 cm gewachsen waren, fingen als Folge der Wuchsstoffzufuhr wieder zu wachsen an. Der Zuwachs betrug 0,8 cm.

Daneben trat auch eine Vergrösserung der Rindendicke ein, namentlich an den flachen Seiten, wo die Wuchsstoffpaste lag. Die Dicke der Rinde betrug an diesen Seiten 0,47 mm. Es hat somit auch in diesem Falle sowohl ein Longitudinal- als ein Transversalwachstum stattgefunden.

Dass Verdickungen in den ersten 24 Stunden entstanden waren, geht daraus hervor, dass an den Flanken, wo IES-Paste nicht gelegt wurde, bei dem Abschluss der Versuche beträchtliche

TABELLE 8. Dekapitierung 24 Stunden; 24 Stunden mit IES-Paste.

1—2) 1 g Lanolin + 5 mg IES, 3—8) 1 g Lanolin + 20 mg IES.

Nr.	Epikotyllänge, cm	Tage	Zuwachs der Zonen in 24 Stunden, cm						Gesamtzuwachs, cm	Grösste Achse			Kleinste Achse mit IES-Paste		
			III	IV	V	VI	VII	VIII		Diameter, cm	Rindendicke mm	Zellwanddicke, μ	Diameter, cm	Rindendicke mm	Zellwanddicke, μ
1.....	6,8	1	0	0	0,1	0	0,1	0	0,2						
		2	0,2	0,2	0,2	0,2	0,2	0,1	1,1	4,1	0,39	3,00	4,8	0,62	0,83
2.....	4,9	1	0	0	0	0,1	0	0	0,1						
		2	0	0,1	0	0	0	0	0,1	4,4	0,47	2,03	3,3	0,51	0,93
3.....	8,0	1	0	0	0	0,1	0,1	0	0,2						
		2	0,1	0,3	0,2	0	0	0	0,6	4,5	0,33	1,41	3,2	0,33	0,77
4.....	4,8	1	0	0	0	0,1	0	0	0,1						
		2	0,4	0,4	0,3	0,2	0	0	1,3	4,4	0,48	1,59	3,5	0,42	0,84
5.....	7,8	1	0,1	0	0	0	0	0	0,1						
		2	0,2	0,3	0,2	0,1	0,1	0	0,9	4,0	0,52	1,82	3,3	0,42	0,77
6.....	4,3	1	0	0	0	0	0	0	0						
		2	0,5	0,3	0,2	0	0,1	0	1,1	5,0	0,41	1,71	3,6	0,43	0,80
7.....	5,4	1	0	0	0	0	0	0	0						
		2	0,1	0,2	0,2	0,1	0,1	0,1	0,8	4,9	0,38	1,25	4,0	0,48	0,65
8.....	6,0	1	0	0	0,1	0	0	0	0,1						
		2	0	0,2	0,1	0	0,1	0	0,4	4,9	0,43	1,17	3,4	0,53	0,74
Durchschnitt			0	0	0	0	0	0	0,1						
			0,2	0,3	0,2	0,1	0,1	0	0,8	4,5	0,43	1,75	3,6	0,47	0,79 ±0,03

Verdickungen noch vorhanden waren. Die durchschnittliche Zellwanddicke betrug hier 1,75 μ. An den flachen Seiten dagegen, unter der IES-Paste, war die Zellwanddicke nur 0,79 μ. Der Wuchsstoff ist somit imstande, die durch Dekapitierung erzeugten Verdickungen zum Verschwinden zu bringen.

Die Ergebnisse der Dekapitierungsversuche stimmen im grossen und ganzen überein mit denjenigen, die bei horizontaler Zwangslage erhalten wurden. Es geht aber auch aus den Versuchen hervor, dass die Verdickungen der Zellwände, die man in dekapitierten Pflanzen erreichen kann, weit hinter den Verdickungen an der Oberseite der Epikotyle in horizontaler Zwangslage zurückbleiben, und dass der Zuwachs bei Wuchsstoffzufuhr

Tabelle 9. Spaltungsversuche.

Nr.	Epikotyllänge, cm	Zuwachs der Zonen in 24 Stunden, cm								Gesamtzuwachs, cm	Rindendicke, mm	Zellwanddicke, μ
		I	II	III	IV	V	VI	VII	VIII			
		ohne IES-Paste										
1	4,0	(0,4)	0,2	0,1	0	0	0	0	..	0,3	0,42	0,98
2	4,3	0,2	0,1	0,1	0,1	0,1	0	0	0	0,6	0,33	1,50
3	5,5	0,1	0	0	0	0	0	0	0	0,1	0,46	1,57
4	6,3	(0,7)	0,5	0,2	0,1	0	0	0	0	0,8	0,42	1,52
Durchschnitt			0,2	0,1	0,1	0	0	0	0	0,5	0,41	1,39
		mit IES-Paste										
5	8,1	0,1	0,2	0,1	0,1	0	0	0	0	0,5		
6	6,4	0,1	0	0,1	0	0	0	0	0	0,2	0,49	1,11
7	5,4	0	0,1	0,1	0,1	0,1	0	0	0	0,4	0,60	0,95
8	4,8	0,1	0,3	0,2	0,1	0,1	0,1	0	0	0,9	0,71	0,93
Durchschnitt		0,1	0,2	0,1	0,1	0,1	0	0	0	0,5	0,60	1,00

zu dekapitierten Epikotylen nur klein ist im Vergleich mit demjenigen der normalen Epikotyle. Die Ursachen dieser verminderten Aktivität wurden schon oben (S. 23) besprochen. Eine bedeutende Rolle spielt dabei die Wunde, die bei der Dekapitierung entsteht, indem von derselben, wie ich 1933 nachweisen konnte, eine Wirkung ausgeht, die die Wachstumsgeschwindigkeit hemmt. Versuche über diese hemmende Wirkung sind in Tab. 9 wiedergegeben.

Es wurde durch die oberen 4 cm der Epikotyle auf der flachen Seite mit einem Augenmesser eine Längsspalte angebracht, so dass sie in zwei oben und unten zusammenhängende Teile getrennt wurden. Auf der flachen Seite wurden wie gewöhnlich 8 Zonen, je 0,5 mm, mit Tuschmarken abgesetzt. In der letzteren Hälfte der Versuche (5—8) wurde ausserdem an den Flanken IES-Paste (1 g Lanolin + 20 mg IES) gelegt. Die Versuchspflanzen standen unter einer Metallstülpe in dampfgesättigter Luft. Aus den Versuchen 1—4 geht hervor, dass der Zuwachs bis auf 0,5 cm vermindert wird. Da die oberen Teile

der Keimpflanzen und der Stofftransport im Epikotyl durch die Längsspalte nicht beeinflusst werden, muss die Verkleinerung der Wachstumsgeschwindigkeit durch eine traumatische Wirkung, die von den Schnittflächen ausgeht, verursacht sein. Durch diese Wirkung werden sowohl der Wuchsstoff als die Zellulosenbildner teilweise inaktiviert. Dass der Wuchsstoffgehalt vermindert worden ist, geht daraus hervor, dass in den Versuchen 1—4 stellenweise ziemlich starke Verdickungen vorhanden sein können. Die Entstehung dieser Verdickungen kann man zwar, wie es aus den Versuchen 5—8 hervorgeht, durch Wuchsstoffzufuhr verhindern, eine Erhöhung des Zuwachses kann man aber nicht erreichen, und man muss daher schliessen, dass auch die Zellulosenbildner zum grossen Teil inaktiviert oder zerstört worden sind.

6. Schlussfolgerungen.

Die Ergebnisse dieser Untersuchungen sind in Tab. 10 zusammengefasst. Bei der Beurteilung derselben sollen vorzugsweise die Versuche mit horizontaler Lage berücksichtigt werden. Die Dekapitierungsversuche haben zwar zu demselben Ergebnis geführt; wegen der komplizierenden Wirkung der Wunde sind sie aber weniger durchsichtig als die erstere Versuchsreihe.

Die Ergebnisse der Versuche mit horizontaler Lage können in folgender Weise zusammengefasst werden:

Wenn die Wuchsstoffkonzentration auf der Oberseite von Epikotylen durch horizontale Zwangslage vermindert wird, tritt eine Verdickung der Zellwände ein. Da die Menge der plastischen Stoffe, die zum Aufbau der Zellwände vorhanden ist, kaum verändert wird, muss gleichzeitig mit der Vergrösserung des Dickungswachstums das Längenwachstum vermindert werden.

Dass die Verdickungen der Zellwände wirklich durch eine Verminderung der Wuchsstoffkonzentration hervorgerufen werden, kann dadurch bewiesen werden, dass sie ausbleiben, wenn Wuchsstoff zugeführt wird.

Die Entstehung der Verdickungen in den Zellwänden ist reversibel. Eine entstandene Verdickung kann durch Erhöhung der Wuchsstoffkonzentration, entweder durch Umkehrung oder durch Wuchsstoffzufuhr, zum Verschwinden gebracht werden.

Man wird hieraus schliessen können, dass die wachstums-

TABELLE 10. Zusammenfassung der Ergebnisse.
(W = Wuchsstoffgehalt).

	Zuwachs cm	Rindendicke cm		Zellwanddicke μ	
		Obers.	Unters.	Obers.	Unters.
Kontrollpflanzen	3,4	0,39		1,00	
Normaler W.					
Horizontale Zwangslage	1,2	0,36	0,57	2,71	0,86
Obers. kleiner W.					
Unters. grosser W.					
Horizontale Lage + IES-Paste	1,7	0,56	0,48	0,74	1,07
Obers. grosser W.					
Horizont. Zwangsl. Umkehrung	1,4	0,56	0,57	1,01	0,73
Unters. grosser W.					
Horizont. Zwangsl. IES-Paste	1,3	0,62	0,60	0,72	0,83
Obers. grosser W.					
Dekapitierung	0,1	0,34		1,73	
Kleiner W.					
Dekapitierung. IES-Paste	1,0	0,54		0,88	
Grosser W.					
Dekapitierung. IES-Paste nach 24 St.	0,8	0,47		0,79	
an den flachen Seiten grosser W.					

regulierende Wirkung des Wuchsstoffes in dem *Phaseolus*epikotyl dadurch zustande kommt, dass mit abnehmender Wuchsstoffkonzentration eine Umschaltung von schnellem Längenwachstum mit der Bildung dünner Zellwände zu langsamem Längenwachstum mit der Bildung dickerer Zellwände stattfindet. Bei zunehmender Wuchsstoffkonzentration geschieht die Umschaltung in entgegengesetzter Richtung.

Es muss jedoch bemerkt werden, dass in den Versuchen nur die Fälle untersucht worden sind, in denen man die grössten Unterschiede hinsichtlich Wachstumsgeschwindigkeit und Zellwanddicke erwarten musste. Man darf jedoch annehmen, dass man durch Schiefstellung der Epikotyle in allen möglichen Winkeln stetige Übergänge zwischen diesen extremen Fällen erhalten würde.

Es soll nun untersucht werden, in welcher Weise diese Vorgänge erklärt werden können.

a. Die Bedeutung der Zellulosenbildner für das Wachstum der Zellwände.

1. *Die Plasmapapillen.* Es ist eine alte Frage in der Pflanzenphysiologie, ob die Zellwände lebend sind oder nicht (vgl. KÜSTER 1956). Es kann wohl nun mit Sicherheit gesagt werden, dass in wachsenden Zellwänden Plasmafortsätze als Papillen oder Kämme in die Zellwände hineinragen oder dieselbe durchsetzen. Die Beweise hierfür sind die folgenden:

a. In plasmolysierten Zellen ist das Plasma häufig mit der Zellwand durch feine Plasmafäden verbunden (HECHTsche Fäden). Die Spannung in diesen Fäden kann in plasmolysierten Wurzelhaaren so gross sein, dass die Plasmakuppe in eine Spitze ausgezogen wird. Es ist daher ausgeschlossen, dass die Fäden durch eine Adhäsion an der inneren Zellwandoberfläche festgehalten werden können, vielmehr muss man annehmen, dass sie sich in die Zellwand hinein fortsetzen, entweder als eine einzige Papille oder als eine Gruppe von Papillen, wo sie dann, wie unten erwähnt werden soll, durch elektrostatische Kräfte oder durch Wasserstoffbindungen festgehalten werden.

b. Schon lange hat man gewusst, dass die Protoplasten benachbarter Zellen durch Plasmodesmen, die die Zellwand durchsetzen, verbunden sind. In neuerer Zeit ist es von SCHUMACHER und HALBSGUT (1939), ULLRICH (1936), LAMBERTZ (1954), und SCHUMACHER (1957) nachgewiesen worden, dass auch in den Aussenwänden solche Plasmafortsätze, Ektodesmen, vorhanden sind. Weil solche Ektodesmen nach LAMBERTZ auch in jungen Zellwänden vorkommen können, darf man es für wahrscheinlich halten, dass sie mit den oben erwähnten Papillen identisch sind, und das es diese Plasmagebilde sind, die, wie später erwähnt werden soll, das Intussusceptionswachstum ermöglichen. Merkwürdigerweise konnten solche Aussenwandplasmodesmen nach LAMBERTZ niemals in Wurzeln nachgewiesen werden. Die Ursache könnte sein, dass eben in den Wurzeln die Plasmapapillen sich sehr leicht aus den Zellwänden zurückziehen. STRUGGER (1957) konnte doch Plasmodesmen in Meristemen von Wurzeln elektronenmikroskopisch nachweisen.

c. Die Zurückziehung des Plasmas von der Zellwand, die weiter unten besprochen werden soll, bildet einen dritten Beweis für das Vorhandensein von Plasmapapillen in der Zellwand.

d. Die Bildung der Höcker, die auswendig auf den Zellwänden, z. B. von vielen Haaren, vorhanden sind, kann wohl nur dadurch erklärt werden, dass sie von Plasmapapillen, die die Zellwände durchsetzen, erzeugt werden.

2. *Die Adhäsion zwischen den Plasmapapillen und der Zellwand.* Zwischen den Plasmafortsätzen und der Zellwand muss eine Adhäsion von bedeutender Grösse vorhanden sein. Diese Adhäsion dürfte durch elektrostatische Kräfte oder durch Wasserstoffbindungen bedingt sein. Während die Zellwände neutral oder schwach sauer sind, sind wahrscheinlich in der Plasmaoberfläche Stoffe mit Basencharakter vorhanden. Man darf daher erwarten, dass das Plasma sich aus der Zellwand herauszieht, wenn man in der einen oder anderen Weise diese Bindungen aufheben kann. Das ist in der Tat der Fall. Wie Klebs (1887) und später Zacharias (1891) gezeigt haben, ruft Kongorot, ein Diaminfarbstoff, der sich an die Zellwände bindet, eine solche Wirkung hervor. Auch Methylenblau und andere basische Farbstoffe, ferner Colchicin, Rhodanammomium und Indolylessigsäure können bewirken, dass das Plasma in Wurzelhaaren sich aus der Zellwand herauszieht, so dass Verdickungen an der inneren Seite der Zellwände entstehen (Boysen Jensen 1954, 1955).

In oberirdischen Pflanzenorganen scheint die Adhäsion zwischen Plasma und Zellwand fester zu sein. Kongorot kann jedenfalls nicht eine Zurückziehung des Plasmas hervorrufen, vielleicht, weil es nicht die Cuticula zu durchdringen vermag. Doch haben Gorter (1951) und Bünning, Hunck und Lutz (1956) zeigen können, dass man in Tomatenspitzen mit Trijodbenzoesäure und bei *Sphagnum*blättern mit Colchicin Verdickungen der Zellwände hervorrufen kann. Es wird wohl in diesen Fällen eine Zurückziehung des Plasmas stattgefunden haben.

Nebenbei sei bemerkt, dass die labile Verbindung zwischen Plasma und Zellwand den Nachteil hat, dass die Pflanzenzelle gegen chirurgische Eingriffe sehr empfindlich ist. Es sind daher die Transplantationen, durch welche die tierische Embryologie so grossartige Erfolge erzielt hat, bei Pflanzen mit sehr grossen Schwierigkeiten verknüpft. Andererseits ist aber der Aufbau des Zellwandmusters wohl der einfachste Gestaltungsvorgang, den man überhaupt kennt, und daher von sehr grossem Wert für Gestaltungsuntersuchungen.

3. *Die Zellulosenbildner.* Das Flächenwachstum kommt, wie ursprünglich NÄGELI vermutete, durch Intussusception, d. h. durch Einlagerung neuer Zellwandelemente zwischen den schon vorhandenen zustande. Dass ein Intussusceptionswachstum tatsächlich vorkommen kann, hat FITTING (1900) in seinen Untersuchungen über das Wachstum der *Selaginella*spore zeigen können. Neben der Intussusception muss doch auch ein Turgordruck vorhanden sein.

Die neuen Zellulosefibrillen, die zwischen den schon vorhandenen eingelagert werden, werden von Enzymen, die ich Zellulosenbildner genannt habe (BOYSEN JENSEN 1950), erzeugt. Diese Enzyme müssen daher in der Zellwand ihren Platz haben, und zwar auf den in die Zellwand hineinragenden Plasmapapillen. Dass solche Zellulosenbildner auf den Plasmapapillen tatsächlich vorhanden sind, kann dadurch erwiesen werden, dass sie, wenn das Plasma in Wurzelepidermiszellen oder in Wurzelhaaren sich aus der Zellwand herauszieht, ihre Wirkung auf der Plasmaoberfläche fortsetzen und Zellwandmaterial auf der inneren Seite der Zellwand ablagern (BOYSEN JENSEN 1950, 1954). Weil die Produkte der Emzymwirkung ausserhalb des Plasmas abgelagert werden, darf man vermuten, dass die Enzyme in der Oberfläche des Plasmas, vielleicht in der Hautschicht, lokalisiert sind. Schon früher hat BURSTRÖM (1941) nachgewiesen, dass Rohrzucker an der Oberfläche des Plasmas in Weizenwurzeln hydrolysiert wird.

Übrigens sind die Verhältnisse sehr verwickelt. Weil es verschiedene Zellwandstoffe gibt, muss man annehmen, dass auch verschiedene Arten von Zellulosenbildnern vorhanden sind. Ferner sind wohl die Enzymmoleküle, die die echte Zellulose bilden, nicht gleichmässig verteilt, sondern sie bilden Gruppen, die je einen Zellulosenfibril erzeugen.

Durch Untersuchungen von STACEY (1950) ist es nachgewiesen worden, dass Zellwandbruchstücke von *Acetobacter xylinum* Zellulose synthetisieren können. Dasselbe konnten HESTRIN und SCHWANN (1954) mit gefriergetrockneten Zellen erreichen. Man darf wohl daher schliessen, dass in *Acetobacter xylinum* ein zellulosenbildendes Enzym vorhanden ist.

b. Die Wirkungsweise des Wuchsstoffes.

Nach der Betrachtung der Zellulosenbildner kehren wir nun zu den Untersuchungen über die Wirkungsweise des Wuchsstoffes in dem *Phaseolus*epikotyl zurück und nehmen an, dass auch in diesem Falle Plasmapapillen, die mit Zellulosenbildnern besetzt sind, sich in die Zellwände hineinstrecken.

1. *Die Wirkung des Wuchsstoffes auf die Verteilung der Zellulosenbildner.* Wie oben erwähnt kann man in Wurzelhaaren durch Colchicin und andere Stoffe eine Zurückziehung des Plasmas und ein Dickenwachstum der Zellwände hervorrufen. In ganz anderer Weise entstehen jedoch die Verdickungen der Zellwände an der Oberseite der *Phaseolus*epikotyle, wenn der Wuchsstoffgehalt durch horizontale Zwangslage vermindert wird. Die Adhäsion zwischen Zellwand und Plasma wird in diesem Falle nicht aufgehoben, und die Plasmapapillen bleiben in der Zellwand, was daraus hervorgeht, dass das Flächenwachstum nicht ganz aufhört und bei Wuchsstoffzufuhr wieder vergrössert wird. Die Verdickungen müssen dadurch entstehen, dass das System der Zellulosenbildner sich zentripetal ausdehnt, so dass es sich über die Plasmaoberfläche an der inneren Seite der Zellwand ausbreitet. Gleichzeitig mit der Dehnung in zentripetaler Richtung tritt eine Kontraktion in einer darauf senkrechten Richtung ein, so dass die Zellulosenbildner, die in Zellen mit hoher Wuchstoffkonzentration über die Papillen der Zellwände gleichmässig verteilt sind, sich an bestimmten Stellen ansammeln, während andere Stellen mehr oder weniger leer werden. Das zentripetale Herausgleiten der Zellulosenbildner muss ein Dickenwachstum der Zellwand hervorrufen, die gleichzeitige tangentiale Verschiebung bewirkt, dass die Verdickungen an den Stellen der Zellwand, wo die Zellulosenbildner sich ansammeln, beschränkt bleiben. Bei Zufuhr von Wuchsstoff treten die umgekehrten Vorgänge ein. Das System der Zellulosenbildner kontrahiert sich in zentrifugaler Richtung und breitet sich gleichmässig über die Plasmapapillen der Zellwand aus.

In Abb. 8 ist es versucht worden, diese Vorgänge zu veranschaulichen. *1* ist ein Querschnitt des Maschenwerkes der Zellwand mit den Papillen, die in dieselbe hineinragen. Die Abstände zwischen den Papillen dürfte nach Lambertz, der angibt dass pro μ^2 etwa 20—30 Papillen vorhanden sein können, von

der Grössenordnung von 0,2 μ sein. Es wurde daher mit einer Zellwanddicke von 0,6 μ, einem Papillenabstand von 0,2 μ, und einem Papillendiameter von 0,1 μ gerechnet. Das Wachstum dieser

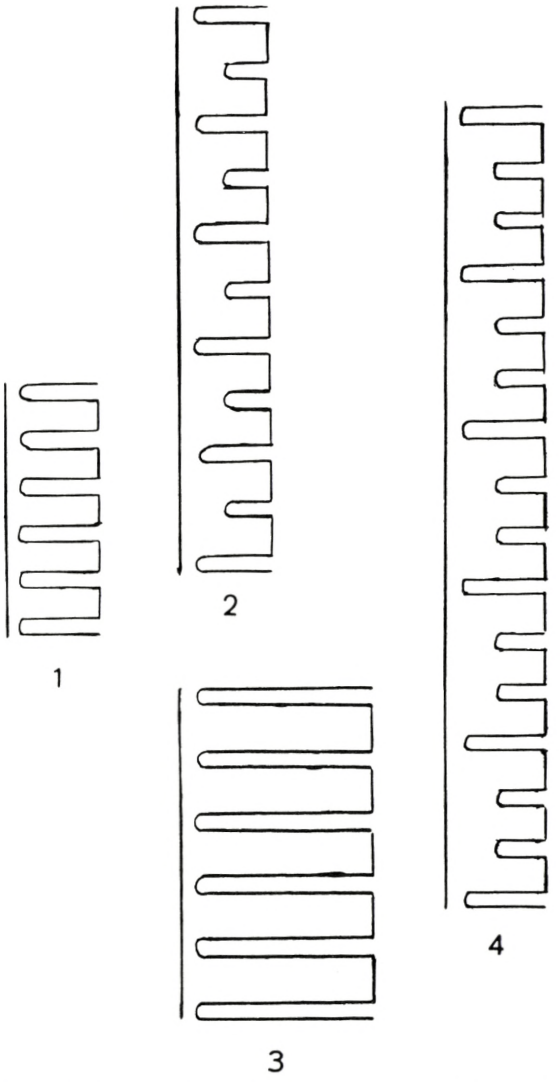

Abb. 8. Schematische Darstellungen des Wachstums der Zellwände bei verschiedener Wuchsstoffkonzentration. *1* Durchschnitt der Zellwand einer Rindenzelle mit Plasmapapillen in einer Kontrollpflanze. *2* Wachstum der Zellwand *1* bei normalem Wuchsstoffgehalt. *3* Wachstum des verdickten Teils derselben Zellwand an der Oberseite einer Pflanze in horizontaler Zwangslage. *4* Wachstum der Zellwand *3* nach Wuchsstoffzufuhr. Wachstum der Zellwände *2*—*4* in 24 Stunden.

Zellwand bei normalem Wuchsstoffgehalt ist in *2* abgebildet. Die Zellulosenbildner an der Oberfläche der Papillen erzeugen Zellulosefibrillen, wodurch die Maschenwände dicker werden und die Zellen daher länger werden. Der Längenzuwachs wurde zu etwa 133 % veranschlagt (vgl. Tab. 1). Gleichzeitig werden wahrscheinlich neue Papillen in die Zellwände hineingeschoben. Das Wachstum der Zellwand 1 bei geringem Wuchsstoffgehalt ist in 3 dargestellt. Ein Teil der Zellulosenbildner ist auf die innere Oberfläche des Maschenwerkes herausgeglitten, und die Zellwände müssen daher sowohl dicker als länger werden. Das Dickenwachstum ist in der Abbildung 100 %, das Längenzuwachstum etwa 33 % (vgl. Tab. 2). Wenn nun der Wuchsstoffgehalt vergrössert wird, wird die Zellwand *3* zu Zellwand *4* umgebildet. Wegen des Turgordruckes wird die Zellwand in *3* plastisch gedehnt, die erhöhten Maschenwände werden ausgeplant und die Dicke der Zellwand wird um 50 % vermindert. Der Längenzuwachs von *3* bis *4* ist zu etwa 150 % veranschlagt worden. Das künftige Wachstum verläuft nun in derselben Weise wie bei *1*.

Wahrscheinlich kommt auch die Kollenchymbildung in Stengeln von Lichtpflanzen in ähnlicher Weise durch Verminderung des Wuchsstoffgehaltes zustande. Warum aber die Blätter bei Lichtzufuhr anfangen zu wachsen, weiss ich nicht.

2. *Die Wuchsstoffmoleküle als Brückenbildner zwischen den Molekülen der Zellulosenbildner.* Es ist somit in diesen Versuchen eine Verknüpfung zwischen dem System der Zellulosenbildner und der Indolylessigsäure nachgewiesen worden. Wenn man sich überlegen will, worin diese Verknüpfung besteht, wird man folgende Tatsachen berücksichtigen müssen. 1. Wuchsstoffänderungen können sowohl Ausdehnung als Kontraktion des Systems der Zellulosenbildner hervorrufen. 2. Der aktive Wuchsstoff kann sehr leicht, z. B. durch Extraktion mit Äther bei Zusatz von Säure vollständig oder durch horizontale Zwangslage teilweise von den Zellulosenbildnern entfernt werden. 3. Für die Entfaltung der Wirkung sind verschwindend kleine Gewichtmengen von Wuchsstoff erforderlich.

Wir nehmen an, dass das System der Zellulosenbildner aus einer sehr grossen Anzahl von Zonen, die je aus einer oder mehreren Schichten von Peptidketten besteht, aufgebaut ist, und wir betrachten nur eine einzelne dieser Zonen.

Damit eine solche Zone sich ausdehnen können soll, müssen die äusserlichen Zonen und vielleicht auch die Zone, die wir betrachten, verschoben werden können. Tatsächlich gleitet das System der Zellulosenbildner, wie es aus zahlreichen Versuchen hervorgeht, leicht über die Oberfläche der Papillen hin.

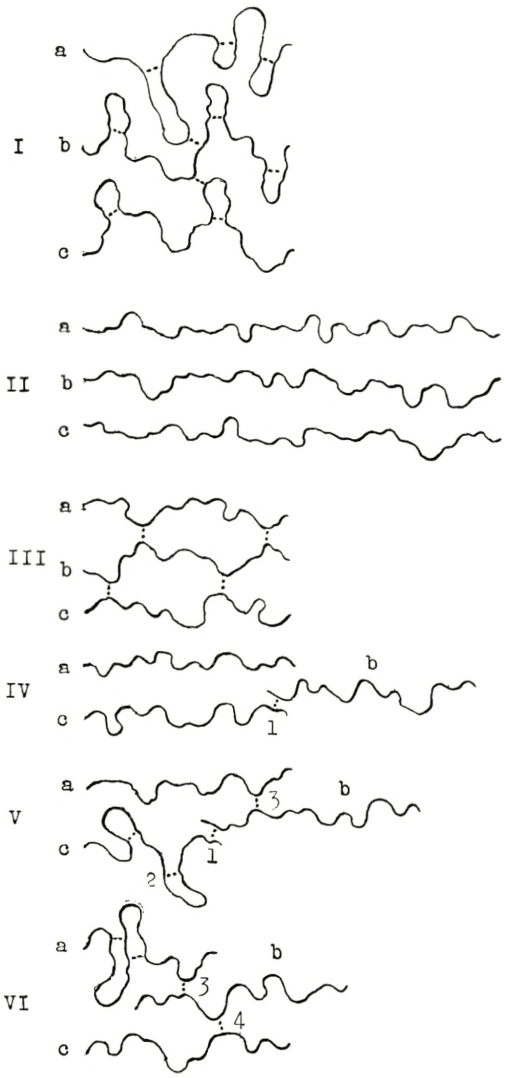

Abb. 9. Schematische Darstellung der Ausdehnung und Kontraktion von 3 Peptidketten. I, II, die Falten der Peptidketten werden aufgelöst, III, IV, V, VI, eine Peptidkette gleitet aus dem Verband heraus und wird wieder eingezogen. I und III entsprechen Abb. $8_{,1}$, II und IV Abb. $8_{,3}$.

Wenn man nun versuchen will zu verstehen, wie es möglich ist, dass eine solche Zone bei Änderung des Wuchsstoffgehaltes sich wechselweise ausdehnen und kontrahieren kann, sind zwei Möglichkeiten vorhanden, die gesondert betrachtet werden sollen.

1. Die erwähnte Zone besteht in kontrahiertem (normalem) Zustande aus gefalteten Peptidketten (Abb. 9, I). Zwischen den Falten sind Bindungen verschiedener Art, hydrophobe Bindungen, Wasserstoffbindungen, vielleicht auch elektrostatische Kräfte, aber keine kovalente Bindungen vorhanden. Wenn der Wuchsstoffgehalt klein ist, reichen diese Bindungen jedoch nicht aus um die Falten zusammenzuhalten. Die Peptidketten strecken sich mehr oder weniger gerade, die Zone dehnt sich in zentripetaler Richtung aus und kontrahiert sich gleichzeitig in tangentialer Richtung (II). Die Wuchsstoffmoleküle müssen daher in der einen oder anderen Weise bei der Aufrechterhaltung der Bindungen zwischen den Falten beteiligt sein, entweder dadurch, dass sie die oben erwähnten Bindungen stärken oder dadurch — und das ist wohl das wahrscheinlichste — dass sie selbst Bindungen, d. h. Brücken, zwischen den Falten bilden. In Abb. 9, I sind die punktierten Linien solche Wuchsstoffbrücken.

Wenn nun Wuchsstoff zu den Peptidketten in Abb. 9 II zugeführt wird, tritt eine Kontraktion ein, d. h. die Anordnung in II wird zu der Anordnung in I zurückgeführt. Das geschieht dadurch, dass in den gestreckten Peptidketten zufälligerweise kleine Falten gebildet werden, die Wuchsstoffmoleküle gewinnen dadurch Möglichkeit, ferner liegende Teile der Peptidketten mit einander zu verbinden, die Falten werden dabei tiefer, und nach und nach entsteht wieder die Anordnung der Ketten, die in I dargestellt ist. Die Zone kontrahiert sich in zentrifugaler Richtung und dehnt sich in tangentialer Richtung aus.

Die beschriebenen Vorgänge entsprechen denjenigen, denen man bei einer Denaturierung und Renaturierung eines globulären Proteins begegnet.

2. In dem anderen Falle besteht die Zone im normalen Zustande aus im grossen und ganzen parallelen Peptidketten, die vielleicht senkrecht auf der Zellwandoberfläche liegen. Sie sind nicht ganz gerade, sondern in zufälliger Weise mehr oder weniger gefaltet (III). Zwischen den Ketten sind Bindungen der oben erwähnten Art vorhanden. Wenn nun der Wuchsstoffgehalt nicht

genügend gross ist, vermögen die Bindungen die Ketten nicht zusammenzuhalten. Wie es in IV dargestellt ist, gleitet die Peptidkette b zwischen a und c aus, und die beiden letzteren rücken enger zusammen. Man muss es für wahrscheinlich halten, dass in diesem Falle die Wuchsstoffmoleküle, wenn sie vorhanden sind, Brücken zwischen den Peptidketten bilden, und in dieser Weise die Anordnung derselben in III aufrechterhalten. Die punktierten Linien in III sind solche Wuchsstoffbrücken zwischen den Peptidketten.

Wenn nun Wuchsstoff zu den Peptidketten in IV zugeführt wird, tritt eine Kontraktion ein, die Anordnung in IV wird zu der Anordnung in III zurückgeführt, d. h. es muss die Peptidkette b zwischen a und c eingezogen werden. Dieses könnte vielleicht in folgender Weise geschehen. Wenn in V die Peptidkette c sich bei Bildung der Wuchsstoffbrücke 2 faltete, würde sie verkürzt werden und dabei die Peptidkette b, mit der sie durch die Wuchsstoffbrücke 1 verbunden ist, eine Strecke einziehen. Es könnte dann eine Brücke 3 mit der Kette a gebildet werden. Wenn nun, nachdem die Brücken 1 und 2 abgebrochen worden wären, die Kette c sich streckte, und die Kette a sich faltete, könnte die Kette b wieder eine Strecke eingezogen werden, und es könnte eine neue Wuchsstoffbrücke 4 zwischen c und b gebildet werden. In dieser Weise könnte die Kette b durch wechselweise Faltung und Streckung der Peptidketten a und c und durch Bildung transitorischer Wuchsstoffbrücken nach und nach ganz eingezogen werden. Natürlich könnten auch Kontraktionen der b-Kette dabei mithelfen. Gleichzeitig mit der Einziehung der Peptidkette b findet eine tangentiale Ausdehnung des Systems statt.

Die Kontraktionen und Streckungen der Peptidketten, die zur Erklärung der Verschiebungen derselben benutzt worden sind, finden sich bei allen fadenförmigen Eiweissmolekülen. Sie entstehen dadurch, dass die einzelnen Teile der Fäden um die Valenzbindungen frei rotieren können, und sie bewirken, dass die Form der Ketten sich unaufhörlich ändert. Wenn aber, wie in dem oben beschriebenen Fall, die Kontraktionen durch transitorische Bindungen an Nachbarketten gesteuert werden, so muss es möglich sein, dass die Peptidkette b zwischen den Peptidketten a und c eingleiten kann oder eingezogen werden kann. Dank

der zufälligen Kontraktionen und der Brückenbildungen sind die Peptidketten im Besitze einer gewissen Beweglichkeit.

Weil die Wuchsstoffbrücken teilweise transitorisch sind, darf man erwarten, dass eine ziemlich begrenzte Anzahl von Wuchsstoffmolekülen genügen würde, um den kontrahierten Zustand zu bewahren. THIMANN und BONNER haben berechnet, dass der Wuchsstoff, wenn er in monomolekularer Schicht über eine neugebildete Zellwand ausgebreitet wurde, nur $\frac{1}{300}$ bis $\frac{1}{400}$ der Zellwand decken können würde. Ich möchte annehmen, dass nach der Brückentheorie eine solche Konzentration genügen würde, um die Funktion des Wuchsstoffes zu ermöglichen.

Ob nun die erste oder zweite Möglichkeit die richtige ist, lässt sich nicht entscheiden. Das Gemeinsame der beiden Möglichkeiten ist, dass die Wuchsstoffmoleküle wahrscheinlich Brücken bilden entweder zwischen ferner liegenden Teilen der einzelnen Peptidketten oder zwischen benachbarten Peptidketten. Es soll nun untersucht werden, wie eine solche Brückenbildung zustande kommen kann.

Nach der Zweipunkttheorie von MUIR and HANSCH (1953) ist die Wuchsstoffwirkung durch zwei Molekülstrukturelemente bedingt, durch einen ungesättigten Ring und durch eine Carboxylgruppe. Nach der Dreipunkttheorie von SMITH and WAIN (1951) ist bei Aryloxyessigsäuren ausserdem ein H-atom in α-Stellung in der Seitenkette notwendig für die Wirkung (2-naphthoxyacetic acid can readily provide the three necessary groupings to the receptors at the side of action). Obwohl nicht alle Tatsachen sich in diese Theorien einfügen lassen, muss man es doch für wahrscheinlich halten, dass ein jeder Stoff, der als Wuchsstoff wirken kann, bestimmte Strukturelemente enthält, die die Bindung des Stoffes an Proteinmoleküle ermöglichen.

MUIR und HANSCH nehmen an, dass z. B. 2,4-Dichlorphenoxyessigsäure sich durch eine Zweipunktsorthoreaktion teils mit dem S-atom teils durch die Carboxylgruppe mit der NH_2 Gruppe in einer Cysteinylgruppe eines Proteins verbindet. Eine solche feste Verbindung wird jedoch kaum gebildet. Wie oben erwähnt, kann die Verbindung zwischen Enzym und Wuchsstoff sehr leicht gelöst werden, kovalente Bindungen kommen daher nicht in Frage.

Es gibt nun viele verschiedene Wuchsstoffe mit sehr verschiedener Konstitution. Die Wirkung derselben ist aber meistens klein im Vergleich mit derjenigen von Indolylessigsäure. Man darf wohl daher annehmen, dass ihre Struktur der Struktur des zellulosebildenden Enzyms angepasst ist. Die Bindungsstrukturen der Indolylessigsäure sind, wie oben erwähnt, die Carboxylgruppe und der Indolring.[1] Die entsprechenden Bindungsstrukturen des zellulosebildenden Enzyms sind eine basische Seitenkette (Arginin, Lysin), mit welcher die Carboxylgruppe des Wuchsstoffes eine salzähnliche Bindung bilden kann, und ferner eine aromatische Ringbildung in einer Seitenkette (Tryptophan, HISTIDIN?[2]), an dem sich der Indolring des Wuchsstoffes legen kann, so dass eine Brücke zwischen zwei Seitenketten der Enzymmoleküle von der Indolylessigsäure gebildet wird. Wegen des Elektronenpaares an dem N-Atom besitzt Indolylessigsäure eine polare Struktur, die vielleicht die Bindung an einen anderen Ring ermöglichen oder verstärken kann. Es wird dadurch verständlich, dass geringe Änderungen des Pyrrolringes (z. B. Ersatz von NH durch CH_2) die Wuchsstoffwirkung sehr stark vermindern können.

Die Grundlage der Brückentheorie ist somit, dass Affinitäten vorhanden sind, einmal zwischen der Carboxylgruppe der Indolylessigsäure und einer basischen Seitenkette, und ferner zwischen dem polaren Indolring und einer anderen aromatischen Ringbildung. Während es nicht bezweifelt werden kann, dass die erstgenannte Affinität besteht, ist die Existenz der letzteren wohl nicht unmittelbar so einleuchtend. Jedenfalls in einem Falle kann doch eine Affinität dieser Art direkt nachgewiesen werden. Wenn Nitrogruppen, die starke Dipole sind, in einen Benzolring eingeführt werden, so üben diese Gruppen eine kräftige Induktion auf die π-Elektronen anderer aromatischer Ringbildungen aus. Trinitrophenol und Trinitrobenzol sind daher imstande, mit Naphthalin Additionsverbindungen zu bilden. Es findet dabei

[1] Während ein H-Atom in einer OH- oder NH_2-Gruppe mit einem O-Atom eine H-Bindung bilden kann, ist dass im allgemeinen nicht der Fall mit einem H-Atom, das mit C verbunden ist. Die H-Atome in der Seitenkette kommen daher wahrscheinlich nicht in Frage als Bindungsmöglichkeiten. Die Bedeutung der CH_2 Gruppe muss daher anderswo gesucht werden.

[2] HISTIDIN kann, wie FITTING gezeigt hat, Plasmaströmung hervorrufen, einen Vorgang, der möglicherweise mit einer Kontraktion von Peptidketten verknüpft ist.

ein Elektronenübergang vom Naphthalin zu den Nitroverbindungen statt.

Die Energiewerte der erwähnten Bindungen der Indolylessigsäure sind ziemlich geringfügig. Wie gross sie sind, ist nicht möglich zu sagen. Wenn aber genügend viele solcher Bindungen vorhanden sind, kann die gesamte Stärke derselben grösser als eine kovalente Bindung sein. Tatsächlich können sie eine Kontraktion der Zellulosenbildner hervorrufen, was die Bindungen, die normalerweise die Proteinmoleküle zu Mizellen zusammenfügen, nicht vermögen.

Wenn nun die Auffassung richtig ist, dass die Funktion des Wuchsstoffes darin besteht, Brücken zwischen Enzymmolekülen zu bilden, um sie dadurch zusammenzuhalten, so wird es, weil die Bindungsmöglichkeiten der Proteinmoleküle so mannigfaltig sind, leicht verständlich, dass diese Funktion mit sehr verschiedenen Mitteln verwirklicht werden kann. Man muss erwarten, dass eine grosse Anzahl von Stoffen mit verschiedener Konstitution im Besitze von Wuchsstoffwirkung sein kann. Das ist tatsächlich, jedenfalls bei der *Avena*koleoptile, auch der Fall. Man kennt gegenwärtig eine grosse Anzahl von Wuchsstoffen, von denen die meisten künstlich hergestellt sind. Der Nutzeffekt derselben ist sehr ungleich, häufig ziemlich klein. Unter Hinweis auf die Darstellung von Audus (1953) sollen einige Eigenschaften dieser Stoffe kurz besprochen werden.

1. Ebenso wie die Indolylessigsäure enthält wohl ausnahmslos ein jeder Wuchsstoff entweder eine Carboxylgruppe, eine saure Gruppe (z. B. Indoxylschwefelsäure) oder eine Gruppe, die in eine Carboxylgruppe umgebildet werden kann (Indolylacetaldehyd, Indolylacetonitril, Ester von Indolylessigsäure).

2. Ebenso wie in der Indolylessigsäure ist in jedem Wuchsstoff (mit einer einzelnen Ausnahme) eine Ringbildung vorhanden. Der Indolring kann aber durch einen anderen aromatischen Ring, einen Naphthol-, einen Anthracen- oder einen Benzolring ersetzt werden. Phenoxyessigsäure ist jedoch wenig aktiv; durch Substitution mit Chloratomen, namentlich in 2,4 Stellungen, wird die Wuchsstoffwirkung sehr stark erhöht, möglicherweise weil dadurch das Dipolmoment vergrössert wird. Wenn dagegen die aromatischen Ringe durch Hydrierung gesättigt werden, wobei

die π-Elektronen verschwinden, wird auch die Wuchsstoffwirkung vernichtet. Während α-Naphthylessigsäure aktiv ist, ist Decahydronaphthylessigsäure inaktiv. Neulich hat man einen Wuchsstoff ohne Ringbildung gefunden, nämlich S-(carboxymethyl)-dimethyl-dithiocarbamat. Die Wuchsstoffwirkung ist jedoch ziemlich gering.

3. Zwischen der COOH-Gruppe und dem Ring muss wie in Indolylessigsäure als Regel mindestens ein C-Atom vorhanden sein. β-Indolylcarbonsäure ist daher inaktiv. Wenn die Anzahl von C-Atomen vergrössert wird, sinkt im allgemeinen auch die Aktivität. — Trotzdem in der Benzoesäure die Carboxylgruppe direkt mit dem Ring verbunden ist, ist diese Verbindung doch aktiv, wenn Wasserstoffatome in dem Ring durch NO_2 oder Halogenatome substituiert werden. Wie oben bemerkt, sind namentlich die NO_2-Gruppen starke Dipole.

Aus dem Angeführten geht es wohl zur Genüge hervor, dass die Wuchsstoffwirkung an Stoffe sehr verschiedener Konstitution verknüpft ist. Es ist daher ausgeschlossen, dass die Wuchsstoffe prosthetische Gruppen eines Enzyms sind. In Übereinstimmung mit der Brückentheorie besitzt jedoch ein jeder einigermassen kräftige Wuchsstoff — und das ist wohl das einzige, das für sämtliche Wuchsstoffe gemeinsam ist — zwei Strukturelemente, eine Carboxylgruppe und einen aromatischen Ring mit polarer Struktur, die es ermöglichen, dass er sich mit zwei verschiedenen Seitenketten in Proteinmolekülen verbinden kann, so dass eine Brücke gebildet wird.

Wegen der Mannigfaltigkeit der Bindungsmöglichkeiten ist es nicht möglich, eine erschöpfende Theorie der Wuchsstoffwirkung aufzustellen. Wir sind nicht imstande, mit Sicherheit zu sagen, ob eine Verbindung Wuchsstoffwirkung besitzt oder nicht, und noch weniger, wie gross die Wirkungsstärke eines Wuchsstoffes sein wird. Die Mannigfaltigkeit der Bindungsmöglichkeiten bewirkt ferner, dass es nicht möglich ist, bestimmte Modelle der Bindungsstrukturen aufzustellen. Selbst für Indolylessigsäure lässt sich dieses Problem nicht lösen.

Wenn nun Indolylessigsäure mit den Molekülen des zellulosebildenden Enzyms Brücken bilden kann, so muss man es ferner für wahrscheinlich halten, dass sie sich auch mit anderen

Proteinen in ähnlicher Weise verbinden kann. Dieses trifft auch zu. Es ist bekannt, dass grosse Wuchsstoffmengen, z. B. in Blättern, vorhanden sein können (vgl. SÖDING 1952). Dieser Wuchsstoff ist an ein Protein, das nach THIMANN ein Globulin sein kann, gebunden. Während der aktive Wuchsstoff sehr leicht in Äther übergeht, ist dagegen der globulingebundene Wuchsstoff schwer extrahierbar. Die Ursache dieses Unterschiedes ist wohl einmal, dass der aktive Wuchsstoff sich an der Oberfläche des Plasmas befindet, während der globulingebundene tiefer in dem Plasma liegt. Ferner ist Globulin ein globuläres Protein, mit einem hohen Dipolmoment, das aus stark gefalteten Proteinketten gebildet wird. Es ist daher verständlich, dass Wuchsstoffmoleküle, die sich in dem Inneren einer solchen Mizelle befinden, selbst wenn sie nicht kovalent gebunden sind, nur langsam freigemacht werden, und dass proteolytische Enzyme oder Alkalihydrolyse, die das Globulin abbauen, bewirken, dass der Wuchsstoff schnell in Lösung geht. Die schlechte Extrahierbarkeit des globulingebundenen Wuchsstoffes könnte jedoch auch darauf beruhen, dass eine kovalente Bindung zwischen Wuchsstoff und Protein, vielleicht eine Esterbindung, vorhanden sein konnte. Eine solche Bindung kann möglicherweise von einem proteolytischen Enzym gespalten werden. Weil man den gebundenen Wuchsstoff auch ohne Abbau des Proteins, obwohl ziemlich langsam, extrahieren kann, ist die letztere Möglichkeit doch wohl weniger wahrscheinlich.

Vielleicht spielt der Wuchsstoff auch eine Rolle bei anderen Vorgängen als dem Zellwandwachstum.

Die Literatur über die Wirkungsweise des Wuchsstoffes ist so überwältigend gross, dass es unmöglich ist alle Seiten dieses Problems zu besprechen. Nur eins möchte ich noch erwähnen.

Eine Voraussetzung der Brückentheorie ist es, dass die Wuchsstoffe eine Anionengruppe besitzen, so dass sie sich mit einer basischen Seitenkette verbinden können. Diese Voraussetzung ist, wie oben erwähnt, erfüllt. Viele Wuchsstofforscher meinen aber, dass die Wuchsstoffwirkung an die undissoziierten Moleküle geknüpft ist. Sie stützen sich auf Versuche, die zeigen, dass die Wirkung bei Abnahme der Dissoziation zunimmt. Weil Änderungen der Wasserstoffionenkonzentration die Wachstumsgeschwindigkeit in sehr verschiedener Weise beeinflussen

können, meine ich nicht, dass man aus den angeführten Versuchen schliessen kann, dass eine Dissoziation die Wuchsstoffwirkung aufhebt. Vielmehr glaube ich, dass die Wirkung an die Wuchsstoffionen geknüpft ist. Andere Forscher sind derselben Meinung.

Es wäre auch sehr interessant, die Bedeutung der Hemmstoffe im Lichte der Brückentheorie zu betrachten. Weil neue Untersuchungen notwendig sind, um diese Probleme zu beleuchten, muss ich es unterlassen.

Auf die Wirkungsweise der Herbizide hoffe ich später zurückzukommen.

3. *Die Wirkungsweise des Wuchsstoffes in anderen Pflanzen.* Es fragt sich nun, ob man eine ähnliche Wuchsstoffwirkung wie in dem *Phaseolus*epikotyl auch in anderen Pflanzen wiederfinden kann. Aus Untersuchungen von Bücher und anderen geht hervor, dass Keimstengel von *Helianthus, Ricinus communis, Vicia faba* und wahrscheinlich vielen anderen Pflanzen sich in ähnlicher Weise wie *Phaseolus* verhalten werden; ob man auch in der *Avena*koleoptile bei Wuchsstoffmangel Verdickungen hervorrufen kann, muss später untersucht werden.

In den Trichoblasten der Wurzelhaare sind die Zellulosenbildner ursprünglich gleichmässig über die Papillen der Zellwände verteilt. Kurz nach dem Beginn der Streckung tritt eine Kontraktion derselben ein, so dass sie sich an dem apikalen Ende ansammeln, wo dann ein Wurzelhaar gebildet wird (Boysen Jensen 1950). In diesem lagern die Zellulosenbildner sich in der Spitze. Sie bilden ein zusammenhängendes System, was daraus hervorgeht, dass sie als ganzes hin oder her verschoben werden können (Boysen Jensen 1957, Abb. 1) oder an der einen Seite herabgleiten können, wo dann eine Verzweigung des Wurzelhaares gebildet wird (Boysen Jensen 1955, Abb. 12 d, e, f). Mann muss daher annehmen, dass die Enzymmoleküle durch Bindungen miteinander verknüpft sind, und dass das ganze System im Besitze einer gewissen Elastizität ist. Diese Bindungen können doch durch verschiedene Gifte (Colchicin, Rhodanammonium) teilweise gelöst werden; die Zellulosenbildner breiten sich dann über grössere Teile des Wurzelhaares aus, und es entstehen Anschwellungen (Boysen Jensen 1955, Abb. 11). Es besteht jedoch immer eine Neigung, das normale Verteilungs-

muster wiederherzustellen, was dadurch erreicht wird, dass eine Kontraktion des Systems der Zellulosenbildner eintritt, so dass sie sich an einem bestimmten, aber zufälligen Ort anhäufen, wo dann ein wurzelhaarähnliches Gebilde entsteht (Boysen Jensen 1955, Abb. 11 g). Wir finden somit in den Trichoblasten und Wurzelhaaren Kontraktionen und Ausdehnungen des Systems der Zellulosenbildner, d. h. ganz dieselben Vorgänge wie in den Rindenzellen des *Phaseolus*epikotyls, ob aber diese Vorgänge in der Wurzel mit Änderungen der Wuchsstoffkonzentration verknüpft sind, lässt sich nicht entscheiden.

c. Zusammenfassung. Die Bedeutung der Zellulosenbildner, des Wuchsstoffes und der Respiration für das Wachstum der Zellwände.

Das Wachstum der Zellwände besteht in der Bildung neuer Zellwandstoffe. Wenn ein Wurzelhaar von *Phleum* 10 μ gewachsen ist, so bedeutet es, dass ein Hohlzylinder von Zellwandstoffen mit einer Wanddicke von etwa 0,6 μ, einer Länge von 10 μ und einem Diameter von 8 μ gebildet worden ist. Nun wissen wir, dass das Längenwachstum der Zellwände in Koleoptilen und Keimstengeln aufhört, wenn kein Wuchsstoff vorhanden ist, und man könnte daher geneigt sein zu schliessen, dass der Wuchsstoff vielleicht bei der Bildung der Zellwandstoffe beteiligt sein könnte. Dem ist aber nicht so. Die Zellulosenbildung ist wie die Stärkebildung ein enzymatischer Vorgang (vgl. Treiber und Stockman in »Die Chemie der Pflanzenzellwand p. 22), und es ist daher nicht ausgeschlossen, dass Wachstum, d. h. Neubildung von Zellwandstoffen, ohne Wuchsstoff stattfindet.

Tatsächlich ist jedoch auch der Wuchsstoff, wie es aus einer Unmenge von Versuchen hervorgeht, notwendig für das Wachstum. Das gilt nicht nur für das Streckungswachstum, sondern auch für das embryonale Wachstum, z. B. für das Kalluswachstum. Wahrscheinlich ist eine geringe Menge von Wuchsstoff auch für das Wachstum der Wurzeln notwendig, obwohl es nicht möglich ist nachzuweisen, dass z. B. Wurzelhaare Wuchsstoff für das Wachstum benötigen. Man wird auch nicht verneinen können, dass in dekapitierten *Phaseolus*epikotylen, wo kein Streckungswachstum, aber Verdickungen der Zellwände stattfindet, mög-

licherweise eine geringe Wuchsstoffmenge vorhanden ist. Die Bedeutung der Wuchsstoffe für das Wachstum ist aber mehr indirekter Natur. Weil, wie später erwähnt werden soll, die Determinations- und Differenzierungsvorgänge wahrscheinlich durch eine Verlagerung der Zellulosenbildner zustandekommen, müssen dieselben einerseits leicht verschiebbar sein, andererseits müssen sie zusammengehalten werden; diese letztere Aufgabe fällt der Indolylessigsäure zu. Wie oben dargelegt, ist der Wuchsstoff in der einen oder anderen Weise bei der Entstehung der Bindungen zwischen den Enzymmolekylen betätigt, oder wirkt vielleicht selbst als Bindung. Der Wuchsstoff kann daher Verschiebungen der Zellulosenbildner hervorrufen; er beeinflusst in dieser Weise die Verteilung und die Anordnung der Zellulosenbildner und dadurch die Wachstumsweise und die Wachstumsgeschwindigkeit der Zellwände.

Es ist, wie oben bemerkt, wahrscheinlich, dass eine gewisse Menge von Wuchsstoff notwendig ist, um die Zellulosenbildner zusammenzuhalten, und dass daher die Zellulosenbildung und das Wachstum aufhören, wenn kein Wuchsstoff vorhanden ist. Es ist jedenfalls sicher, dass Zellulosenbildung und Wachstum vergrössert werden, wenn die Konzentration der Zellulosenbildner durch Kontraktion erhöht wird. Das ist z. B. der Fall bei der Bildung der Wurzelhaare in den Trichoblasten.

Die Bedeutung der Respiration für das Wachstum ist wahrscheinlich darin zu suchen, dass durch dieselbe die Bildung der plastischen Stoffe, aus welchen die Zellulose entsteht, ermöglicht wird. Die Rohstoffe für die Zellulosenbildung sind wahrscheinlich Glykose-1-phosphat oder Uridin-diphosphat-glycose (Aldermatt und Neisch 1956); vielleicht werden sie vor dem Aufbau teilweise gespalten. Wahrscheinlich ist die bei der Respiration freigemachte Energie auch notwendig um osmotisch wirksame Stoffe in die Vakuole hinein zu transportieren, so dass der Turgordruck während des Wachstums aufrechterhalten werden kann.

d. Die Bedeutung der Zellulosenbildner und des Wuchsstoffes für den Aufbau des Zellwandmusters.

Dieser Aufbau kann auf gleichmässiges oder ungleichmässiges Flächen- und Dickenwachstum der Zellwände und auf die Bil-

dung neuer Zellwände zurückgeführt werden. Die Anordnung der neuen Zellwände wird durch die Lage der Zellkerne und die Orientierung der Kernspindel bestimmt (Boysen Jensen 1957). An dieser Stelle soll nur die Bedeutung des Wachstums der Zellwände für den Aufbau des Zellwandmusters besprochen werden.

Es geht aus dem oben Erwähnten hervor, dass die Zellulosenbildner die Wuchsstoffe im eigentlichen Sinne des Wortes sind. Die Stoffe, die man Wuchsstoffe nennt, sind zwar unentbehrlich für das Wachstum, aber sie sind doch eher von sekundärer Bedeutung. Wenn man daher die Bedeutung des Wachstums der Zellwände für den Aufbau des Zellwandmusters zu verstehen wünscht, wird man vornehmlich die Bildung und Verteilung der Zellulosenbildner untersuchen müssen.

Es ist wahrscheinlich, dass Verlagerungen der Zellulosenbildner in ähnlicher Weise wie in den Trichoblasten eine bedeutende, vielleicht eine ausschlaggebende Rolle spielen bei den Differenzierungsvorgängen in Sprosspitzen und Blüten. Das Kennzeichnende dieser Vorgänge ist es, dass benachbarte, gleichartige Zellen (d. h. primär die Zellwände derselben) ungleichartig oder überhaupt nicht wachsen. Nun kann das Wachstum der Zellwände entweder durch Wuchsstoff oder durch die Menge der Zellulosenbildner begrenzt sein. Da in den Sprosspitzen und jungen Blüten der Wuchsstoffgehalt gross ist, wird es wahrscheinlich, dass das ungleichartige Wachstum dadurch zustande kommt, dass die Zellulosenbildner, die ursprünglich gleichmässig über die Zellwände verteilt sind, sich an bestimmten Stellen ansammeln, wo die Zellen dann anfangen zu wachsen.

Diese Anhäufung der Zellulosenbildner an einem bestimmten Ort könnte in folgender Weise zustandekommen. Man muss sich vorstellen, dass zwei Arten von Bindungen an das System der Zellulosenbildner geknüpft sind, einmal solche, die die Enzymmolekyle des Systems zu einer Ganzheit vereinigen, und ferner solche, die das System mit dem Plasma verbinden. Wenn nun die Zellulosenbildner ursprünglich über die ganze Fläche in Abb. 10 verteilt wären, so würden sie, wenn die Bindungen, die das System mit dem Plasma verknüpfen, in dem weissen Feld in der einen oder anderen Weise gelöst werden, als Folge der zwischenmolekularen Bindungen im System sich elastisch zu-

sammenziehen und sich an dem schraffierten Ort im Zentrum des Feldes anhäufen. An dieser Stelle könnte dann ein kleiner Höcker gebildet werden, der unter fortgesetzten Differenzierungen sich zu einem Organ der einen oder anderen Art, zu einem Blatte, einem Staubgefässe u. s. w. entwickeln könnte, in ähnlicher

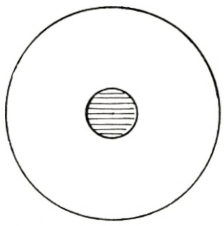

Abb. 10. Schematische Darstellung der Kontraktion der Zellulosenbildner an einem bestimmten Ort, wo dann ein Organ gebildet werden kann.

Weise wie ein Wurzelhaar als Folge einer Anhäufung von Zellulosenbildnern in dem apikalen Ende eines Trichoblasten auswächst.

Es dürften die zwischenmolekulären Kräfte und nicht die Membranpotentiale sein, die bei der Verlagerung der hochmolekularen Verbindungen (und der Organellen?) während der Differenzierungsvorgänge beteiligt sind. Es wird eine bedeutungsvolle Aufgabe sein, die Ergebnisse der modernen Elektronentheorie der Valenzkräfte in die Gestaltungs- und Bewegungsphysiologie einzuführen.

Dem pflanzenphysiologischen Laboratorium der Universität Kopenhagen und dem Carlsbergfond, die mir die für die Untersuchungen notwendigen Instrumente zur Verfügung gestellt und mich auch in anderer Weise unterstüzt haben, spreche ich meinen besten Dank aus.

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Summary.

When epicotyls of *Phaseolus* are fixed in a horizontal position in a glass-tube, the amount of growth substance will be greater on the lower than on the upper side of the stem. At the same time the increase diminishes and an anatomical difference between the two sides arises, the cells on the upper side becoming small and thick-walled and the cells on the lower side large and thin-walled. It is an obvious conclusion that these anatomical changes are caused by the decrease of growth substance on the upper side and the increase on the lower side. The correctness of this view is checked by the fact that by covering the upper side of epicotyls in a horizontal position with an IAA-paste the thickening of the cell walls will be curtailed. Further, thickenings present can be removed by a supply of growth substance. When epicotyls after 24 hours in a horizontal position are turned 180°, the amount of growth substance will increase on the lower side (the former upper side) and the thickenings will disappear during the following 24 hours. A similar result was obtained when the upper side of epicotyls after 24 hours in a horizontal position was covered with an IAA-paste.

We can therefore conclude that growth substances regulate growth in epicotyls in the following way. When the amount of growth substance diminishes, a rapid growth with formation of thin-walled cells is changed to a slower growth with formation of thick-walled cells. An increase of growth substances will have the opposite effect.

In previous publications it has been demonstrated that elongation arises through a cooperation of two factors, viz. turgor pressure and intussusception, i. e. production of new cellulose fibrils by cellulose-building enzymes on the surface of plasma papillae protruding into the cell wall. From the investigations mentioned above it may be concluded that the system of cellu-

lose building enzymes, when the concentration of growth substance is diminished, will expand centripetally and be spread over the surface of plasma on the inner side of the cell wall. At the same time a contraction of the system will take place in a tangential direction. The consequence is, that a localized thickening of the cell walls sets in, and a collenchyma-like tissue arises.

Hence it appears that a connection exists between the system of cellulose-building enzymes and the growth substance, the latter being able to produce a contraction of the enzyme system.

It is concluded that the mode of action of the growth substances is to make bridges between the molecules of the cellulose-building enzyme to hold them together. In the presence of growth substance the enzyme system is contracted, in the case of scarcity it expands.

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CONSTRUCTION AND PROPERTIES OF COMMA-FREE CODES

BY

S. W. GOLOMB, L. R. WELCH, AND M. DELBRÜCK

København 1958
i kommission hos Ejnar Munksgaard

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CONTENTS

Synopsis.

The sequence of bases in deoxyribonucleic acids is assumed to represent a coded message, embodying information concerning the sequence of amino acids in proteins. Crick *et al.* [7] suggested that the code might be a "comma-free triplet" code. This means that each amino acid is coded by a triplet of bases, and that the triplets are chosen such that no overlap between any pair of triplets codes for an amino acid. In such a code the triplets do not have to be separated from each other by some kind of comma; they can be run together without causing ambiguities in the message.

This paper concerns the following aspects of comma-free codes:

1) Procedures for the construction of all comma-free triplet codes involving the maximum number (20) of triplets. It is shown that there are five classes of such codes and a total of 408 codes.

2) It is shown that no message written with any of these codes ever contains a fourfold repeat of any base, and that in some of the codes certain threefold repeats are excluded.

3) Certain misprints in the coded message will produce nonsense (the resulting triplet does not code for any amino acid), other misprints will produce missense (the resulting triplet codes for a different amino acid). The codes were studied with respect to missense/nonsense ratio produced by various classes of misprints.

4) DNA has a directional symmetry. The basic structure is such that the message could be read in either direction. The question is posed whether codes could be devised such that if they are read in the wrong direction they give nonsense everywhere, i. e., no triplet or overlap between triplets read in reverse corresponds to any amino acid. Such codes are termed transposable codes. It turns out that a transposable triplet code can code for at most 10 amino acids, which is too few. Therefore quadruplet codes were taken into consideration. These are mathematically more difficult to handle and only a few fragmentary results have been obtained so far.

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Part I.

Origin of the Problems, Summary and Discussion of Results.

By M. Delbrück.

The discovery that genetic information in many organisms is transmitted from parent to offspring through desoxyribonucleic acid (*DNA*) and the discovery of the structure of *DNA* by Watson and Crick [1] have raised the problem as to the nature of the code used to carry this information and as to the mechanism by which the code is read. It is believed that one of the intermediate steps of the translation consists in the synthesis of specific proteins and that the essential element of this specificity consists in the sequence of the amino acids in the proteins. Both the *DNA* and the protein are linear polymers. The *DNA* molecule is a duplex of two chains containing principally four bases (adenine = *A*, cytosine = *C*, guanine = *G*, and thymine = *T*). In addition, there are one or more bases occurring in very small proportion which may or may not have any particular significance. The two chains have base sequences which are complementary, *A* always opposite *T*, and *C* always opposite *G*. The duplex as well as the single chain may therefore be looked upon as a message written in a code involving four symbols. In the case of the single chain, the symbols are *A*, *C*, *G*, and *T*; in the case of the duplex, the symbols are the base pairs *A*–*T*, *T*–*A*, *C*–*G*, and *G*–*C*. The proteins are polypeptide chains of some 20 amino acids and can thus be looked upon as messages written in a code containing some 20 symbols. The problem is thus reduced to one of coding the information contained in a message employing some 20 symbols in a code employing only four symbols and to finding the mechanism for its translation.

The *DNA* as a message container confronts us at once with a peculiar duality feature: it contains *two* complementary messages which are chemically quite different. This is so whether the single chain or the duplex as a whole is the message container. If a single chain is the container, then the complementary chain contains a message which differs from the first one by two operations: (1) reading backward, (2) substitution of the complementary symbol X' for each of the symbols X (A for T, T for A, G for C, and C for G). If the duplex as a whole is the message container, with base pairs as symbols, then we are still dealing with two complementary messages. This is so because the duplex as a whole is symmetric, it can be read in either direction, and the two messages so obtained differ exactly by the same operations as those on the two chains: reversal of direction and substitution of the complementary symbol (base pair $A-T$ for $T-A$, etc.).

By the time the message is translated into protein this duality is apparently gone, as there is no evidence that one piece of genetic material is regularly responsible for two different proteins. Along the path from *DNA* to protein one of the messages is therefore eliminated. The last chapter of this paper arose from a specific suggestion as to the nature of this elimination. To introduce this notion, as well as those which prompted the other mathematical questions and answers dealt with in this paper, it will be necessary to insert a few comments on the biochemical aspects of the problem.

It seems fairly certain that the centers of protein synthesis in the cell are the microsomes, particles which contain no *DNA* but which do contain ribonucleic acid (*RNA*), a linear polynucleotide also containing four bases. It is a likely conjecture that the *RNA* represents an intermediate translation of the code. If so, it would be of the greatest interest to know whether at this point of the translation process the duality of the message has already been eliminated. The fact that the base ratios of *RNA* in some cases deviate from those imposed in *DNA* by the complementarity feature would seem to speak in favour of the idea that the duality has been removed, but neither this fact, nor, if it is a fact, the method by which it is accomplished are clear.

Regarding the actual synthesis of proteins it is now believed [2, 3, 4] that the amino acids are first activated, in two steps: in

the first step a complex is formed between amino acid and adenosine monophosphate (*AMP*); while in a second step the amino acid is transferred from *AMP* to a soluble *RNA* fraction. More precisely, there is a specific enzyme for each amino acid which couples only this amino acid to *AMP*. The amino acid is then transferred to a specific site on the soluble *RNA*. At first sight it would seem surprising that for each amino acid there should be a specific enzyme to couple it to *AMP*, since no confusion, i.e., no false synthesis, would occur if this step were unspecific. It is conceivable, however, that in vivo not soluble *RNA* molecules but tri- or tetra-nucleotides play the role of intermediate amino-acid carriers, supplying a specific adaptor to each, this adaptor serving the purpose of fitting it to the code letters in the message. If the same enzyme which couples the amino acid to *AMP* were responsible for the transfer of the amino acid to the specific adaptor then the specificity of the enzyme would make sense. Obviously, the same enzyme could not be charged with the duty of coupling each amino acid to its specific adaptor.

Four years ago GAMOW [5] published in these proceedings an important paper in which a first attempt was made to "break the code". The main characteristics of Gamow's attempt were the following: first, it assumed a *direct* translation from *DNA* into protein; second, it assumed an *overlapping* code, the piece of *DNA* determining one amino acid (a diamond shaped structure extending over three base pairs) and the piece of *DNA* determining the next neighboring amino acid overlapping by two thirds of their length; third, it assumed a *degenerate* code, in that several different triplets of base pairs coded for the same amino acid. The reason why it was assumed that a triplet of three pairs code for one amino acid was simple. Two base pairs give only 16 possibilities, which is not enough to code for some 20 amino acids. Three base pairs give 64 possibilities which is more than necessary and therefore permits degeneracy. The overlap feature was introduced for geometrical reasons: with this amount of overlap the spacing from one amino acid to the next would correspond roughly to the spacing from one base pair to the next and this seemed reasonable on structural grounds.

With the increase in our knowledge of amino acid sequences in proteins Gamow's particular scheme, and several others, have

been demonstrated to be untenable, and in fact BRENNER has given [6] an elegant proof of the impossibility of all overlapping triplet codes. In 1957 CRICK, GRIFFITH and ORGEL [7] introduced a new idea into the problem. If it is true that groups of base pairs, say triplets, code for one amino acid, and that these triplets are not overlapping, and if the message is formed simply by tacking these triplets end to end, how do we know where one triplet ends and the next begins? Either the message would have to be read strictly in sequence starting at one end, or the triplets might be chosen such that no overlap makes "sense". The triplets which code for amino acids might form a "dictionary" of "words" such that no overlapping triplet in a message written from these words is a word in this dictionary. This is the idea of the comma-free code, and CRICK *et al.* proved that in the case of four symbols and words of length three the maximum size of such a dictionary is 20. They also constructed some of the dictionaries of this size. In such a code, then, the freedom resulting from the fact that there are 64 possible triplets and only 20 amino acids to code for is used to select a comma-free dictionary. GOLOMB, GORDON and WELCH [8] addressed themselves to a mathematically interesting generalization of this problem: what is the maximum size of a comma-free dictionary in the case of an arbitrary number of symbols and an arbitrary length of the words? They were able to obtain a partial solution of this problem, and to develop methods which are useful also for the mathematical developments presented in this paper.

These developments were motivated directly by certain aspects of the *DNA*-protein problem. It is the purpose of this Part I to explain the biological interest of these questions and to summarize and discuss the results. It is hoped that they will stimulate new experimental approaches and point the direction which further mathematical analysis might take, to be of the greatest interest to the biologist.

The overlapping codes considered by previous authors implied no restriction on base neighbours, but did imply certain restrictions, statistical or absolute, regarding amino acid neighbors. It had been hoped that these restrictions would afford clues for breaking the code without any actual knowledge of base sequences in the *DNA*. As it turned out, these restrictions only

served to *eliminate* the overlapping codes. The comma-free codes present the opposite situation. They are non-overlapping codes, and as such they contain no restrictions, statistical or absolute, regarding amino acid neighbors, but they do imply restrictions on base sequences. If this is the type of code actually used, then the study of amino acid sequences by itself will be useless for breaking the code. However, information on the neighbor relations among the *bases* may be indicative as to the specific code used, and it becomes of interest to examine these codes closely to see what they imply, statistically or absolute, with respect to base neighbor relations.

A prerequisite for such a study is a method for constructing all possible comma-free codes. This is accomplished for the three letter codes in the first three chapters of Part II of this paper. The key to this construction is the important Theorem 2 which states a surprisingly simple condition which is both necessary and sufficient for a collection of 20 triplets to constitute a comma-free dictionary. With the help of this theorem it can be shown that there are five types of maximal comma-free dictionaries using triplets. Of each type there are a large number of different dictionaries, dictionaries of the same type differing from each other by permutations of letters and by reversals of sections of the dictionary. Chapter 3 is devoted to a study of these permutations and reversals.

Being in possession of these dictionaries we would like to enquire into properties of these dictionaries which might find expression in an experimentally verifiable manner and might thus serve the diagnostic purpose of differentiating between various codes.

The most direct route for breaking the code would of course be available if the conjecture of a specific oligonucleotide adaptor for each amino acid were found to be valid, and if these adaptors should stand in a complementarity or identity relation to the words of the coded message. It would then only be necessary to isolate and characterize the amino acid-adaptor complexes.

It is more likely that a less direct approach will be needed, involving properties of the *DNA* by itself or of the *RNA* by itself, and here a promising attack would lie in the examination of oligonucleotide fragments prepared by hydrolytic procedures with known specificities. Here it is important, before comparing ex-

perimental data with the mathematical properties of the codes, to take proper account of the duality feature.

We will illustrate this by examining three rules, proved in Part II of this paper, concerning forbidden symbol combinations.

1) In stochastic messages written from some of the triplet codes one and only one of the triplets of type XXX does not occur.

This is not a property which is verifiable by fragment analysis if the material studied still has the duality feature. Indeed, since the triplet $X'X'X'$ (the complement of XXX) will occur in the *message*, the triplet XXX will occur in the complementary message. If one of the single chains is the message, and does not contain XXX, then its complement will contain it, and its absence from the message proper will not be apparent from a hydrolyzate which does not distinguish the breakdown products of the real message from those of the complement. Similarly, if the duplex, read in one direction, is the real message, and this excludes of the triplets only the triplet XXX (X representing, say, the base pair $A-T$), then one chain excludes the triplet AAA and the other TTT, but each chain will contain the triplet which the other excludes, and the exclusion will not be observable by fragment analysis.

In fact, what is observable is not any rule as expressed in terms of the symbols of the message, but only such a rule after "mixing" it with the complementary rule. Thus, the rule "the triplet XXX does not occur and the triplet $X'X'X'$ does occur" is to be mixed with the complementary rule "triplet XXX does occur and triplet $X'X'X'$ does not occur." The mixed rule then says that both triplets occur.

2) No quadruplet of type $XXXX$ can occur in any message written from any maximal comma-free triplet code.

This rule is not affected by mixing with the complementary rule, since in this case the original and the complementary rule are identical.

3) In two of the five types of triplet code *two* triplets are excluded from the messages. If the two excluded triplets are complementary, then the rule is not changed by mixing, while if they are not complementary, then there are no exclusions after mixing. Whether the two triplets are complementary or not depends on the identification of the symbols with the bases or base pairs.

Up to this point we have been talking about absolute rules: complete exclusions of certain symbol combinations. We now turn to statistical rules. Here the first rule (theorem 1) is that in each maximal comma-free dictionary every letter occurs equally frequently and in the same number of words. Any deviation from equality between the four symbols therefore indicates an unequal frequency of the words, as is indeed obvious from the inequalities between the frequencies of the different amino acids. This inequality is of course insufficient to characterize the type or the specific dictionary. It follows further that it would be very difficult to make predictions concerning the frequencies of permitted digrams and longer combinations. For digrams, for instance, it is easy to calculate the frequencies with which they occur in the different dictionaries, and wide differences will be found between different dictionaries. However, the observable digrams include the overlaps, and their frequencies depend critically on the relative word frequencies which are not known in terms of the words of the code, even though they may be ascertainable, in some cases, in terms of the amino acids. For these reasons it does not seem worth while at this stage to go into a detailed analysis of the statistical relations.

The comma-free codes differ in another important respect from those previously considered: every misprint of necessity alters the message. This is in contrast to degenerate codes, where a large class of misprints produces no alteration at all in the message. The non-degenerate codes are therefore more vulnerable. The errors which occur, may, moreover, be divided into two classes: those which change a word of the dictionary into another word of the dictionary, and therefore one amino acid into another amino acid, one polypeptide into an altered one; and those which change a word of the dictionary into a combination of letters which is not in the dictionary, and therefore one amino acid into no amino acid, one polypeptide of length n into two polypeptides of total length $n-1$. Let us call these two classes of misprints the mis-sense class and the non-sense class. Presumably the non-sense class represents on the average a more severe functional change. It seems likely that the mis-sense to non-sense ratio is an important characteristic of each code and might have played a role in its natural selection. Moreover, it may be that

among the individual symbol errors those that interchange similar ones (the purines among themselves and the pyrimidines among themselves) are much more probable than the others.

The mis-sense to non-sense ratios are shown in Tables III and IV for the five types of dictionaries and for various restricted classes of misprints. There exist only slight differences between the five types of dictionaries when the types of misprints are unrestricted. In a maximal comma-free dictionary using four symbols and three-letter words there are sixty letters (20 words with three letters each) and each of them can be misprinted in three different ways, giving a total of 180 different misprints. The class of non-sense misprints ranges in size from 80 (in type I) to 92 (in type V). The differences become more pronounced when restricted classes of errors, of the types alluded to above, are considered. In this case, each letter can be misprinted in only one way (each pyrimidine by the other pyrimidine and each purine by the other purine). The total number of possible misprints is now sixty, and the class of non-sense misprints ranges in size from 16 (for type I) to 32 (for type V).

We turn now to the problem of how the duality of the message in the *DNA* is handled in the translation process. Here we put to ourselves the question whether the dictionary might not be so constructed that it automatically eliminates the duality. This would be accomplished if one of the two complementary sequences of letters contained nowhere letter combinations which occur in the dictionary. This question is examined in the last section of Part II of this paper. It turns out that it is indeed possible to construct dictionaries such that the complement to any message composed of words of the dictionary contains nowhere, neither as the complements of words nor as complements of the overlaps, a word of the dictionary. Such dictionaries are called *transposable* dictionaries and the very strong constraint which they fit does not reduce their size inordinately. A general theorem (theorem 15) about transposable dictionaries is given, establishing an upper bound as to their sizes. In the case of four symbols and three letter words, this maximum size is 10, as compared to 20 in the absence of the constraint. Several such dictionaries are given. Ten words is too few to code for all the amino acids and the simplest way to increase the number of words is by increasing the length of the words to four letters. In this case the upper bound turns

out to be 27 and an actual transposable dictionary of size 26 has been constructed. [Added in proof: numerous examples of 27 word dictionaires are now known.]

We wish to emphasize that we consider the postulate of comma-freedom and the postulate of transposability to be almost on the same footing. Indeed the principal virtue of comma-freedom is that any message can be read unambiguously starting at any point, with the proviso, however, that one must know in advance *in which direction to proceed.* Since the equivalence of the two opposite directions in a structural sense seems to be one of the more firmly established features regarding the *DNA* molecule the advance knowledge as to the direction in which to read cannot come from the basic structure. Comma-freedom would therefore seem to be a worthless virtue unless it is coupled with transposability.

Transposable comma-free dictionaries, in contrast to ordinary comma-free dictionaries, are strongly asymmetric with respect to symbol frequencies and symbol combinations. This is due to the fact that in the ordinary comma-free dictionaries every complete equivalence class[1] is represented, whereas in the transposable ones at least one half of these classes are not represented. This may be illustrated by the 26 word dictionary using 4-letter words exhibited in the last chapter. In this dictionary the pairs A and B, and C and D are complementary. The four symbols occur in the dictionary with the frequencies 21, 31, 41, 11. The complementary pairs A, B and C, D occur equally frequently in the dictionary. This is a general rule for transposable dictionaires in which each non-symmetric equivalence class[1] is represented. In the example given each class, with one exception, is, in fact, represented. This exception is the class $ADBC$. Its omission does not lead to a violation of the equality rule for complementary pairs, since it contains an equal number of each pair.

In terms of the bases this rule implies that in the dictionary the pair of bases Adenine-Thymine occurs as frequently as the pair of bases Guanine-Cytosine. For *DNA* molecules this equality in general does not hold. The inequality would have to be due to the unequal frequencies of the words (amino acids) of the dictionary in the coded message (*DNA* molecule).

A strong asymmetry may be inherent in the dictionary with

[1] for definition of this term see Part II, chapter 1.

respect to *purines* vs. *pyrimidines*. This we will illustrate again with the 26 word dictionary. Let us make the identification:

A = adenine
B = thymine
C = cytosine
D = guanine

Thus A and D are the purines (collectively designated as *Pu*), and B and C are the pyrimidines (collectively designated as *Py*). The dictionary is thus seen to contain 32 *Pu* and 72 *Py*. The symbol frequencies in the message will of course depend on the word frequencies, and this may affect the letter frequencies appreciably. However, even for equal word frequency, there would be on one of the chains a large excess of *Py*. Let us call this the *Py* chain. This would be compensated by a corresponding excess of *Pu* on the other chain, the *Pu* chain, and would not be observable as an asymmetry in the total hydrolysate. The asymmetry would be observable, however, in an experiment of the type described by MESELSON and STAHL [9], where N^{15} labelled *DNA* is permitted to duplicate once in the presence of N^{14}, producing "hybrid" *DNA* molecules, half labeled with N^{15}. If the "halves" in these experiments are indeed single polynucleotide chains (of which there is still doubt), then we should obtain two classes of hybrids, those whose *Py* chain is N^{15} labelled and *Pu* chain N^{14} labelled, and vice versa, and these two types of hybrids would differ sufficiently in density (by 0.2 %) to be resolvable by the equilibrium density gradient technique.

The unequal distribution of the purines and pyrimidines over the two chains will lead also to a phenomenon recently reported by SHAPIRO and CHARGAFF [10]. These authors found that the pyrimidines are bunched, i.e., that runs of pyrimidines of length one and two, flanked by purines, occurred less frequently than would be expected for random sequences in which purines and pyrimidines occur equally frequently. Messages constructed from a given dictionary are certainly not to be equated statistically with random letter sequences. The statistical proportion of the symbol relationships in such messages cannot be assessed in detail without a knowledge of the statistics of words. It is clear, however, that a strongly unequal distribution of the pyrimidines

over the two chains will introduce a bias in the direction of bunching.

It is perhaps unlikely that the words in the dictionary are of equal length. Indeed if economy with respect to total length of the coded message were the overriding principle in the choice of the code, nature would certainly have selected short words for frequently occurring amino acids and vice versa. We know at present too little about other aspects, particularly those of steric convenience, that might have more decisive influences affecting the selective advantages of various possible codes. The arguments and mathematical results presented in this paper can do no more than to help clarify ideas and direct attention to the types of data on base sequences that would be most helpful.

Part II.

Mathematical Developments.

By S. W. Golomb and L. R. Welch.

1. Definitions and General Theorems.

Let n be a fixed positive integer, and consider an alphabet consisting of the numbers 1, 2 . . . n. With this alphabet form all possible k-letter words $(a_1 a_2 \ldots a_k)$, where k is also fixed. There are evidently n^k such words in all.

Def. A set L of k-letter words is called a *comma-free dictionary* if whenever $(a_1 a_2 \ldots a_k)$ and $(b_1 b_2 \ldots b_k)$ are in L, the "overlaps" $(a_2 a_3 \ldots a_k b_1)$, $(a_3 \ldots a_k b_1 b_2)$, ... , $(a_k b_1 \ldots b_{k-1})$ are not in L.

Let $W_k(n)$ denote the greatest number of words that such a dictionary can contain. In [8] the upper bound

$$W_k(n) \leq \frac{1}{k} \sum_{d/k} \mu(d)\, n^{k/d} \tag{1}$$

was obtained, where $\mu(d)$ is the Möbius function, and the summation is extended over all divisors d of k. It was further shown that the upper bound (1) is actually attained by some dictionary for all values of n when $k = 1, 3, 5, 7, 9, 11, 13, 15$, and this is *conjectured* to extend to all *odd* k. On the other hand, if k is *even*, the upper bound (1) is *not* attained by $W_k(n)$ for any $n > 3^{k/2}$. Specifically, for $k = 2$ it was demonstrated that $W_2(n) = \left[\frac{n^2}{3}\right]$, whereas (1) merely asserts that $W_2(n) \leq \frac{n^2 - n}{2}$. The treatment of the case $k = 2$ actually contained the method for finding

all possible comma-free dictionaries containing the maximum number of words, $W_2(n) = \left[\frac{n^2}{3}\right]$.

The primary objective here is to provide a constructive method for finding all maximum comma-free dictionaries for $k = 3$. Here the size of such a dictionary is $W_3(n) = \frac{n^3 - n}{3}$.

Before confining attention to the case $k = 3$, certain observations are appropriate concerning all dictionaries for which the upper bound (1) is attained.

Def. Two k-letter words are in the same *equivalence class* if they differ only by a cyclic permutation of the letters. (Thus *ABC*, *BCA*, *CAB* are all in the same equivalence class.) An equivalence class is *complete* if it contains k distinct members. (The example *ABC*, *BCA*, *CAB* is complete. The equivalence class of *AAA* is *degenerate*, containing only one member.)

Degenerate equivalence classes are never represented, and complete equivalence classes are represented at most once, in any comma-free dictionary, as shown in [7] and [8].

Theorem 0. The upper bound (1) is attained by a comma-free dictionary if and only if every complete equivalence class has *exactly* one representative in the dictionary.

Theorem 1. In any comma-free dictionary for which $W_k(n)$, the size of the dictionary, attains the upper bound (1), all n letters of the alphabet occur equally often among the $kW_k(n)$ letters in the dictionary. Also, each letter occurs in the same number of different words.

Proof. The dictionary contains one representative from each complete equivalence class. *Which* representative is inconsequential insofar as the letters comprising the individual words and the entire dictionary are concerned. Since any permutation of the alphabet leaves the class decomposition of the k-letter words invariant, permutation of the alphabet does not affect the distribution of letters in the dictionary, nor the frequency with which letters appear in distinct words. Therefore, these distributions and frequencies must be the same for all n letters of the alphabet.

Corollary. If all words containing a given letter are dropped from a maximum comma-free dictionary L attaining the upper bound (1) for $W_k(n)$, the remaining words form a maximal

comma-free dictionary L' for the $(n-1)$-letter alphabet. The number of different words of L in which a given letter appears is thus $W_k(n) - W_k(n-1)$.

Proof. The deletion of certain words from a comma-free dictionary cannot destroy the comma-free property. Thus expurgation of all words of L containing the letter X leaves a comma-free dictionary L' involving only an $(n-1)$-letter alphabet. Moreover, every complete equivalence class for these $n-1$ letters had a unique representative in L, which has not been dropped during the expurgation process. Thus L' is maximal by Theorem 0.

2. Classes of Dictionaries for $k = 3$.

The fundamental result concerning the structure of maximal comma-free dictionaries for $k = 3$ is expressed in the following theorem. Unless otherwise specified it will henceforth be assumed that $k = 3$.

Def. The first two letters of a word are an *initial digram*; the last two letters are a *final digram.*

Theorem 2. For $n > 2$, the necessary and sufficient condition that a collection of $(n^3 - n)/3$ words constitute a maximal comma-free dictionary is that no initial digram ever occurs as a final digram.

Proof. The condition is *sufficient* for maximal comma-freedom, since $(n^3 - n)/3$ is the correct dictionary size, and violation of comma-freedom from $(ABC)(DEF)$ could only occur if either BCD or CDE were in the dictionary along with ABC and DEF. But BCD begins with the final digram of ABC, and CDE ends with the initial digram of DEF.

To show *necessity*, suppose that ABC and BCD were both in the maximum comma-free dictionary L. To prevent conflicts, A must never end a word, nor D begin a word, and in particular, $A \neq D$. Therefore, the equivalence class $\{ AAX, AXA, XAA \}$ must be represented by AAX, and the class $\{ YDD, DYD, DDY \}$ must be represented by YDD, for all $X \neq A$ and $Y \neq D$. For any Z distinct from both A and D, consider the class $K = \{ ADZ, DZA, ZAD \}$. (Here the hypothesis $n > 2$ is required.) Observe that ADZ is contained in the overlap of $(AAX)(YDD)$ when $X = D$, $Y = Z$; while ZAD is in the overlap of $(AAX)(YDD)$ when $X = Z$,

$Y = A$. Also, DZA ends in A. Thus L can contain no representative of the class K, and by Theorem 0, L is not maximal. This contradiction completes the necessity proof.

Notes. 1) The case $n = 2$ is truly exceptional. Thus $\{110, 100\}$ is a maximal dictionary, although 10 occurs both initially and finally.

2) The proof of Theorem 2 can be used to show that a comma-free dictionary in which a digram occurs both initially and finally falls short of the maximum by at least $n-2$, corresponding to the $n-2$ choices of Z in (ADZ).

3) The method given in [8] to obtain maximum comma-free dictionaries for $k = 3$ uses all words XYZ satisfying $X < Y \geq Z$, where $<$ and $\geq$ refer to alphabetical ordering. It is clear that no initial digram could then be a final digram; and since the number of words satisfying the inequality is $(n^3-n)/3$, the dictionary is maximum comma-free by Theorem 2.

The following result may be remembered as a "law of the excluded middle".

Theorem 3. 1) In a maximum comma-free dictionary, every letter with one possible exception occurs in the middle position of some word.

2) If every letter is sometimes a middle letter, then every digram occurs, either initially or finally.

3) If A is the excluded middle letter, then every digram except AA occurs either initially or finally.

Proof. 1) If neither A nor B occurs as a middle letter, then the complete equivalence class AAB, ABA, BAA is unrepresented in the dictionary, contradicting maximality.

2) Suppose every letter can occur in the middle position, and CD is neither an initial nor a final digram. Let XCY be a word with C in the middle. Then XCD could be added into the dictionary, because XC is a legitimate initial digram, and CD, having never occurred initially, is a legitimate final digram. But this contradicts the assumption that the dictionary was already maximal.

Note. The corresponding result concerning the absence of initial and final letters is as follows:

In a maximum comma-free dictionary, every letter occurs both initially and finally, with at most one exception. If D is

exceptional, it occurs *either* initially *or* finally, and also occurs $n(n-1)$ times as a middle letter.

The proof of this assertion is inherent in the proof given subsequently for Theorem 9.

3) Suppose A never occurs in the middle position. Then the digram AA cannot occur initially or finally. However, any digram $CD \neq AA$ has one member (or both) unequal to A, and that member can be made to occur in the middle of a word, exactly as in 2) above.

Def. A *section* of a comma-free dictionary is the set of all words having a given middle letter.

Theorem 4. In a maximum comma-free dictionary, the following conditions hold for the sections.

1) The number s of sections is either n or $n-1$.
2) The number of words in each section has the form ij, with $i \leq n$, $j \leq n-1$.
3) $\sum_{\sigma} ij = (n^3-n)/3$, where σ runs through all sections.
4) $\sum_{\sigma} (i+j-1) = n^2-n$.

Proof.

1) This restates Theorem 3, part 1.
2) The words having a given middle letter D involve certain initial digrams ending with D, and certain final digrams beginning with D. By Theorem 2, all linkages of such initial and final digrams are consistent with comma-freedom, hence must occur in the interest of maximality. The size of the section is thus the number of initial digrams ending in D times the number of final digrams beginning with D. Each of these factors is at most n; and they cannot both equal n, since DD cannot occur both initially and finally.
3) The sum of the sizes of the parts equals the size of the whole.
4) In the section of size ij, the number of digrams used either initially or finally is $i+j$. By Theorem 3,

$$\sum_{\sigma} (i+j) = \begin{cases} n^2 & \text{if } s = n \\ n^2-1 & \text{if } s = n-1, \end{cases}$$

since there are n^2 possible digrams.

Therefore

$$\sum_{\sigma} (i+j-1) = n^2 - n.$$

Corollary 4.1. To obtain all maximum comma-free dictionaries for $k = 3$ and fixed n, it suffices to consider the dictionary size $(n^3 - n)/3$ partitioned into n or $n-1$ sections, each of size ij, with $i \leq n$, $j < n$, where the sum of the *weights* $(i+j-1)$ of the individual sections equals $n^2 - n$.

Corollary 4.2.

$$\sum_{\sigma} (i-1)(j-1) = 2\binom{n}{3}.$$

Example. When $n = 4$, the dictionary size is $(n^3 - n)/3 = 20$, and the sum of the weights of the sections is $n^2 - n = 12$. The only partitions of 20 into three or four sections of total weight 12 are:

I.	$20 = 12 + 6 + 2$,	$12 = 6 + 4 + 2$.
II.	$20 = 12 + 6 + 1 + 1$,	$12 = 6 + 4 + 1 + 1$.
III.	$20 = 12 + 4 + 4$,	$12 = 6 + 3 + 3$.
IV.	$20 = 9 + 9 + 2$,	$12 = 5 + 5 + 2$.
V.	$20 = 9 + 9 + 1 + 1$,	$12 = 5 + 5 + 1 + 1$.

Dictionaries of all five types exist, as shown (by sections and digrams) in Table I. [Added in proof: in a paper just published (Proc. Acad. Sci. Amsterdam, Series A, **61**: 253–258) H. Freudenthal obtained the same five types without use of our Theorem 2.]

TABLE I.

The five types of maximum comma-free dictionary for $k = 3$, $n = 4$. Braces indicate the reversible subcollections of Theorem 7.

I	II	III	IV	V
AD DA	*AD DA*	*AD DA*	*AD DA*	*AD DA*
BD DB	*BD DB*	*BD DB*	*BD DB*	*BD DB*
CD DC	*CD DC*	*CD DC*	*CD DD*	*CD DD*
DD	*DD*	*DD*		
AC CA	*AC CA*	*AC CA*	*AC CA*	*AC CA*
BC CB	*BC CB*	*BC CC*	*BC CB*	*BC CB*
CC	*CC*		*DC CC*	*DC CC*
AB BA	*BB BA*	*AB BA*	*AB BA*	*BB BA*
BB		*CB BB*	*BB*	
	AA AB			*AA AB*

3. Reversible Portions of Dictionaries.

Once the basic types of maximum comma-free dictionaries for $k = 3$ and fixed n are constructed, it is important to determine those transformations which preserve maximality, comma freedom, and type. Two obviously permissible transformations are permutation of the alphabet, and reading all words of the dictionary backwards. CRICK *et al.* [7] observed that certain *portions* of a dictionary may sometimes be read backwards. The aim of this chapter is a precise mathematical formulation of the circumstances under which portions of the dictionary may be so reversed, and a criterion for the number of *different* dictionaries which result from a given dictionary by the reversal process. The basic result appears as Theorem 6.

Def. Reversal of a word means writing it backward. *Reversal of a set* means writing every word in the set backward.

Def. A palindromic set is a subset of the dictionary which is setwise invariant under reversal. A *palindromic word* (or *palindrome*) is a word invariant under reversal.

Examples. The reverse of ABC is CBA.
The set (ABC, CBA) is palindromic.
The word ABA is a palindrome.

Def. A *reversible set* W is a subset of a maximum comma-free dictionary L such that simultaneous reversal of all members of W leaves maximality and comma-freedom intact.

Lemma A. Given a dictionary L,

1) Every palindromic set is reversible.
2) If ABC and CBA are distinct words in L, a reversible set containing either must contain both.
3) The intersection of a reversible set and a palindromic set is palindromic.

Proof.

1) The reversal of palindromic sets leaves L invariant.
2) If ABC is reversed and CBA is not, the dictionary size is reduced by one word, violating maximality.
3) Let M be palindromic and let W be reversible.

If ABC is in $M \cap W$, then CBA is in M by palindromicity, and in W by 2) above, so that CBA is in $M \cap W$.

Def. Two reversible sets are called *congruent* if they differ only by a palindromic set. (The effect on L of reversing the one is the same as reversing the other.)

Def. A subset T of L is *digram-closed* if for any digram AB occurring (either initially or finally) in some word of T, the digrams AB and BA never occur outside of T.

Example. In the dictionary,

$L = \{010, 011, 020, 021, 022, 120, 121, 122\}$, the subset $T_1 = \{010, 011\}$ is digram-closed, since none of the digrams 01, 10, 11 occur outside of T_1. However, the subset $T_2 = \{120, 121, 122\}$ is not digram-closed, because 20 occurs in T_2 while 20 and 02 occur outside of T_2.

Def. For any subset S of L, $\bar{S}$ will denote the complement of S in L. The largest palindromic set in L will be denoted by Q. (It is shown in Theorem 8 that $Q \neq L$)

Lemma. If S is reversible, if $S_1 = S \cap \bar{Q}$, and if T is the union of S_1 with the set of all palindromes and palindromic pairs having a digram in common with a word of S_1, then T is a digram-closed subset of L which is congruent to S.

Proof. Since T is congruent to S_1 and S_1 is congruent to S, T is congruent to S.

To prove that T is digram-closed, we first establish: *i*) If the digrams AB and BA (not necessarily distinct) are both initial or both final in L, then either no word of L or all words of L containing such digrams are in T.

Without loss of generality, suppose AB and BA are both initial, and ABC is in T. Then CBA is in the new dictionary Γ, so that BA is final in Γ. Therefore, every word of L beginning with BA belongs to T by Theorem 2. Since BA is assumed initial in L, some word BAD does exist in L, hence belongs to T. By repetition of the foregoing argument, using BAD instead of ABC, every word of L beginning with AB belongs to T, and *i*) is proved.

The lemma is next proved for the following case: *ii*) Let AB occur in a word of S_1, without loss of generality *initially*. Thus ABC is in S_1, for some C. Suppose that BA occurs as a digram in some word of $\bar{T}$. (The alternative is that BA occurs only in words

of T, which is treated later.) By *i*), the word of $\bar{T}$ in which BA occurs can be written XBA.

Since AB is initial and BA is final, maximality requires ABA to be in L. Whether or not ABA is reversed, AB is initial in Γ. By the construction of S_1, ABC in S_1 implies that CBA is not in L. But BA is final in L. Therefore, CB is not initial in L, and by Theorem 3 must be final. By *i*), every word containing BC or CB must be reversed. In particular, XB is initial and BC is final, so that XBC is in L, and must be reversed. Therefore BX is final in Γ.

Since Γ is maximal, with BX final and AB initial, it follows that ABX is in Γ. Since XBA was assumed to remain unreversed in the transition from L to Γ, ABX is in $\bar{T}$. But XBA and ABX cannot belong simultaneously to $\bar{T}$, by the definition of T. Therefore, every word containing BA is in T.

If $A = B$, case *ii*) is concluded, while if $A \neq B$ then BA occurs at least once, and repetition of the argument proves that every occurrence of AB is in T.

iii) It remains to consider ABC in $T \cap \bar{S}_1$ and neither AB nor BA is a digram in any word of S_1. Then by construction, CBA is in $T \cap \bar{S}_1$ and BC or CB occurs as a digram in some word of S_1. By *ii*), every occurrence of BC or CB is reversed in the transition from L to Γ. By Theorem 3, BB is a digram of L. Since CB is initial and BC is final, either BBC or CBB is in L, and is reversed. Because BB cannot be both initial and final, neither BBC nor CBB is in Q, and the one reversed is therefore in S_1. Case *ii*) now implies that every occurrence of BB is reversed, which covers both ABB and BBA. But not both of these are in L, so that one or the other is in S_1. This contradicts the assumptions of *iii*).

Theorem 5. A subcollection S of L is reversible if and only if S is congruent to a digram-closed subset T of L.

Proof. If S is reversible, it is equivalent to a digram-closed subset T of L by the foregoing Lemma.

Conversely, if T is digram-closed, reversing T will preserve the dictionary size, and will keep initial and final digrams separate. By Theorem 2, comma-freedom is thereby preserved. Thus if S is congruent to T, S is reversible.

Def. The palindromic sets in a dictionary L, and their complements relative to L, constitute the *palindromic algebra* P in L.

Theorem 6. The reversible sets of a dictionary L form a Boolean algebra R of subsets. The palindromic algebra P is a Boolean subalgebra of R which is in fact an ideal. The distinct dictionaries obtainable from L by reversing reversigle sets are in two-to-one correspondence with the elements of the quotient algebra R/P.

Proof. Any collection of subsets of a set L, closed under $\cup$ and $\cap$, containing L and $\emptyset$ and the complement of every set in the collection, is a Boolean algebra [11]. That both R and P contain L, $\emptyset$, and complements is clear.

The closure of P under unions and intersections is also obvious (cf. Lemma A, part 3). The closure of R under unions and intersections follows from Theorem 5. The fact that reversible sets are essentially closed with respect to digrams is preserved under union and intersection operations. Finally P is an ideal of R, because it is clearly a subring, and moreover if $M \in P$ and $W \in R$, them $M \cap W \in P$. The two-to-one correspondence arises because L itself may be reversed at will.

Theorem 7. To preserve comma-freedom, maximality, section sizes and weights under reversal of subsections of the dictionary L, it suffices to operate on the unmatched digrams in the individual sections of L.

Proof. This is a direct corollary of Theorems 4 and 5.

Example. See Table I for the reversible subsections corresponding to $k = 3$, $n = 4$.

Note. The group of reversals of Theorem 7, times the group of permutations on the n-letter alphabet, generate the complete set of comma-free dictionaries from the representative ones obtained in Corollary 4.1. For example, the five representative dictionaries for $n = 4$, shown in Table I, correspond to 408 distinct maximum comma-free dictionaries in all, as shown in Table II.

Theorem 8. For $k = 3$, no maximum comma-free dictionary is palindromic.

Proof. In a palindromic dictionary, if the digram XX occurs initially it must also occur finally. By Theorem 2, this is impossible for $n > 2$. Hence none of the digrams XX can occur, precluding maximality by Theorem 3. The case $n = 2$ is settled separately by observing that the only palindromic possibility, $\{ABA, BAB\}$, is not comma-free.

TABLE II.

Numbers of distinguishable maximum comma-free dictionaries for $k = 3$, $n = 4$.

Basic Dictionary	Permutations of Alphabet	Reversals	Product
I	24	8	192
II	12	8	96
III	12	4	48
IV	12	4	48
V	6	4	24
Totals: 5	66	28	408

4. Characteristics of Messages.

For the case $k = 3$, $n = 4$, it is important, for the biological applications, to obtain results concerning the possible messages written with words selected stochastically from the maximum comma-free dictionaries. The investigation here will assume $k = 3$, but n will remain free.

Theorem 9. In a message written from a maximum comma-free dictionary, every triple XYZ is capable of occurring unless $X = Y = Z$. The triple XXX *fails* to occur if and only if either

1) X is the excluded middle letter or
2) the section with X in the middle contains $n(n-1)$ words. (Thus all n^3 triples are capable of appearing in the message with at most two exceptions.)

Proof. Unless $X = Y = Z$, the equivalence class XYZ, YZX, ZXY is nondegenerate, hence represented in the dictionary. In a message, the representative word can appear consecutively, so that the other two members of its class appear in the overlaps.

If A is the excluded middle letter, it is clearly impossible to find AAA in the message. Suppose D is not an excluded middle letter, but DDD never occurs in messages. The digram DD occurs, without loss of generality, initially. Should any word end in D, the message could contain DDD. Thus every digram XD occurs initially.

Next, every digram DY occurs finally. For consider the effect of expurgating from the dictionary all words containing D. The residue is a maximum comma-free dictionary for $n-1$ letters (see

Corollary to Theorem 1). It contains $n(n-1)$ fewer words than the original dictionary. Adding the D-words back, there are $n(n-1-\alpha)$ words in the section for D, where α is the number of digrams DY not occurring finally. These occur initially in other sections, and contribute at most $\alpha(n-1)$ words. But $n(n-1-\alpha) + \alpha(n-1) < n(n-1)$ for $\alpha > 0$. Thus the section for D must contain the full $n(n-1)$ words, and there is at most one such section.

Examples. In the five dictionaries of Table I, all possible situations are illustrated. Thus patterns I and III exclude both AAA and DDD; pattern II excludes only DDD; pattern IV excludes only AAA; and in pattern V no triples are excluded.

Theorem 10. In a message written from a maximum comma-free dictionary, the sequences $XXXX$ will never appear.

Proof. In order for $XXXX$ to appear in the message, either XXX is a word in the dictionary, which is impossible, or XX ends one word and begins another, which is likewise impossible.

Theorem 11. Let L contain I initial digrams, F final digrams, and $N = \dfrac{n^3-n}{3}$ words in all. Of the n^4 possible quadruples of letters from the n-letter alphabet, exactly $IF + (i+f)N$ quadruples are capable of occurring in messages written from L. (Here i and f are the number of initial and final letters in L, respectively.)

Proof. An overlap quadruple $VXYZ$ breaks either as $(VXY)(Z\cdot\cdot)$ or $(\,.\,.VX)(YZ.\,.)$ or $(\,.\,.V)(XYZ)$. These possibilities are mutually exclusive by Theorem 2. The second case leads to IF quadruples. The first and third cases combine to yield $(i+f)\,N$ quadruples.

Corollary. Let $Q(n)$ denote the possible quadruples. Then

$$\frac{11\,n^4-8\,n^2}{12} \geqq Q(n) \geqq \frac{11\,n^4-4\,n^3-17\,n^2+10\,n}{12}.$$

Moreover, $Q(n)/n^4 \sim 11/12$ as $n \to \infty$.

Proof. As in Theorem 9, there is at most one letter which fails to occur both initially and finally.

Thus

$$(2\,n-1)\left(\frac{n^3-n}{3}\right) \leqq (i+f)\,N \leqq 2\,n\left(\frac{n^3-n}{3}\right).$$

Also, $I+F$ is either n^2 or n^2-1, and it is easily seen that

$$\left(\frac{n^2+n-2}{2}\right)\left(\frac{n^2-n}{2}\right) \leqq IF \leqq \left(\frac{n^2}{2}\right)^2.$$

Adding inequalities,

$$\frac{11\,n^4 - 4\,n^3 - 17\,n^2 + 10\,n}{12} \leqq Q(n) \leqq \frac{11\,n^4 - 8\,n^2}{12}.$$

The asymptotic relation follows immediately.

Example. When $n = 4$, $194 \leqq Q(4) \leqq 224$.

Both extremes are attained among the 408 dictionaries of Table II.

5. Missense and Nonsense.

Def. If one letter A of a word W is misread as B, the resulting word W^1 may be either in or out of the dictionary. In the former case the word W^1 is called *missense*, and in the latter case, *nonsense*.

Def. If for each individual occurrence of the letter A in a dictionary L, A is misread as B, the missense-to-nonsense ratio $\frac{M}{N}(AB)$ is the number of times $M(AB)$ that missense results, divided by the number of times $N(AB)$ that nonsense results.

Theorem 12. In any comma-free dictionary L which attains the size (1),

1) $\frac{M}{N}(AB) = \frac{M}{N}(BA)$,

2) $M(AB) + N(AB) = \frac{k}{n} W_k(n)$,

3) $M(AX) = M(AY)$ whenever X and Y occur symmetrically in L.

Proof. By Theorem 1, A occurs a total of $\frac{k}{n} W_k(n)$ times in L. Since misreading A as B produces either missense or nonsense disjunctively, $M(AB) + N(AB) = \frac{k}{n} W_k(n)$, which is 2). By the symmetry of missense, $M(AB) = M(BA)$ independent of the comma-free hypothesis. By 2), $M(AB) + N(AB) = M(BA) + N(BA)$. Combining these relations yields 1). Statement 3) is self-evident.

Example. For the five dictionaries of Table I, the missense-to-nonsense structure is shown in Table III.

TABLE III.

Missense/Nonsense for the Five Patterns with $k = 3$, $n = 4$.

Dictionary	Transitions											
	AB	*AC*	*AD*	*BA*	*BC*	*BD*	*CA*	*CB*	*CD*	*DA*	*DB*	*DC*
I	13/2	9/6	3/12	13/2	11/4	5/10	9/6	11/4	9/6	3/12	5/10	9/6
II	12/3	10/5	4/11	12/3	10/5	4/11	10/5	10/5	9/6	4/11	4/11	9/6
III	11/4	11/4	3/12	11/4	8/7	7/8	11/4	8/7	7/8	3/12	7/8	7/8
IV	13/2	6/9	6/9	13/2	8/7	8/7	6/9	8/7	4/11	6/9	8/7	4/11
V	12/3	7/8	7/8	12/3	7/8	7/8	7/8	7/8	4/11	7/8	7/8	4/11

These figures can be summed over certain sets of errors, corresponding to probable misprints. The results of four such summations are shown in Table IV.

TABLE IV.

Missense/Nonsense Summed over Classes of Transitions.

Dictionary	Transitions			
	Unrestricted	$(AB)(CD)$	$(AC)(BD)$	$(AD)(BC)$
I	100 / 80	44 / 16	28 / 32	28 / 32
II	98 / 82	42 / 18	28 / 32	28 / 32
III	94 / 86	36 / 24	36 / 24	22 / 38
IV	90 / 90	34 / 26	28 / 32	28 / 32
V	88 / 92	32 / 28	28 / 32	28 / 32

Remarks. Reversals of the type in Theorem 7, as well as permutations of the alphabet, leave the total missense-to-nonsense ratio of the dictionary unchanged. The numbers shown in Table IV are invariant under the permutation-and-reversal group which generates 408 dictionaries from the five patterns in Table I.

6. Extensions to Larger Values of *k*.

Theorem 2 furnishes the key to the structure of maximum comma-free dictionaries when $k = 3$. Some partial analogues to Theorem 2 for the case $k = 5$ and beyond will now be presented.

Theorem 13. A necessary condition that L be a maximum comma-free dictionary for $k = 5$ is that no initial tetragram be also a final tetragram. (This holds for *all* values of n).

Proof. Suppose $A\gamma$ and γB are both in L, where γ is a tetragram. Then as in Theorem 2, no word can end in A nor begin in B, and $A \neq B$. Thus the words $AABBB$, $AAABB$, and $AAAAB$ must be in L as the representatives of their classes. The equivalence class $ABABB$, $BABBA$, $ABBAB$, $BBABA$, $BABAB$ is then unrepresented, because $BABBA$, $BBABA$, and $BABAB$ begin in B, while $ABABB$ is found in the overlap of $(AAAAB)$ $(ABBBB)$, and $ABBAB$ is found in the overlap of $(AAABB)$ $(ABBBB)$. Thus L could not be maximal.

Note. As in Theorem 2, the constraint that no initial digram be a final digram, nor any initial tetragram a final tetragram, suffices for comma-freedom. It is not likely that the digram condition is *necessary* when $k = 5$. However, the upper-bound dictionary for $k = 5$ given in [8] consisted of all words $ABCDE$ from the ordered n-letter alphabet $1, 2, \ldots, n$ satisfying either

$$A < B \geqq C,\ D \geqq E \text{ or } A < B < C < D \geqq E,$$

wherein initial digrams and initial tetragrams cannot occur finally.

Theorem 14. For all $k \geq 5$, a necessary condition for L to be a comma-free dictionary which attains the upper bound (1) is that no initial $(k-1)$-gram occur finally.

Proof. Let L be maximum comma-free, and suppose γ is a $(k-1)$-gram such that both $A\gamma$ and γB are in L. Then as in Theorem 13, all "duplex" words of the type $ABB \cdots BB$, $AAB \cdots BB$, $\cdots$, $AAA \cdots AB$ must be in L. Consider the equivalence class containing $ABABB \cdots B$. The only cyclic permutations not beginning with B, and hence possible candidates for inclusion in L, are $ABABB \cdots B$ itself, and $ABB \cdots BAB$. Each of these, however, occurs in the overlaps of successive "duplex" words.

Speculation. All dictionaries in [8] which attained the upper bound (1) for odd k have the property that no initial i-gram is a final i-gram for any even $i < k$. To what extent this characterizes the maximum comma-free dictionaries is not known. Theorem 2 shows that for $k = 3$, this property is characteristic, *except* when $n = 2$. It is reasonable to hypothesize the possibility of finding maximum dictionaries satisfying the condition of non-ambiguity of even-grams for *all* odd k. The conjecture that for odd $k > 3$ these are the *only* maximum comma-free dictionaries is less likely.

7. Transposable Dictionaries.

The chemical structure of deoxyribonucleic acid (*DNA*) is known to comprise two oppositely polarized strands, here viewed as sequences whose terms are the four bases (here labelled *A*, *B*, *C*, *D*) in seemingly random order, except that *A* in one strand is always opposite *B* in the other, and *C* in one strand is always opposite *D* in the other. (This situation is illustrated in Figure 1.)

It is quite possible that a 180° rotation of this configuration would give rise to new ambiguities, so that in addition to the comma-free condition, an auxiliary restriction should be imposed

Fig. 1. The two oppositite polarized strands of *DNA*.

on the dictionary structure. These considerations motivate the definitions and theorems which follow.

Def. In an alphabet in which there is an even number n of letters, to each letter X can be assigned a *transpose* letter X' such that $X \neq X'$ and $(X')' = X$. There are then $\frac{n}{2}$ *pairs* of transpose letters.

Def. Given the word or letter sequence $AB \cdots G$, the *transpose* is defined to be $(AB \cdots G)' = G' \cdots B'A'$. A word or collection of words transformed back into itself under transposition is called *symmetric*.

Def. Given a comma-free dictionary L, the *transpose dictionary* L' contains the transposes of the words of L. Further, L is called *transposable* provided that no word of L can appear as a word of L' nor as an overlap of two words of L'.

Theorem 15. The number of words in a comma-free transposable dictionary L, containing k-letter words from an n-letter alphabet, does not exceed one-half the number of non-symmetric complete equivalence classes.

Proof. Every complete class $(AB \cdots FG,\ B \cdots FGA, \cdots, GAB \cdots F)$ has a transpose class $(G'F' \cdots B'A',\ A'G'F' \cdots B', \cdots, F' \cdots B'A'G')$. If $AB \cdots FG$ is in L, then $G'F' \cdots B'A'$ is in L',

and looking at the overlaps of $(G'F'\cdots B'A')$ $(G'F'\cdots B'A')$ the entire transpose class is seen to occur. Thus, representing one class in L precludes the representation of its transpose class. If the class is symmetric, it is its own transpose class, and cannot be represented at all. Otherwise, either the original class, the transpose class, or neither, but not both, may be represented in L. Hence the theorem.

Corollary. For odd k, the size $S_k(n)$ of a comma-free transposable dictionary satisfies

$$S_k(n) \leq \frac{1}{2k} \sum_{d/k} \mu(d)\, n^{k/d}. \tag{2}$$

For even k, (2) holds with strict inequality.

Proof. The statement for odd k simply combines the inequality (1) with Theorem 15. The strict inequality for even k devolves from the fact that there are always symmetric, complete classes—e.g. the class of $AA\cdots AA'A'\cdots A'$, which is clearly symmetric, and complete because $A \neq A'$.

Note. A careful count of symmetric classes shows

$S_2(n) \leq (n^2-2n)/4$ and $S_4(n) \leq (n^4-3n^2+2n)/8$. [Added in proof: The general result is

$$S_k(n) \leq \frac{1}{2k} \sum_{d/k} \mu(d)\, n^{k/d} - \frac{1}{4} \sum_{2d/k} \mu(d)\, n^{k/2d}.]$$

Theorem 16. For $k = 3$ and even n, there exist comma-free transposable dictionaries which attain the size $\dfrac{n^3-n}{6}$, which corresponds to the upper bound (2) when $k = 3$.

Proof. In each transpose pair X, X', designate one member as *primary* and the other as *secondary*. (This designation can be performed in any of $2^{n/2}$ ways.) With the $\dfrac{n}{2}$ primary letters, form a maximum comma-free dictionary of

$$\frac{\left(\frac{n}{2}\right)^3 - \frac{n}{2}}{3} = \frac{n^3}{24} - \frac{n}{6}$$

three-letter words. This is Part I of the new dictionary. Adjoin Part II, consisting of all sequences of primary-secondary-primary

letters. There are $\frac{n}{2}$ choices for each of the three positions, hence $\frac{n^3}{8}$ words in Part II. Part I and Part II are clearly disjoint, and their union is a dictionary L containing $\frac{n^3-n}{6}$ words.

No initial digram of Part II can occur finally in L, because no word of L ends in a secondary letter. No final digram of Part II occurs initially in L, because no word of L begins with a secondary letter. Thus the only violations of comma-freedom in L are within Part I, but Part I was comma-free by hypothesis.

It remains to show that L is transposable. Transposes of words from Part I consist entirely of secondary letters. Transposes of words from Part II have the pattern secondary-primary-secondary. Hence any three consecutive letters in a message written using words of the transpose dictionary L' will include at least two secondary letters. Since words of L contain at most one secondary letter each, L is transposable.

Caution. If $n = 4$, there are only 2 primary letters. A comma-free dictionary using these two letters and not satisfying the digram condition of Theorem 2 must not be used as Part I of a transposable dictionary. Not only does the proof of Theorem 16 break down in such cases, but the conclusion itself is false.

Examples. For the four-letter alphabet A, B, C, D, with $A' = B$, $C' = D$, suppose that A and C are designated as primary. The

TABLE V.

The three patterns for transposable comma-free dictionaries, for the case $k = 3$, $n = 4$.

	1.	2.	3.
Part I	*A A C*	*A A C*	*A A C*
	C A C	*C C A*	*C A C*
Part II	*A B A*	*A B A*	*A B B*
	A B C	*A B C*	*A B C*
	C B A	*C B A*	*C B B*
	C B C	*C B C*	*C B C*
	A D A	*A D A*	*A D B*
	A D C	*A D C*	*A D C*
	C D A	*C D A*	*C D B*
	C D C	*C D C*	*C D C*

first two dictionaries of Table V are formed in accordance with Theorem 16, while the third dictionary is an independently discovered example.

Given any transposable comma-free dictionary in Table V, a symmetry group H containing sixteen operators can be applied to obtain new dictionaries. This group is generated by the three alphabetic permutations (AB), (CD), and $(AC)(BD)$, and by the operation of reading all words backward. The eight alphabetical permutations form a group isomorphic to the dihedral group of the square with consecutive vertices $ACBD$. Under the operations of H, dictionaries 1. and 3. in Table V lead to sixteen dictionaries each. However, dictionary 2. leads to only eight dictionaries, because the permutation $(AC)(BD)$ leaves dictionary 2. unchanged. Thus, Table V yields a total of forty distinct transposable comma-free dictionaries for the case of $k = 3$, $n = 4$, and these forty are believed to exhaust the possibilities.

All forty of the ten-word dictionaries just described satisfy the digram condition of Theorem 2. Moreover, each is embeddable in one or more of the 408 twenty-word dictionaries obtainable from Table I. The extent to which these digram and embeddability properties are fortuitous is not known.

For $k = 4$, considerably less is known. For $k = 4$, $n = 4$, the note following Theorem 15 yields 27 as an upper bound to the size of a transposable comma-free dictionary. No such example has been constructed. In Table VI, however, a 26-word dictionary for this case is exhibited. Part I of this dictionary was obtained from an 18-word maximal comma-free dictionary using only A, B and C, from which two words were omitted because of the transposability constraint. In Part II, representatives of ten of the remaining eleven complete non-symmetric classes appear. They were chosen to represent their classes according to the following criteria:

1) D never begins or ends a word,
2) D occurs as the second letter,
3) In case of competing candidates, the one with the fewest D's is chosen.

The 27th class, which contains $A\ D\ B\ C$, cannot be added consistently to the dictionary already formed.

TABLE VI.

A comma-free transposable dictionary for $k = 4$, $n = 4$, containing 26 words. (One non-symmetric complete class is unrepresented.)

Part I		Part II	
A B B B	*A C C B*	*C D C B*	*B D C C*
A C B A	*A C C C*	*C D B C*	*C D D B*
A C B B	*B A C A*	*C D C A*	*B D B C*
A C B C	*B B C A*	*C D C C*	*C D B B*
A A C A	*B B C B*	*A D C C*	*B D C B*
A B C A	*B C C A*		
A B C B	*B C C B*		
A C C A	*B C C C*		

In contrast to the equal frequency of letters (Theorem 1) in maximum comma-free dictionaries, the dictionary of Table VI contains *A*, *B*, *C*, *D*, with the respective frequencies 21, 31, 41, 11. The dictionaries of Table V are also seen to deviate significantly from equal letter frequency. The proper analog of Theorem 1 for the case of transposable dictionaries asserts that every *transpose pair* of letters is represented equally often in a dictionary containing words from all the non-symmetric complete equivalence classes. The sharing of representation by members of the same pair is less rigidly constrained.

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UNTERSUCHUNGEN ÜBER DETERMINATION UND DIFFERENZIERUNG

6. ÜBER DEN AUFBAU DES ZELLWANDMUSTERS DES BLATTES VON *HELODEA DENSA*

VON

P. BOYSEN JENSEN

With an English Summary

København 1959
i kommission hos Ejnar Munksgaard

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Synopsis.

Der Bau des *Helodea*blattes ist sehr einfach. Es besteht mit Ausnahme des Mittelnervs nur aus zwei Schichten von kistenförmigen Zellen.

Die Initialzellen der Blattanlagen treten auf dem Vegetationskegel als horizontale erhabene Wülste hervor. Ein Längsschnitt durch den Vegetationskegel zeigt, dass ein solcher Wulst aus zwei Zellreihen besteht, die sich je zu der oberen und unteren Zellschicht des Blattes entwickeln.

Die Initialzellen entstehen aus den Epidermiszellen durch Änderung der Wachstumsrichtung der Zellwände und zwar in folgender Weise:

Epidermiszellen		Blattzellen	
Tangentiale Zellwände	Diffuses Wachstum (Longit. + tangent. W.)	Transversale Antikline	Orientiertes, transversales Wachstum
Radiale Zellwände	Orientiertes longitudinales Wachstum	Longitudinale Antikline	Orientiertes, longitudin. Wachstum
Transvers. Zellwände	Orientiertes tangential. Wachstum	Obere, mittlere und untere Zellwände	Diffuses Wachstum (Longit. + transvers. Wachstum).

Die Veränderungen der Wachstumsrichtung entstehen wahrscheinlich durch eine Umlagerung der Zellulosenbildner an der Oberfläche der Papillen, die in die Zellwände hineinragen.

Die Blattanlagen bilden ein bestimmtes Muster, so dass schliesslich 4-gliedrige Quirle hervorgehen.

Die Entwicklung der einzelnen Zellen während der Blattbildung am Vegetationskegel kann nicht durch die Einwirkung des schon vorhandenen Musters in Verbindung mit der Reaktionsfähigkeit der Zellen erklärt werden. Man wird zu der Annahme gedrängt, dass ein ganzheitschaffender Faktor von nicht physikalischer Natur bei den Differenzierungsvorgängen im Vegetationskegel beteiligt ist.

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1. Einleitung.

Wenn man wünscht zu verstehen, was Leben ist, muss man versuchen, sich den eigentlichen Lebensäusserungen so viel wie möglich zu nähern. Als eigentliche Lebensäusserungen betrachten wir solche, die mit dem Organismus an sich, d. h. mit der Struktur desselben, verknüpft sind, und die nicht *in vitro* verlaufen können.

Die Lebenserscheinungen sind natürlich an das Plasma geknüpft. Damit aber dieselben in planmässiger Weise sollen verlaufen können, muss bei den Pflanzen das Plasma in einem zweckdienlichen[1] Zellwandgerüst untergebracht sein. Dieses Gerüst wird von dem Plasma gebildet. Der Aufbau des Zellwandmusters gehört zu den eigentlichen Lebensäusserungen in dem oben erwähnten Sinne. Zwar ist es möglich, dass der Aufbau von Zellulose *in vitro* wird verlaufen können, es ist aber ausgeschlossen, dass der Aufbau des Zellwandmusters in einem leblosen Medium stattfinden kann. Die Erforschung dieses Aufbaues ist daher sehr wohl geeignet, uns zu einem näheren Verständnis der Arbeitsweise des Plasmas zu verhelfen.

Tatsächlich sind die Differenzierungsvorgänge in den Pflanzen hauptsächlich mit dem Aufbau des Zellwandmusters verknüpft. Die Inhaltsstoffe und die Organellen sind im grossen und ganzen dieselben in allen lebenden Zellen. Sowohl über- als unterirdische Organe können Chlorophyll, Carotin, Stärke u.s.w. bilden. Der

[1] Eine jede Pflanze ist in eine bestimmte Umwelt eingebaut. Damit eine Pflanze sich soll behaupten können, muss ihr Zellwandmuster so gebaut sein, dass sie die notwendigen Nährstoffe und die notwendige Energie von der Umgebung aufnehmen kann, dass sie ferner diese und andere von ihr selbst erzeugten Stoffe in den Körper herumtransportieren kann, und schliesslich dass sie sich gegen zu starken Wasserverlust schützen kann. Um sich fortpflanzen zu können, muss eine Pflanze häufig durch sehr komplizierte Vorgänge Spermazellen von einer anderen Pflanze derselben Art zugeführt bekommen. Auch diese Vorgänge werden durch Ausbildung besonderer Organe, der Blüten, mit sehr komplizierten Zellwandmustern ermöglicht (vgl. Boysen Jensen 1939).

wichtigste Unterschied zwischen den verschiedenen Plasmasorten ist, dass das Wurzelplasma ein Wurzelzellwandmuster, das Sprossplasma ein Sprosszellwandmuster und das Blütenplasma ein Blütenzellwandmuster aufbaut. Diese Differenzierungen sind nicht mit Änderungen des Gengehaltes in den Zellen verknüpft, was daraus hervorgeht, dass sie in gewissen Fällen reversibel sind. Es können z. B. bei gewissen Pflanzen Sprosse an Wurzeln gebildet werden.

Die spezifische Gestalt einer Pflanze ist somit vornehmlich durch den Aufbau des Zellwandmusters ihrer Organe bestimmt. Es sind ferner die Unterschiede zwischen den spezifischen Gestalten der 150.000 verschiedenen Arten von Samenpflanzen letzten Endes durch den verschiedenartigen Aufbau ihrer Zellwandmuster verursacht. Es geht wohl hieraus zur Genüge hervor, dass Untersuchungen über den Aufbau des Zellwandmusters für das Verständnis der Gestaltungsprobleme innerhalb der Pflanzenwelt von fundamentaler Bedeutung sind.

2. Der Aufbau des Zellwandmusters bei *Helodea densa.*

Als Beispiel des Aufbaues eines Zellwandmusters, soll die Entwicklung einer sehr einfach gebauten Blütenpflanze, nämlich *Helodea densa*, in Kürze beschrieben werden (vgl. Abb. 1).

Die Sprossachse. Der Vegetationskegel oberhalb der Blattanlagen besteht, wie es aus Abb. 2,1 hervorgeht, aus einer zweischichtigen Tunica und dem Korpus.

Die Epidermis, die den Scheitel des Kegels bedeckt, ist in Abb. 2,2 dargestellt. In dem unteren Teil befindet sich eine grössere Zelle, die sich soeben geteilt hat, übrigens sind die Zellen ungefähr gleich, eine bestimmte Anordnung derselben ist nicht zu beobachten; es besteht doch vielleicht eine Neigung zur Bildung von longitudinalen Zellreihen.

In den Abb. 2,3,1, 3,2 finden sich Flächen-, Längs- und Querschnitte der Epidermis, die den übrigen Teil des Vegetationskegels bedeckt. Es geht aus diesen Abbildungen hervor, dass die Epidermis aus Zellen besteht, die unmittelbar vor der Zellteilung ungefähr kubisch, nach der Zellteilung prismatisch sind. Die Zellen sind im grossen und ganzen in longitudinalen Reihen angeordnet, doch kaum so regelmässig wie in der Wurzelepidermis.

Abb. 1. Medianer Längsschnitt durch die Sprossachse von *Helodea densa*. Zwischen den Blättern sind Schleimschuppen (S). Die eckigen Figuren im Stengel sind Interzellularräume. Zeichenapparat 36/1.

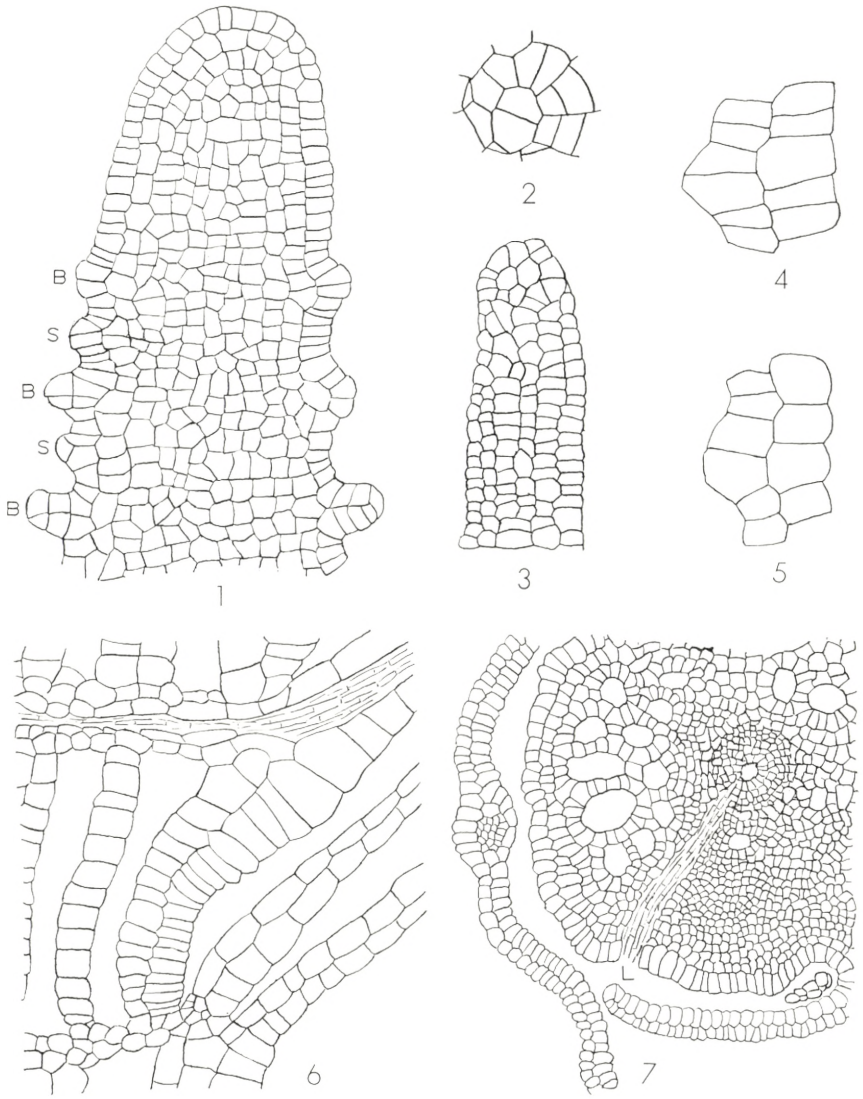

Abb. 2. *1* Medianer Längsschnitt durch den Vegetationskegel. B Blattanlagen. S Schleimschuppen. *2* Scheitel des Kegels. *3* Epidermis des Kegels. *4, 5* Junge Blattanlagen. *6* Längsschnitt der Sprossachse, weiter unten. *7* Querschnitt der Sprossachse. Der Schnitt geht oben rechts durch ein Internodium, unten links durch eine Querscheibe. L Leitbündel.

Zeichenapparat 1, 2, 3 235/1; 4, 5 500/1; 6 98/1; 7 36/1.

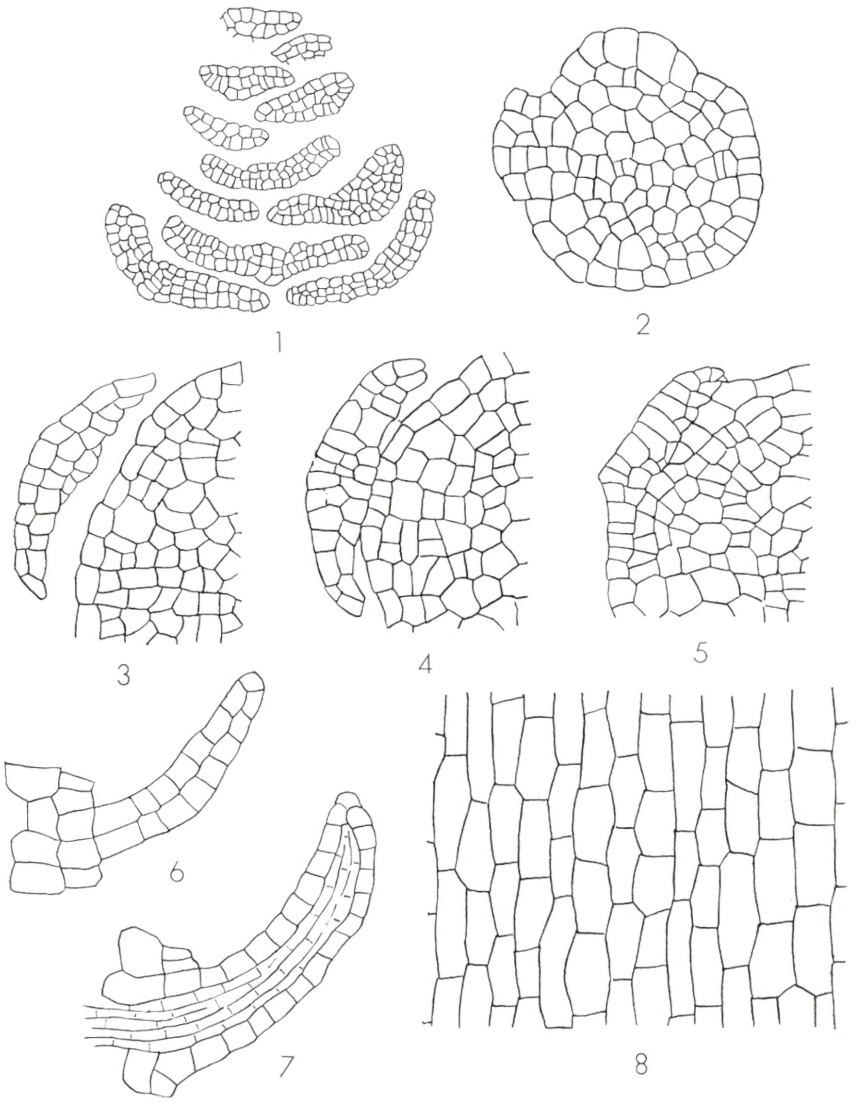

Abb. 3. *1* Flächenschnitt des Vegetationskegels mit Blattanlagen. *2* Querschnitt des Kegels mit Blattanlagen. *3*, *4*, *5* Dasselbe, weiter unten. *6* Junges Blatt, seitlicher Längsschnitt. *7* Junges Blatt, medianer Längsschnitt. *8* Obere Zellschicht eines fertigen Blattes.

Zeichenapparat 1 98/1; 2, 3, 4, 5, 6, 7 235/1; 8 98/1.

Für das Verständnis der Blätterbildung ist es von Wichtigkeit, über das Wachstum der verschiedenen Zellwände der Epidermiszellen klar zu sein. Die tangentialen Zellwände (die senkrecht auf den Radien des Kegels stehen) wachsen sowohl longitudinal als tangential (weil das Durchschnittsareal des Kegels fortwährend zunimmt). Die radialen Zellwände wachsen in longitudinale, aber nicht in radiale Richtung. Die transversalen Zellwände wachsen in tangentiale, aber nicht in radiale Richtung (vgl. Abb. 7 a). Bei den Zellteilungen werden neue radiale und transversale, aber keine tangentialen (periklinen) Zellwände gebildet.

Die Zellen der subepidermalen Zellschicht wachsen und teilen sich in ähnlicher Weise wie die Epidermiszellen.

Der Korpus besteht aus kubischen oder prismatischen Zellen, die mehr oder weniger regelmässig in Längsreihen angeordnet sind. Sie teilen sich durch tangentiale, radiale und transversale Zellwände.

In einem Abstande von 0,2 mm vor der Spitze des Kegels beginnen die Blattanlagen, deren Entwicklung später besprochen werden soll. Die Anordnung und Grösse der Zellen in dem Korpus werden nun nach und nach unregelmässiger. Einige Zellen teilen sich in derselben Weise wie in dem Vegetationskegel, andere Zellen wachsen ohne sich zu teilen, behalten aber ihre isodiametrische Form, und wieder andere Zellen strecken sich, häufig in transversale Richtung. Ferner entstehen durch wiederholte Teilungen innerhalb einer grösseren Zelle Leitbündelelemente, die sich zu Leitbündeln zusammenfügen. Auffallend ist es, dass das Querschnittareal der Sprossachse trotz der Ungleichartigkeit der Zellen regelmässig zunimmt.

Weiter unten, etwa 0,9 mm von der Spitze der Kegels, treten in der Rinde der künftigen Internodien kleine, schizogene, isodiametrische Interzellularen auf. Bald beginnen die Zellen der Internodien in longitudinale Richtung zu wachsen, indem sie sich gleichzeitig durch transversale Wände teilen. Die Interzellularen werden dadurch zu Zylindern, die durch ein- oder mehrschichtige Wände, die aus isodiametrischen Zellen gebildet werden, voneinander getrennt sind (Abb. 2, 6, 7). Zwischen den Internodien liegen dünne, transversale Scheiben, die von Parenchymzellen gebildet werden (Abb. 2, 6 und 2, 7 links unten).

Der zentrale Teil der Sprossachse besteht aus Parenchymgewebe. In der Mitte desselben liegt ein stark reduzierter Leitbündel, der aus kurzen, dünnen, ungefähr zylindrischen, plasmagefüllten Zellen gebildet wird. Zwischen diesen kommen dünne Tracheiden mit spiralförmigen Verdickungen vor. Man kann das Leitbündel nach oben bis zu 0,8 mm von der Spitze verfolgen. In den dünnen Scheiben von parenchymatischem Gewebe, die die Internodien voneinander trennen, gehen Verzweigungen des Leitbündels zu den Blättern aus (Abb. 2,7 links unten).

Die Bildung der Blätter. Auf einem Flächenschnitt des Vegetationskegels (Abb. 3,1) findet man in verschiedener Höhe horizontale, nach oben schwach konkave, erhabene Wülste, die je aus zwei horizontalen Zellreihen gebildet sind. Weil der äusserste Teil der Wülste nach oben gebogen ist, sind jedoch in einigen derselben mehr als zwei Zellreihen von dem Schnitt getroffen worden. Die Zellen dieser Wülste sind die Initialzellen der Blätter, die mit Ausnahme des Mittelnervs nur zwei Zellschichten enthalten. Die Blätter stehen in Quirlen von vier (oder drei), die in ungefähr derselben Höhe sitzen. Von jedem Quirl sieht man doch in dem Flächenschnitte nur ein oder zwei Blattanlagen, die übrigen stehen an den Flanken oder an der Rückseite des Kegels. Die einzelnen Blattanlagen liegen wie Inseln auf dem Vegetationskegel, sie sind durch Epidermiszellen, die nicht zur Entwicklung kommen, voneinander getrennt. Die Blätter der Quirle und somit auch die Gruppen von Initialzellen alternieren miteinander.

Einen näheren Einblick in die Entwicklung des Blattes erhält man aus Längs- und Querschnitten des Vegetationskegels. Am Längsschnitte (Abb. 2,1,4,5)[1] sieht man in einem Abstande von etwa 0,2 mm von der Spitze zwei Zellen, die etwas grösser als die Nachbarzellen nach oben und unten sind, und deren Spitze über die Oberfläche des Kegels hervorragen. Diese Zellen sind zwei Zellen des kleinsten Wulstes in Abb. 3,1. Wie es aus dem Längsschnitt hervorgeht, entstehen die Wülste dadurch, dass die drei transversalen Wände der künftigen Initialzellen in zentrifugale Richtung wachsen; sie werden später zu der oberen, der mittleren und der unteren Zellwandfläche des Blattes. Die Zellen des

[1] Vgl. die Abbildungen von Herrig 1914—15. Es fehlen in seiner Zeichnung die Schleimschuppen. Wahrscheinlich sind sie weiter unten am Vegetationskegel entwickelt.

Vegetationskegels, die in derselben Höhe wie die Initialzellen liegen, entwicklen sich ferner zu den Scheiben, die die Internodien voneinander trennen (vgl. S. 8). Wie es aus Abb. 2,6 hervorgeht, bestehen sie bisweilen wie die Wülste nur aus zwei Zellschichten.

Ein Querschnitt durch eine ganz junge Blattanlage gibt ein entsprechendes Bild (Abb. 3,2). Oben sieht man den ersten Anfang einer Blattanlage. Es haben sich nur die radialen Wände gestreckt, Zellteilungen sind noch nicht entstanden. In der Blattanlage links ist die Entwicklung weiter fortgeschritten. Die Zellen haben sich geteilt, aber man kann noch sehen, dass die radialen Zellwände der Epidermiszellen des Kegels sich in die Blattanlagen hinein fortsetzen; sie entwickeln sich später zu einigen der longitudinalen Antikline in dem Blatte. An Querschnitten, die weiter unten liegen, erhält man, weil die jungen Blätter nach oben gebogen sind, Querschnitte sowohl des Vegetationskegels als der Blätter. An Schnitten, die aufeinander folgen, kann man feststellen, dass die jungen Blattanlagen ungefähr herzförmig sind. Sie beginnen oben mit einer Spitze, dann breitet die Blattfläche sich aus, und schliesslich geht sie in die Epidermis der Sprossachse über (Abb. 3,3,4,5).

In der subepidermalen Zellschicht kann eine Zellreihe, die unmittelbar unter den Initialzellen liegt, anfangen zu wachsen, und sich bisweilen durch Zellwände zu teilen, so dass die junge Blattanlage schwach hervorgewölbt wird. Die Blattbildung verläuft am häuftigsten in dieser Weise (Abb. 2,1, die Blattanlage rechts unten). Es kann jedoch auch ein Blatt entstehen ohne jegliche Veränderung in der subepidermalen Zellschicht (Abb. 3,6). Wenn Wachstum und Zellteilungen in der subepidermalen Zellschicht entstehen, so treten die neugebildeten Zellen jedoch normalerweise nicht in das Blatt ein, das wie oben bemerkt nur zwei Zellschichten enthält und somit ausschliesslich aus Epidermiszellen gebildet ist. Nur in der Mitte des Blattes läuft ein Nerv in das Blatt hinein, und es wird dadurch an diesen Stellen mehrschichtig.

Die weitere Entwicklung der Blattanlage geschieht nun in folgender Weise (Abb. 3,6). Von den zwei Zellreihen von Initialzellen teilt die eine sich häufig durch eine schiefe Querwand, so dass eine Scheitelzelle gebildet wird. Das Blatt wächst jedoch nicht, oder jedenfalls nicht ausschliesslich, in der Spitze. Man kann in

dem basalen Teil des sich entwickelnden Blattes Mitosen finden. Während der Entwicklung des Blattes werden sowohl longitudinale als transversale Antikline gebildet.

Schon wenn die Blattanlage eine Länge von 1—2 mm erreicht hat, sind die meisten Zellen des Blattes angelegt, und die Zellen fangen an sich zu strecken. Die Streckung beginnt in der Spitze der Blätter und schreitet allmählich gegen die Basis fort. Gleichzeitig entsteht eine Differenzierung, indem die antiklinen Zellwände in die Höhe wachsen, an der Oberseite etwas mehr als an der Unterseite.

Wie es aus dem Querschnitt eines Blattes in Abb. 2,7 hervorgeht, findet sich in der Mitte des Blattes ein Leitbündel. In dem medianen Längsschnitt eines jungen Blattes in Abb. 3,7 (2,6) sieht man, wie die Leitbündelzellen sich in das Blatt hineinstrecken.

Das fertige Blatt ist sehr einfach gebaut. In der Spitze und an einigen Stellen an den Flanken finden sich dornähnliche Haare die durch Ausstülpung aus den Randzellen und durch Verdickung der Zellwände entstanden sind. In der Spitze des Blattes sind die Zellen polyedrisch und liegen etwas unregelmässig in 5—7 Zellreihen. Bald aber nimmt die Anzahl der Zellreihen stark zu, und das Blatt erreicht eine konstante Breite. Die Zellen werden kistenförmig und liegen in regelmässigen Längsreihen (Abb. 3,8). Die Zellen der Unterseite sind kleiner als diejenigen der Oberseite, sind aber in ähnlicher Weise angeordnet.

Wenn die Internodien anfangen zu wachsen, strecken die Epidermiszellen derselben und die Zellen auf der Unterseite des basalen Teiles der Blätter sich senkrecht zur Oberfläche. Es wird dadurch ein Kissen gebildet, das dazu dient, die Ansatzstelle des Blattes zu unterstützen (Abb. 2,6).

Zwischen den Blättern entstehen die Schleimschuppen. Wenn man die Blätter in der Spitze eines Sprosses mit einer Augenpinzette entfernt, bleiben sie zurück, und man kann sie dann mit einer schwachen Vergrösserung *in situ* beobachten, wenn man den Stengel unter das Mikroskop legt. Längsschnitte der Schleimschuppen finden sich in Abb. 1, 2,1. Die Schleimschuppen werden in ähnlicher Weise wie die Blätter als zwei Initialzellen im Epidermis angelegt, der Entwicklungsverlauf ist aber ein ganz anderer. Die fertigen Schuppen sind ziemlich kleine, kreis- oder eiförmige,

zweischichtige Gebilde. Die Determination derselben muss somit eine ganz andere sein als diejenige der Blätter. Der Name Schleimschuppe, den ich in der Literatur gefunden habe (RAUNKIÆR 1895—99) gibt ihre Funktion an. In einem Längsschnitt durch die Sprossachse, der auf Gefriermikrotom hergestellt war, konnte ich sehen, dass die Spitze des Vegetationskegels mit einer dünnen Schleimschicht bedeckt war. Dieser Schleim ist wahrscheinlich von den Schleimschuppen gebildet.

Die Schleimschuppen werden ungefähr in der Mitte zwischen zwei Blättern angelegt. Die Internodien entstehen zwischen den Schleimschuppen und den obenstehenden Blättern. Die Schuppen enthalten daher ihren Platz in den Blattachseln der untenstehenden Blätter.

Ausser den erwähnten Organen kann die *Helodea*pflanze auch Wurzeln und Blüten bilden. Die Entwicklung dieser Organe soll nicht besprochen werden.

Es geht aus diser kurzen Darstellung der Entwicklung der *Helodea*pflanze hervor, dass selbst bei dieser sehr einfach gebauten Pflanze eine sehr grosse Anzahl von Gestaltungsproblemen mit dem Aufbau des Zellwandmusters verknüpft ist. Von diesen Problemen soll nur eines heraus gegriffen werden, nämlich die Bildung der Blätter.

Um dieses Problem zu erhellen, soll zunächst untersucht werden, in welcher Weise die Blattbildung durch Indolylessigsäure beeinflusst wird.

3. Die Einwirkung von Indolylessigsäure auf die Entwicklung des *Helodea*blattes.

Die *Helodea*sprosse wurden von einer Gärtnerei, die Aquarienpflanzen kultiviert, bezogen. Sprossspitzen mit einer Länge von 6—10 cm wurden in Krystallisierschalen mit einem Diameter von 12,5 cm gezüchtet. In jede Schale kamen 3 Sprosse. Die Spitzen der Sprosse 2 und 3 wurden über die Basis des vorhergehenden Sprosses in der in Abb. 4 dargestellten Weise gelegt, so dass man das Wachstum jedes einzelnen Sprosses verfolgen konnte. Um die Sprosse reichlich mit Kohlensäure zu versorgen, wurde die Schale nur mit so viel Flüssigkeit beschickt, dass die Spitze der Sprosse

eben in der Wasseroberfläche lag. Die Sprossen wuchsen in horizontaler Richtung, geotropische Krümmungen traten nicht ein, doch konnte bisweilen eine schwache Krümmung des Vegetationskegels beobachtet werden. Die Schalen standen in dem Fenster eines Nordzimmers. Die Versuche wurden im Sommer und Herbst ausgeführt.

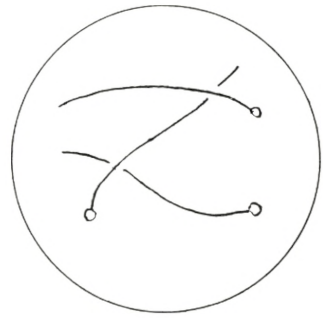

Abb. 4. Krysrallisierschale mit *Helodea*pflanzen.

Eine jede Versuchsserie durchlief 3 Perioden von etwa 10 Tagen, eine a-Periode in reiner Nährlösung, eine b-Periode mit Nährlösung, zu welcher Indolylessigsäure zugefügt wurde, und drittens eine c-Periode wieder mit reiner Nährlösung. Die letztere bestand aus 1 l Leitungswasser + 10 ccm A + 5 ccm B + 10 ccm C. A enthielt in 1 l Leitungswasser 100 g $Ca(NO_3)_2$, 25 g KCl, 25 g $MgSO_4$ und 5 g KH_2PO_4; B enthielt pro l 8 g Ferrizitrat und C 0,1 g Borsäure, 0,1 g $MnSO_4$, 0,01 g $CuSO_4$ und 0,01 g $ZnSO_4$, gleichfalls pro l. Auf der Oberfläche der Nährlösung wurde häufig eine dünne Schicht von nicht gelösten Salzen ausgeschieden. Diese wurde mit Filtrierpapier entfernt.

Es wurde in einigen Fällen sowohl der Zuwachs der ganzen Sprosse als auch derjenige der äussersten Spitzen gemessen. Um diese letztere Messung ausführen zu können, wurde am Schlusse der a-Periode ein dünner Zwirn um die Sprossachse so nahe der Spitze wie möglich gewickelt.

Der Zuwachs in der a-Periode beträgt etwa 1,6 mm pro Tag. Bei dem Vergleich des Zuwachses in den verschiedenen Perioden, muss man vielleicht berücksichtigen, dass die Länge der Sprosse sich ändert, und dass diese Änderungen möglicherweise die Grösse des Zuwachses beeinflussen können.

Nach dem Abschluss der verschiedenen Perioden wurden die Sprosse mit CRAF (eine von RANDOLPH modifizierte NAVASHIN Lösung) fixiert, in Paraffin eingebettet, und in Serienschnitte mit einer Dicke von 13 μ zerlegt. Die Schnitte wurden mit Methylviolett 6 B gefärbt.

Es ist leider nicht möglich, die Wachstumsvorgänge der Zellwände direkt im Mikroskope zu verfolgen. Es ist zwar leicht mit

Tabelle 1. Wirkung von Indolylessigsäure.

Zuwachs pro Tag in mm.

	Total			Spitze		
IES pct.	a	b	c	a	b	c
0,001	1,4	5,6	2,6			1,8
0,0003	1,7	4,8	3,1			1,0
—	1,6	2,8	2,5		1,8	2,0
0,0001	1,8	5,1	3,2			1,7

einer Augenpinzette den Vegetationskegel freizulegen. Derselbe ist aber so dick, dass man nur die Wülste der Initialzellen, aber nicht die einzelnen Zellen beobachten kann.

Die Wirkung von IES auf das Wachstum der Sprosse ist in Tab. 1 dargestellt. Es geht aus der Tabelle hervor, dass der Zuwachs in IES-Lösungen (in der b-Periode), selbst in starken Konzentrationen vergrössert wird. Nach Überführung in normaler Nährlösung sinkt die Wachstumsgeschwindigkeit ungefähr zu dem normalen Wert. Die Sprosse sind somit durch die Behandlung mit IES nicht geschädigt worden.

Eine mikroskopische Untersuchung zeigt, dass die Differenzierung im Vegetationskegel, wie es aus der Zuwachsgeschwindigkeit zu erwarten ist, normal verläuft. Es sind doch gewisse kleinere Unterschiede zwischen den normalen und den mit IES behandelten Sprossen vorhanden. In den letzteren ist die Entwicklung der Blätter etwas verzögert, während die Streckung der Internodien bisweilen, aber nicht immer, früher eintreten kann als bei normalen Pflanzen (vgl. Abb, 5).

Das wichtigste Ergebnis dieser Untersuchungen ist jedoch, dass die Differenzierungsvorgänge, die mit der Bildung der Blätter verknüpft sind, durch die Wuchsstoffkonzentration gar nicht beeinflusst werden.

4. Schlussfolgerungen.

a. Die Faktoren, die das Wachstum der Zellwände beeinflussen.

Ehe wir auf die Bildung der Initialzellen des *Helodea*blattes näher eingehen, ist es notwendig zu untersuchen, in welcher Weise das Wachstum der Zellwände zustandekommt. Dieser Vorgang

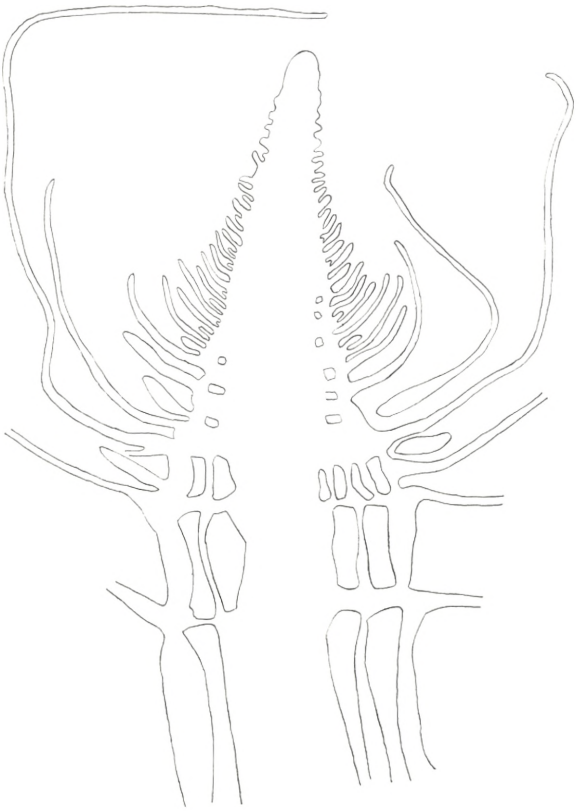

Abb. 5. Medianer Längsschnitt durch eine mit IES behandelte *Helodea*pflanze. Zeichenapparat 36/1.

besteht vornehmlich in der Bildung neuer Zellwandstoffe. Der Aufbau derselben dürfte von den folgenden Komponenten bedingt sein: 1) Rohstoffe, aus denen die Zellwandstoffe gebildet werden, wahrscheinlich entweder Glykose-l-phosphat oder Uridin-diphosphat-glykose. 2) Enzyme, die diese Rohstoffe in Zellwandstoffe umbilden können. Ich habe diese Enzyme Zellulosenbildner genannt. 3) Wuchsstoffe (IES), die die Verteilung und die Anordnung der Zellulosenbildner ändern und dadurch die Wachstumsweise und Wachstumsgeschwindigkeit der Zellwände beeinflussen können (Boysen Jensen 1958).

Ausserdem ist für das Wachstum der Zellwände ein Turgordruck notwendig. Dieser wird dadurch aufrechterhalten, dass osmotisch wirksame Stoffe in die Vakuole hineingepresst werden.

Hinsichtlich der Anordnung der Zellulosenbildner habe ich in einer früheren Abhandlung (Boysen Jensen 1958) dargelegt, dass man annehmen muss, dass dieselben auf den Ektodesmen oder Plasmodesmen, d. h. auf den Plasmapapillen, die in die Zellwände hineinragen oder dieselben durchsetzen, liegen, so dass die neugebildeten Zellulosefibrillen zwischen den schon vorhandenen eingeschoben werden.

Es ist nun von Wichtigkeit zu wissen, welcher von den oben genannten Faktoren das Wachstum der Zellwände in dem Vegetationskegel begrenzt. Man darf vermuten, dass es entweder die Menge der Zellulosenbildner oder die Wuchsstoffkonzentration ist. Aus den oben angeführten Versuchen geht hervor, das die Differenzierung in dem Vegetationskegel von IES gar nicht beeinflusst wird. Man kann hieraus schliessen, dass in dem Meristem Wuchsstoff in genügender Menge vorhanden ist. Es müssen somit die Differenzierungsvorgänge in dem Vegetationskegel in der einen oder anderen Weise mit den Zellulosenbildnern verknüpft sein[1].

b. Die Wachstumsweise der Zellwand.

Ich habe früher geglaubt (Boysen Jensen 1958), dass eine Vermehrung der Menge der Zellulosenbildner genügen könnte, um eine Organbildung, z. B. die Entwicklung eines *Helodea*blattes, hervorzurufen. Eine nähere Überlegung zeigt jedoch, dass der Verlauf dieses Vorgangs weit mehr kompliziert ist.

Um dieses zu erhellen, muss man die Wachstumsweise der Zellwände betrachten. Wir wählen als Beispiel ein Prothallium von *Pteris*, das, wie bekannt, einschichtig ist. Das Zellwandsystem besteht aus einer oberen und einer unteren, horizontalen Zellwandfläche, die mit vertikalen periklinen und antiklinen Zellwänden verbunden sind. Während die oberen und unteren Zellwände in allen Richtungen wachsen, so wachsen die periklinen und antiklinen Zellwände in die Länge, dagegen nicht in die Höhe. Wir können somit zwei Haupttypen der Wachstumsweise der Zellwände unterscheiden: die diffuse Wachstumsweise, die in allen Richtungen vorgeht, und die orientierte Wachstumsweise,

[1] Es hätte vielleicht auch die Wirkung des Kinetins untersucht werden sollen (vgl. Skoog and Miller 1957). Es steht jedoch fest, dass lokale Veränderungen des Wachstums der Zellwände kaum durch lösliche Stoffe, sondern nur durch verschiebbare, unlösliche Verbindungen hervorgerufen werden können.

die nur in einer oder zwei Richtungen verläuft. Wenn nun eine Zelle, z. B. eine Epidermiszelle in dem Vegetationskegel von *Helodea*, von tangentialen, radialen und transversalen Zellwänden umgeben ist, wachsen diese häufig in drei verschiedenen Weisen.

c. Die Entstehung und Entwicklung des *Helodea*blattes.

1. *Die Entstehung der Initialzellen.* Wir wenden uns nun zu den Vorgängen, die zur Entwicklung der Initialzellen des *Helodea*blattes führen.

Eine schematische Darstellung der Entstehung der Initialzellen findet sich in Abb. 6 (vgl. den Abb. 2,1,4,5, 3,1,2). 1 und 2 sind zwei Epidermiszellen des Vegetationskegels. Wenn die beiden Zellen in longitudinale Richtung wachsen und sich teilen,

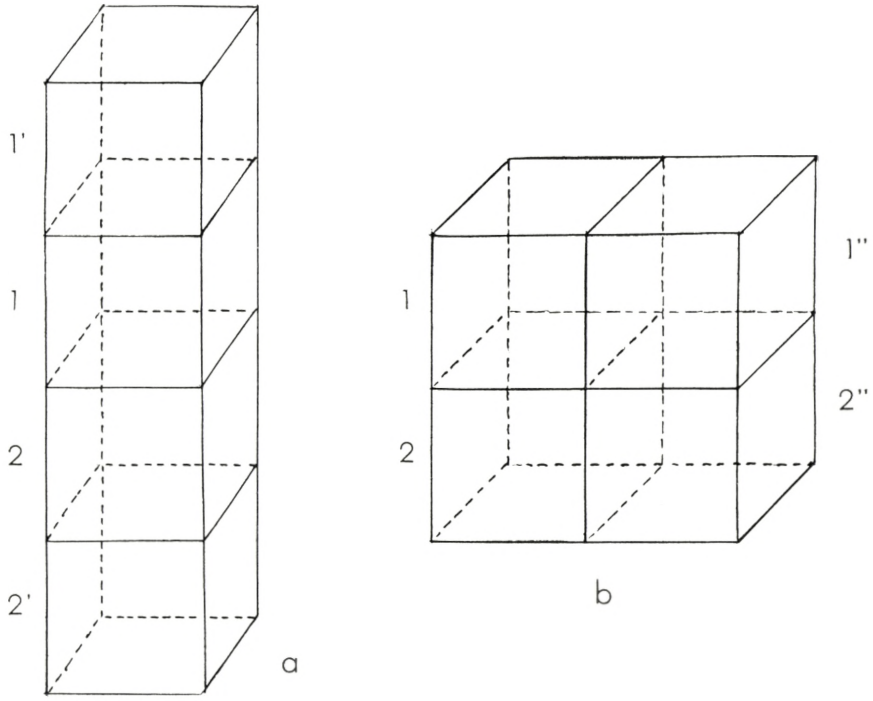

Abb. 6. Schematische Darstellung des Wachstums der Epidermiszellen bei normalem Längenwachstum (a) und bei der Bildung der Initialzellen eines Blattes (b).

Tabelle 2.

Epidermiszellen		Blattzellen	
Tangentiale Zellwände	Diffuses Wachstum (Longit. + tangent. W.)	Transversale Antikline	Orientiertes, transversales Wachstum
Radiale Zellwände	Orientiertes longitudinales Wachstum	Longitudinale Antikline	Orientiertes longitudin. Wachstum
Transvers. Zellwände	Orientiertes, tangential. Wachstum	obere, mittlere und untere Zellw.	Diffuses Wachstum (Longit. + transv. Wachstum)

entstehen zwei neue Epidermiszellen 1′ und 2′. Sie können aber auch in zentrifugale Richtung wachsen, und es entstehen dann die Initialzellen eines Blattes 1″ und 2″. Es geht aus der Abb. hervor, dass die Initialzellen durch Änderungen der Wachstumsrichtungen der Zellwände der Epidermiszellen entstehen.

In der schematischen Abb. 6 sind die Wachstumsänderungen der Zellwände bei der Bildung der Initialzellen dargestellt, wie sie aus einem Längsschnitt hervorgehen. In den Epidermiszellen findet doch neben dem longitudinalen Wachstum auch ein tangentiales Wachstum statt, und in dem sich entwickelnden Blatte ist neben einem longitudinalen Wachstum auch ein transversales Wachstum vorhanden. Alle diese Wachstumsvorgänge sind in Tab. 2 zusammengefasst.

In der Abb. 7 ist eine Zelle der Epidermis und eine Zelle der unteren Zellschicht des Blattes gezeichnet. Die Pfeile stellen die Wachstumsrichtungen dar.

Gleichzeitig mit der Änderung der Wachstumsrichtung der Zellwände muss wahrscheinlich auch eine Änderung der Orientierung der Zellulosefibrillen eintreten. Namentlich in den radialen Zellwänden, wo die Orientierungsrichtung vollkommen umschlägt, sollte die Möglichkeit vorhanden sein, diese Änderungen polarisationsmikroskopisch nachzuweisen. Weil aber die Doppeltbrechung in den embryonalen Zellwänden sehr schwach ist, sind die Untersuchungen mit grossen Schwierigkeiten verknüpft, und es ist noch nicht gelungen, entscheidende Ergebnisse zu erhalten. Wenn ich aber bessere Instrumente bekommen habe, hoffe ich, dass ich auf diese Untersuchungen zurückkommen kann.

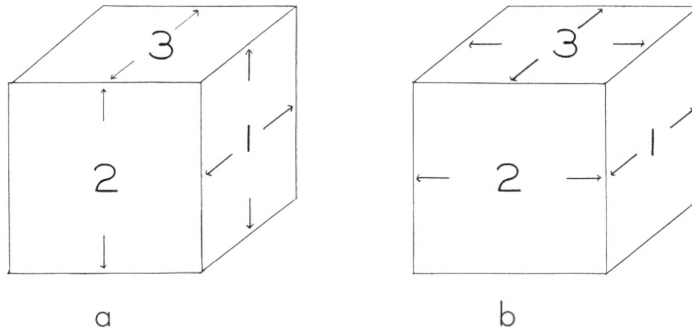

Abb. 7. Die Wachstumsrichtungen der Zellwände in einer normalen Epidermiszelle (a) und in einer Blattzelle (b). (a) 1) tangentiale, 2) radiale, 3) transversale Zellwand. (b) 1) transversale Antikline, 2) longitudinale Antikline, 3) mittlere Zellwand.

Es ist wohl auch möglich, dass diese Probleme nur durch elektronenmikroskopische Untersuchungen gelöst werden können.

Dass aber eine Umschaltung der Wachstumsrichtung der Zellwände stattfindet, ist sicher. Es erhebt sich daher die Frage, wie eine solche Umschaltung stattfinden kann. Wie oben erwähnt, müssen die Differenzierungsvorgänge im Vegetationskegel, d.h. die Umschaltung der Wachstumsrichtung der Zellwände, in der einen oder anderen Weise mit den Zellulosenbildnern verknüpft sein. Soll nun in einer Zellwand mit einer bestimmten Wachstumsrichtung dieselbe verändert werden, ist es wahrscheinlich, dass diese Änderung durch eine Verlagerung der Zellulosenbildner an den Plasmapapillen erzeugt wird. Man darf vermuten, dass die Zellulosenbildner in Zellwänden, die in allen Richtungen wachsen, gleichmässig rings um die Papillen verteilt sind. Soll nun ein solches diffuses Wachstum in ein orientiertes Wachstum verändert werden, muss wahrscheinlich eine Verschiebung der Zellulosenbildner stattfinden, so dass sie vorzugsweise an den Flanken der Papillen zu liegen kommen.

Dass eine Verlagerung von Zellulosenbildnern stattfinden kann, geht aus Versuchen mit Wurzeln hervor. Man kann nachweisen, dass die Zellulosenbildner sich an dem apikalen Ende der Trichoblasten ansammeln, bevor ein Wurzelhaar gebildet wird (Boysen Jensen 1950). In Wurzelhaaren kann man Verschiebungen der Zellulosenbildner durch Colchicin, IES und andere Stoffe hervorrufen (Boysen Jensen 1955). Man kann

sogar beobachten, dass das System der Zellulosenbildner hin und her pendeln kann (Boysen Jensen 1957).

Aus Versuchen über die Wirkungsweise des Wuchsstoffes in *Phaseolus*epikotylen (Boysen Jensen 1958) geht hervor, dass das System der Zellulosenbildner sich ausdehnen und kontrahieren kann, je nachdem Wuchstoff fehlt oder vorhanden ist. Das System der Zellulosenbildner ist daher kontraktibel, und man kann daher vermuten, dass die Verlagerung der Zellulosenbildner in den Trichoblasten und vielleicht auch an den Papillen bei der Bildung der Initialzellen durch Kontraktion der Zellulosenbildner zustande kommt. Es ist doch von Wichtigkeit hervorzuheben, dass die Verlagerungen der Zellulosenbildner bei der Bildung der Initialzellen des *Helodea*blattes weit mehr kompliziert sind als in den Trichoblasten und in den Zellen des *Phaseolus*epikotyls. In den Zellen des Vegetationskegels von *Helodea* muss die Verlagerung in den verschiedenen Gruppen von Zellwänden ungleichartig verlaufen, und in jeder Gruppe muss sie gleichzeitig in gleicher Weise an allen Papillen der Zellwände eintreten.

Es ist in diesem Zusammenhang von Interesse, dass man in Prothalien von *Pteris longifoloa* eine »Drehung« der Wachstumsrichtung der Zellwände hervorrufen kann. Die anti- und periklinen Zellwände wachsen normalerweise nur in die Länge und nicht in die Höhe, durch Colchicin kann man bewirken, dass sie in die Höhe wachsen, so dass senkrechte Prothallïumteile gebildet werden.

2. *Der weitere Verlauf der Entwicklung des Helodeablattes.* Damit nun aus den Initialzellen ein Blatt entstehen können soll, muss doch nicht nur eine qualitative Umschaltung der Wachstumsrichtungen der Zellwände stattfinden, sondern es müssen auch die Wachstumsgeschwindigkeiten der verschiedenen Zellwände quantitativ aufeinander abgestimmt sein, so dass das Wachstum des Zellverbandes harmonisch verläuft. Da die Wachstumsgeschwindigkeit durch die Menge der Zellulosenbildner begrenzt wird, darf man vermuten, dass die Steuerung derselben durch eine Regelung der Neubildung und Anordnung von Zellulosenbildnern zustandekommt. Erst wenn die Blätter eine gewisse Grösse erreicht haben, wird eine grössere Menge von Zellulosenbildnern erzeugt, es tritt nun eine Streckung der Blattzellen ein, wobei der Wuchsstoff als begrenzender Faktor wirkt.

3. *Die Musterbildung.* Die Musterbildung entsteht einmal da-

durch, dass nur bestimmte Bezirke sich zu Initialzellen und ferner zu Blättern entwickeln, während die Entwicklung in den umgebenden, anscheinend vollkommen gleichartigen Zellen ausbleibt. Die Blattanlagen entstehen ferner an bestimmten Orten, sie fügen sich zu einem Muster zusammen, so dass schliesslich 4-gliedrige alternierende Blattquirle gebildet werden.

d. Ausblick.

Die Blattbildung bei *Helodea* findet in einem embryonalen Gewebe statt, und man darf es daher für wahrscheinlich halten, dass auch andere Seitenorgane, die in embryonalem Gewebe entstehen, in ähnlicher Weise wie die *Helodea*blätter gebildet werden.

Wir untersuchen zunächst die Bildung exogener Seitenorgane. Diese können entweder aus der Epidermis oder den subepidermalen Zellschichten hervorgehen.

Die Blätter von *Helodea* sind mit Ausnahme des Mittelnervs reine Epidermisbildungen. In ähnlicher Weise entstehen auch Sporangien (Wilson 1958) und Haare (vgl. Abb. 7, 11 in Esau 1953) durch Umschaltung der Wachstumsrichtung antikliner Wände in den Epidermiszellen.

Die Bildung der Blätter bei der Hauptmenge der Blütenpflanzen verläuft prinzipiell in derselben Weise wie bei *Helodea*. Der Unterschied besteht darin, dass die Entwicklung in einer oder mehreren der subepidermalen Zellschichten beginnt, indem die transversalen und radialen Wände derselben in zentrifugale Richtung wachsen. Die Zellen werden dadurch senkrecht zur Oberfläche des Stengels gestreckt und teilen sich durch perikline Zellwände. Es entsteht in dieser Weise ein kleiner Höcker. Die Rolle des Epidermis ist in diesem Falle mehr passiv; durch Flächenwachstum folgt sie dem Wachstum des Höckers nach, so dass derselbe fortwährend von der Epidermis überzogen wird (vgl. die Abb. 5.5 in Esau 1953). Wie es aus der Form des Blattes von *S. Tübingense* hervorgeht, ist die Epidermis doch nicht ein rein passiver Überzug der inneren Gewebe, sondern sie ist bei der Gestaltung der Blätter — wenn auch in beschränktem Umfang — aktiv beteiligt.

Wahrscheinlich werden auch Knospen und Blütenteile in ähnlicher Weise wie die Blätter gebildet.

Endogene Organe, z. B. Seitenwurzeln, entstehen in ähnlicher Weise durch Umschaltung der Wachstumsrichtung der transversalen und radialen Zellwände in dem Pericykel (vgl. Abb. 17.11 in Esau 1953).

e. Zusammenfassung.

Hinsichtlich der Differenzierungsvorgänge in dem Vegetationskegel von *Helodea densa* und wohl auch in anderen embryonalen Geweben sind wir somit zu den folgenden Ergebnissen gelangt.

1. Die Differenzierung in embryonalem Gewebe ist mit den Wachstumsvorgängen der Zellwände verknüpft.

2. Die Wachstumsvorgänge der Zellwände in embryonalem Gewebe wird durch die Menge und die Anordnung der Zellulosenbildner geregelt.

3. Die Entstehung der Initialzellen der Blätter von *Helodea* geschieht durch eine Umschaltung der Wachstumsrichtung der Zellwände in horizontalen, scharf begrenzten Bezirken, die durch Zellen, die sich nicht entwickeln, voneinander getrennt sind.

4. Die Umschaltung der Wachstumsrichtung der Zellwände entsteht wahrscheinlich durch eine Umlagerung der Zellulosenbildner an den Plasmapapillen.

5. Die weitere Entwicklung des Blattes geschieht dadurch, dass das Wachstum der Zellwände der einzelnen Zellen qualitativ und quantitativ geregelt wird, so dass sie sich zu dem fertigen Blatt zusammenfügen.

6. Die Bezirke, die sich zu Blätter entwickeln, fügen sich zu einem bestimmten Muster zusammen, so dass die Blätter in viergliedrigen Quirlen, die durch Internodien voneinander getrennt sind, zu stehen kommen.

Das entscheidende bei der Blattbildung von *Helodea* ist somit nicht — oder jedenfalls nicht ausschliesslich — eine ungleiche Verteilung der Menge der Zellulosenbildner in den verschiedenen Zellen, sondern eine Umlagerung der Zellulosenbildner in den verschiedenen Zellwänden der einzelnen Zellen, wodurch ihre Wachstumsrichtung verändert wird.

Für den Aufbau des Zellwandmusters sind jedoch nicht allein das Wachstum der Zellwände, sondern auch Zellteilungen und Bildung neuer Zellwände von grosser Bedeutung. Bei den Differenzierungsvorgängen in *Helodea* und den S. 21 besprochenen Pflanzen ist jedoch das Wachstum der Zellwände der primäre Vorgang. Die Lage der neuen Zellwand ist vornehmlich durch die Wachstumsrichtung der sich teilenden Zelle bestimmt.

Die mit dem Aufbau des Zellwandmusters verknüpften Gestaltungsvorgänge dürften die einzigen sein, bei denen es möglich sein wird, die Analyse bis auf lokalisierte und orientierte enzymatische Vorgänge zurückzuführen.

f. Der determinierende Faktor.

Die Vorgänge, die zu einer Blattbildung bei *Helodea* führen, sind anscheinend von überraschender Einfachheit. Die Schwierigkeiten fangen aber sofort an, wenn man zu untersuchen beginnt, warum diese Vorgänge eintreten, und in welcher Weise sie geregelt werden.

Man nimmt im allgemeinen an, dass die Entwicklung einer Zelle einmal durch die Reaktionsfähigkeit die Zelle und ferner durch die Einwirkung der umgebenden Zellen bestimmt ist. Im Einklang mit dieser Auffassung könnte man sich vorstellen, dass die Entstehung der Initialzellengruppen an dem Vegetationskegel von *Helodea* durch die Einwirkung der älteren Blattanlagen hervorgerufen (induziert) würde. Der induzierende Faktor müsste wohl dann chemischer Natur sein.

Ist es nun möglich, dass chemische Stoffe eine derartige Umschaltung der Wachstumsrichtungen der Zellwände hervorrufen können?

Hierauf muss man wohl antworten, dass es nur möglich ist, wenn die Umschaltung der Wachstumsrichtungen als ein Bereitschaftskomplex in den Initialzellen vorhanden ist, so dass sie als Folge einer chemischen Einwirkung zwangsläufig eintreten muss.

Als Beispiel eines solchen Bereitschaftskomplexes kann die Kernteilung dienen. Diese besteht aus einer Reihe von Vorgängen, die zwangsläufig aufeinander folgen. Dieser Komplex liegt im Protoplasma bereit, und kann entweder autonom eintreten oder durch verschiedene Stoffe in Gang gesetzt werden. Man könnte sich vorstellen, dass die Möglichkeit einer Umschaltung des Wachstums der Zellwände in ähnlicher Weise in den Epidermiszellen des Vegetationskegels der *Helodea*pflanzen vorhanden sein könnte, und dass sie durch einen spezifischen Stoff, der von den älteren Blattanlagen abgegeben würde, ausgelöst würde.

Mit dieser Hypothese würde man doch nur das Problem verschieben, aber nicht lösen. Weil man sich schwierig vorstellen kann, dass dieser Bereitschaftkomplex, wie es mit der Fähigkeit zur Kernteilung der Fall ist, in allen jungen Zellen der *Helodea*pflanze vorhanden ist, wird man sich sofort fragen müssen, wie er in den Epidermiszellen des Vegetationskegels entstanden ist.

Aber selbst wenn man diese Frage vernachlässigen wollte, bleiben andere, grosse Schwierigkeiten übrig.

Die einzelne Initialzelle ist nur ein kleiner Teil des sich entwickelnden Blattes. Selbst wenn dieses, wie oben erwähnt, ziemlich einfach gebaut ist, sind doch gewisse Differenzierungen vorhanden. Die Zellen an der Ober- und Unterseite, an der Spitze und am Blattrande sind verschieden gebaut. Es genügt daher nicht, dass die Wachstumsrichtungen der Zellwände in den Initialzellen umgeschaltet werden, sondern es muss die Entwicklung jeder einzelnen Zelle während des Wachstums des Blattes qualitativ und quantitativ gesteuert werden, so dass die Zellen sich schliesslich zu dem Zellwandmuster des fertigen Blattes zusammenfügen. Diese Steuerung ist während der ganzen Entwicklung des Blattes tätig, ihr Verlauf kann nicht »vorausgesehen« werden, sie kann nicht als ein Bereitschaftskomplex vorhanden sein.

Aber noch andere Schwierigkeiten sind vorhanden. Wie oben erwähnt, werden zwischen den Blättern Schleimschuppen gebildet. Diese werden in derselben Weise wie die Blätter angelegt, aber die Entwicklungsfolge ist ganz verschieden von derjenigen der Blätter. Es gibt somit verschiedene Formen von Steuerungen, die Steuerung bei der Entwicklung der Schleimschuppen ist eine andere als diejenige bei der Entwicklung der Blätter.

Und schliesslich haben wir dann das Problem der Musterbildung. Die Blattanlagen entstehen dadurch, dass bestimmte Bezirke von Epidermiszellen sich zu Initialzellen entwickeln. Wenn nun die älteren Blätter Stoffe erzeugen, die diese Umwandlung der Epidermiszellen hervorrufen können, warum reagieren dann nicht alle Epidermiszellen auf diesen Reiz, und werden zu Initialzellen umgebildet, und warum ordnen sich die Blattanlagen in ein bestimmtes Muster, so dass schliesslich Blattquirle entstehen?

Noch schwieriger ist es die Entwicklung der Seitenknospen zu verstehen. Diese entstehen entweder endogen oder exogen. In beiden Fällen bestehen sie ursprünglich aus gleichartigen Zellen. An diesen entstehen dann gleichzeitig zwei Gruppen von Initialzellen, die sich zu Blättern entwickeln. Es kann weder das eine dieser Blätter das andere induzieren, noch können sie von anderen Organen induziert sein. Das Muster muss somit autonom geschaffen oder zu Entfaltung gebracht werden.

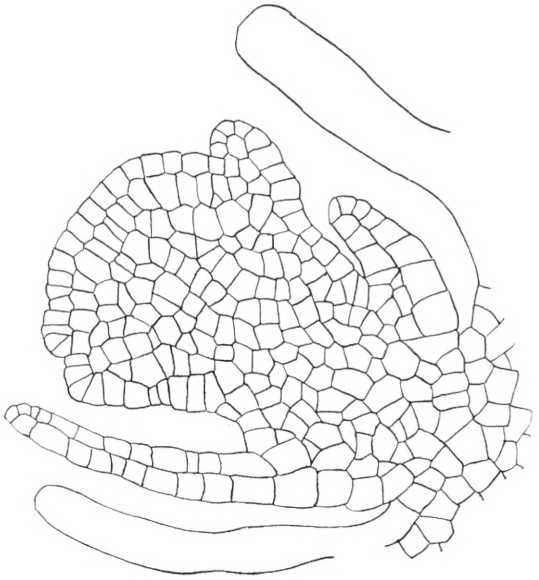

Abb. 8. Bildung einer Seitenknospe. Zeichenapparat 235/1.

Der Ausgangspunkt unserer Überlegungen war die Annahme, dass die Anlage und Entwicklung der Blätter durch die älteren an dem Vegetationskegel vorhandenen Blätter hervorgerufen sein könnten. Es war doch nicht möglich, diese Auffassung durchzuführen. Um nun dem Gestaltungsproblem etwas näher zu kommen, wollen wir die Blattbildung bei *Helodea* vorläufig verlassen und uns zu Differenzierungsvorgängen in Zellkomplexen, wo überhaupt kein Muster vorhanden ist, wenden.

Man kann in verschiedener Weise Zellkonglomerate herstellen oder in der Natur finden, bei denen das Wachstum der Zellwände der verschiedenen Zellen vollkommen unabhängig von demjenigen der Nachbarzellen ist. Es sollen hier drei Beispiele genannt werden: 1) Gewebekulturen, 2) Kallusprothallien von *Pteris*, das bei Behandling mit 2,4 D entstanden ist, 3) Kallusgewebe in *Coleus*stengeln, das durch Behandlung mit IES erzeugt worden ist. Es ist von Bedeutung, darüber klar zu sein, dass die Zellen wirklich autonom, d. h. unabhängig voneinander, in gleicher Weise wie bei Einzelligen wachsen (Steward 1958).

In diesen Zellkonglomeraten können, gleichfalls autonom, Differenzierungen eintreten, es können in den obengenannten

drei Fällen beziehungsweise Leitbündel, Wurzeln und Sprosse (Gautheret 1957, Steward et al. 1958 II), normale Farnprothallien (Boysen Jensen 1957) und Wurzeln (Moureau 1940) gebildet werden. Die Differenzierung aüssert sich dadurch, dass die einzelnen Zellen sich ungleichartig entwickeln, und zwar so, dass eine Gruppe von Zellen in Gemeinschaft ein ganzheitsgeprägtes Gebilde (d. h. ein Gebilde mit einer festen, artsgeprägten Struktur, ein Prothallium, eine Wurzel u.s.w.) hervorbringt. Die Entwicklung der einzelnen Zellen ist somit dieser Ganzheit untergeordnet, d. h. jede Zelle entwickelt sich so, wie sie sich entwikkeln muss, wenn die Ganzheit, das fertige Organ, entstehen soll.

Damit dieses möglich sein soll, muss die Entwicklung der einzelnen Zellen durch einen überzellulären, ganzheitschaffenden Faktor, den determinierenden Faktor, gesteuert werden.

Wenn nun dieser ein physikalischer Faktor ist, muss er irgendwo lokalisiert werden können.

Der Faktor kann nicht in den Genen oder in der Struktur der sich entwickelnden Zelle liegen, denn diese sind ja nur ein kleiner Teil der Ganzheit, die erzeugt wird. Ebensowenig kann der Faktor in den Nachbarzellen liegen, denn auch diese sind wieder nur Teile, die in Gemeinschaft mit anderen Zellen bei der Bildung der Ganzheit beteiligt sind. Der ganzheitschaffende Faktor kann somit nicht mit einem Teil des Zellkomplexes identifiziert werden. Er liegt so zu sagen ausserhalb des Organismus, er kann aber die Vorgänge im Organismus steuern, so dass Ganzheiten hervorgehen.

Die hier angestellten Überlegungen gelten gleichfalls für die Differenzierungsvorgänge im Vegetationskegel von *Helodea*. Auch in diesem Falle ist es nicht möglich den Faktor, der die Wachstumsvorgänge der Zellwände steuert, so dass ein Blatt hervorgeht, zu lokalisieren.

Es dürfte wohl möglich sein, dass man die Vorgänge in dem anorganischen Bereich durch Kausalitäts- und Wahrscheinlichkeitsgesetze erschöpfend beschreiben kann. Diese Gesetze machen sich auch in den lebenden Organismen geltend, die Wahrscheinlichkeitsgesetze z. B. bei der Verteilung der Chromosomen während der Meiose. Aber diese Gesetze reichen nicht aus zur Beschreibung der Lebenserscheinungen. Man muss schliessen, dass der Verlauf der Einzelvorgänge während der Ontogenese nicht

allein durch die Eigenschaften der Zelle und die Umweltbedingungen bestimmt ist, sondern dass er durch einen nicht physikalischen Faktor, der nur in dem lebenden Organismus tätig ist, der aber nicht an einem bestimmten Ort in demselben lokalisiert werden kann, gesteuert wird. Es ist überhaupt das Leben ein Geschehen einer ganz anderen Art, als man sich im allgemeinen vorstellt.

Obwohl der determinierende Faktor nicht physikalischer Natur ist, kann man sich doch gewisse Vorstellungen über seine Wirkungsweise und Eigenschaften bilden.

Ein lebender Organismus ist eine dynamische Ganzheit, die durch ein harmonisches Zusammenspiel zwischen einer ungeheuer grossen Anzahl von Einzelvorgängen entsteht und aufrechterhalten wird. Z. B. muss bei dem Aufbau des Zellwandgerüstes in jeder einzelnen der Millionen von Zellen, die während der Entwicklung einer höheren Pflanze gebildet werden, die Verteilung der Zellulosenbildner so gesteuert werden, dass schliesslich eine Pflanze mit einem bestimmten Zellwandmuster gebildet wird. Wenn der Same gesund ist, schlägt die Entwicklung selbst unter sehr verschiedenen äusseren Bedingungen niemals fehl. Die Ontogenese der Organismen ist durch eine Determination und Planmässigkeit gekennzeichnet, die wir in dem anorganischen Bereich nicht antreffen. Es ist die Aufgabe des determinierenden Faktors alle die Vorgänge, die zusammen die Ortogenese ausmachen, z. B. die Anordnung der Zellulosenbildner an den Papillen in den Zellwänden, so zu steuern, dass schliesslich eine Ganzheit, ein Organismus mit einer spezifischen, artsgeprägten Struktur, hervorgeht. Das Muster, das entsteht, ist von Ort zu Ort qualitativ verschieden, die Wirkungsweise des determinierenden Faktors ist daher mehr qualitativer als quantitativer Art.

Von den Eigenschaften des determinierenden Faktors fallen zwei besonders in die Augen.

Weil die Gestaltung der Individuen einer Art in der Natur von Generation zu Generation hinsichtlich aller wesentlichen Eigenschaften unveränderlich ist, muss auch der Faktor, der die Gestaltung hervorruft, d. h. der determinierende Faktor, unveränderlich sein. Wir verfügen überhaupt nicht über Mittel diesen Faktor für dauernd zu ändern, was daraus hervorgeht, dass wir

nicht imstande sind, eine Art in eine andere umzuwandeln (Boysen Jensen 1957).

Der determinierende Faktor ist wie oben bemerkt ganzheitschaffend, er vermag auch, wenn die Ganzheit eines Organismus vernichtet ist, innerhalb gewisser Grenzen die Ganzheit wieder herzustellen. Die Wege, auf welchen dieses Ziel erreicht wird, können aber, selbst wenn die Organismen anscheinend gleichartig sind, verschieden sein. Z. B. bilden Sporen von *Pteris longifolia*, wenn sie auf Lösungen von 2,4 D keimen, Konglomerate von gleichartigen Zellen. Werden solche Kallusprothallien in Nährlösung übergeführt, so tritt im Laufe von einigen Tagen Bildung normaler Prothallien ein. Aus jedem Kallusprothallium entsteht normalerweise nur ein normales Prothallium. Dasselbe kann entweder von einer grösseren Menge von Zellen ausgehen (Boysen Jensen 1957, Abb. 5 b, 15) oder es kann aus einer einzelnen Zelle im Stiel oder an der Spitze entstehen (Abb. 5 b, 16 und 5 a, 12). Ob nun das normale Prothallium in der einen oder anderen Weise gebildet wird, so ist seine Gestalt doch immer dieselbe.

Es erhebt sich nun die Frage, was man eigentlich mit dem determinierenden Faktor in der biologischen Forschung anfangen soll. Wenn es richtig ist, dass ein nicht physikalischer Faktor in den lebenden Organismen tätig ist, so ist das einzige, was man nicht tun kann, denselben zu vernachlässigen. Man würde dann ein lückenhaftes, d. h. ein falsches, Bild von den Organismen erhalten. Im Gegenteil muss man versuchen festzustellen, wo der Faktor wirkt und wie er wirkt.

Wenn man die Wirkungsweise eines äusseren Faktors, z. B. der Temperatur, auf eine Lebenserscheinung, die quantitativ bestimmt werden kann, z. B. die Respiration, untersuchen will, geht man im allgemeinen in der Weise vor, dass man den äusseren Faktor variiert, und die Intensität der Lebenserscheinung bei verschiedenen Werten des äusseren Faktors untersucht. Man stellt somit die Lebensäusserung als eine Funktion des äusseren Faktors dar, im Einklang mit der Auffassung von Mach: »Die Naturgesetze sind Gleichungen zwischen den messbaren Elementen $\alpha, \beta, \gamma \ldots \lambda, \mu, \nu \ldots \ldots$ der Erscheinungen«.

Der determinierende Faktor ist von ganz anderer Art als die physikalischen Faktoren. Er wirkt nicht auf einzelne Lebens-

erscheinungen ein, sondern er vermittelt die Koordination derselben, so dass ein Muster hervorgeht. Er ist ferner nicht messbar. Es ist daher nicht möglich das Verfahren, das man bei Untersuchungen über die Wirkung der Temperatur benutzt, bei Untersuchungen über die Wirkung des determinierenden Faktors zu verwenden. Man kann aber einen anderen Weg einschlagen. Die lebenden Organismen sind stoffliche Systeme, und der determinierende Faktor greift somit in das stoffliche hinein. Das stoffliche im Organismus kann man aber in beschränktem Umfange verändern, ohne das Leben zu vernichten. Solche Änderungen kann man durch physische Faktoren (z. B. Lichtfarbe) und durch chemische Stoffe (IES, 2,4 D, Colchicin etc.) hervorrufen, oder man kann die Ganzheit des Organismus durch Amputationen zerstören. In diesen Fällen kann man untersuchen, in welcher Weise der determinierende Faktor die Ganzheit wiederherstellt, d. h. man kann dem determinierenden Faktor Probleme vorlegen und untersuchen, in welcher Weise sie gelöst werden.

In dieser Weise kann man vielleicht zu dem Schnittpunkt vordringen, wo die physikalischen Faktoren nicht mehr ausreichen, und wo somit der determinierende Faktor eingreifen muss, um die Vorgänge (wahrscheinlich ohne Energiezufuhr) hervorzurufen oder zu regeln. Man wird dann — vielleicht — feststellen können, an welchem Orte der determinierende Faktor in das stoffliche System eingreift, und — vielleicht — in welcher Weise er wirkt.

Der determinierende Faktor ist wahrscheinlich bei vielen verschiedenen Vorgängen im Organismus betätigt; hier soll nur seine Wirkung bei der Bildung des Zellwandmusters berücksichtigt werden.

Für das Verständnis dieses Problems ist es von grosser Bedeutung, dass man die Wirkung des determinierenden Faktors transitorisch ausschalten kann, während das Wachstum der Zellwände (und die Zellteilungen) fortgesetzt werden. Es sollen hierfür einige Beispiele, die schon teilweise oben erwähnt sind, angeführt werden. Man kann von Pflanzen Gewebekulturen herstellen, die jahrelang wachsen können, ohne sich zu differenzieren. Durch bestimmte Einwirkungen (Kinetin, Auxin (Skoog and Miller 1957)) können sie Sprosse bilden. Wenn ferner Sporen von *Pteris longifolia* in rotem Licht kultiviert werden,

entwickeln sich Schläuche, die bei Zufuhr von weissem oder blauem Licht normale Prothallien bilden. Schliesslich entstehen aus *Pteris*sporen, wenn sie in 2,4 D-Lösung gezüchtet werden, Kallusprothallien, die, wenn sie in reine Nährlösung versetzt werden, normale Prothallien erzeugen können.

In allen drei Fällen kann somit der determinierende Faktor die Bildung eines normales Zellwandmusters hervorrufen. Seine Wirkungsweise besteht in diesen Fällen darin, dass er die Wachstumsweise der Zellwände verändert, so dass ein gleichartiges Wachstum in ein ungleichartiges, differenziertes Wachstum umgewandelt wird.

Wie es in dieser Abhandlung gezeigt wurde, ist eine Umschaltung der Wachstumsweise der Zellwände wahrscheinlich mit einer Umlagerung der Zellulosenbildner an den Plasmapapillen verknüpft. Man darf wohl dann schliessen, dass der determinierende Faktor in der einen oder anderen Weise bei Verlagerungen der Zellulosenbildner beteiligt sein kann.

Weiter können wir in diesem Augenblick nicht kommen.

Diese Untersuchungen sind mit Hilfe von Unterstützungen von dem pflanzenphysiologischen Laboratorium der Universität Kopenhagen und dem Carlsbergfond ausgeführt. Ich möchte diesen Institutionen meinen besten Dank für diese Unterstützungen aussprechen.

Ohne die getreue Hilfe meiner Tochter, Frau Margrete Ehlers, wäre es mir nicht möglich gewesen, diese Untersuchungen durchzuführen. Für ihre Hilfe möchte ich ihr auch an dieser Stelle herzlich danken.

Summary.

The structure of the leaves of *Helodea densa* is very simple: they consist of two layers of prismatic cells (fig. 3,8); only the vein contains a greater number of layers (fig. 3,7).

The initiating cells of the leaf develop on the apex of the shoot as horizontal protrusions, arranged in a pattern, so that whorls with four leaves arise (fig. 3,1). A median longitudinal section through the apex of the shoot demonstrates that the protrusions consist of two rows of cells, one developing to the upper, the other to the lower layer of cells in the leaf (fig. 2,1,4,5).

The initiating cells arise through changes in the direction of growth of the cell walls in the epidermis in the following way (figs. 6,7).

Epidermal cells		Leaf cells	
Tangential cell walls	Longitudinal + tangent. growth	Transversal anticlines	Transversal growth
Radial cell walls	Longitudinal growth	Longitudinal anticlines	Longitudinal growth
Transversal cell walls	Tangential growth	Upper, middle, lower cell walls	Longitudinal + transvers. growth

The changes in the direction of growth probably arise through a rearrangement of the cellulose-building enzymes on the surface of plasma papillae protruding in the cell wall.

During the development of the initiating cells into the leaf the growth of the individual cells must be regulated qualitatively and quantitively, so that the fusion of the cells to an organized entity, the leaf, is secured.

The question now arises how the harmonious development of the leaves on the apex of the shoot can be explained.

It is often supposed that the development of a cell is determined by the reactivity of the cell and the influences of the sur-

rounding cells. In accordance with this view one might imagine that the building of the initiating cells and their further development was induced by the older leaf primordia on the shoot apex.

An analysis of this interpretation shows that it does not hold good. We must conclude that besides the laws of causality and probability also an entity-creating factor, that cannot be localized anywhere, is acting in the living organisms and in them alone. This factor cannot be derived from elements in the physical world.

For the understanding of the mode of action of this factor it is significant that growth of the cell walls can continue, whereas differentiation, e.g. the development of the normal cell-wall pattern, is discarded. As the differentiation according to this paper is connected with a rearrangement of the cellulose-building enzymes on the plasma papillae, it is probable that the determining factor is active in the said rearrangement.

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der Universität, Kopenhagen.

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ON THE HABITS OF THE MIGRATORY BUTTERFLY *ASCIA MONUSTE* L.

BY

ERIK TETENS NIELSEN

København 1961
i kommission hos Ejnar Munksgaard

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During the first six months of 1949 my wife and I made observations on the habits of *Ascia monuste* as part of a research program in field physiology initiated by Dr. LAURENCE IRVING. Base for this work was at the Archbold Biological Station, Lake Placid, Florida. The basic life-history was worked out, and a number of characteristic elements of the migratory habit were observed (NIELSEN and NIELSEN, 1950).

It seemed likely, however, that a continuation of the study might render a considerably better insight into the subject; and I am, therefore, deeply indebted to the Director and the Advisory Board of the Archbold Biological Station for giving us the oppotunity of continuing this investigation for another year. Above all I want to express my most sincere gratitude to the Director, Mr. RICHARD ARCHBOLD, for placing at our disposal all the excellent facilities of the Station, and for all the kindness and help which he and the personnel extended to us.

After this year I joined the Florida State Board of Health to work with Dr. MAURICE W. PROVOST in his research on mosquitoes. It was found that our understanding of the habits of the migratory salt-marsh mosquitoes was considerably enhanced by our previous studies of the butterflies emigrating from the same marshes. As a number of problems relating to the migratory flight of *Ascia* required further study, Dr. PROVOST granted permission to use time, equipment and personnel of the Entomological Research Center of the Florida State Board of Health to continue these investigations in 1954, 1957, and 1960. My best thanks are due to Dr. PROVOST both for his permission to do this work and for his own interested participation in it.

I would also like to cite the names of the many people who in various ways have contributed to this work: Mr. W. L. BIDLING-

MAYER, Mrs. HELEN BOURINOT, Mrs. NINA BRANCH, Mrs. MARILYN CHESSWICK, Mrs. BARBARA DEMPSEY, Mr. W. B. DEMPSEY, Mr. F. SHERWOOD EVANS, Mr. J. S. HAEGER, Mr. JAMES HAEGER, Mr. R. HAEGER, Mr. and Mrs. KARL HODGES, Mr. W. JANSE, Miss KIRSTEN TETENS NIELSEN, Mr. W. PROVOST, Mr. and Mrs. SEXAUER, Mrs. JOAN WOOD, and Mr. W. F. WOOD.

Special mention should be given to Miss HEDVIG TETENS NIELSEN who has taken part in all phases of the work; Mr. L. M. BOURINOT, instrument maker of the Entomological Research Center, for his skillful help in developing the technique which made it possible to mark migrating butterflies; and last, but not least, Mr. FRANK RINALD of the Archbold Biological Station for his help in correcting my faulty English and preparing the manuscript.

This paper reporting on our work on the migratory butterfly *Ascia monuste* is dedicated to the memory of my wife and partner, Mrs. ASTRID TETENS NIELSEN, née SEHESTED.

I. Introduction

Under certain conditions many insects will become migratory: insect migrations are best defined as a special type of active locomotion by which the individual moves away from its natural habitat. Contrary to birds, insects only once in their life make a migration, and this habit could more correctly be called an emigration. There have only been a few cases reported of a return of the same individual insect to the point of origin.

There is a great variation in the appearance of the migrations in different insects: Aphids migrate only a few meters, locusts hundreds of kilometers; some insects migrate as single individuals and such movements will be detected only by a trained observer, others migrate in countless numbers upsetting the welfare and way-of-living of man. But whatever the appearance of the migration may be, this habit is, according to our present knowledge, always the effect of a basic urge unrelated to other habits. Insects do not migrate for want of food or to fulfill any other direct purpose known to us.

In some insects, such as aphids and locusts, much work has been undertaken to analyse the factors releasing the migratory habit. In butterflies most studies have been simple descriptions of migratory flights reported by stationary observers, a method which is hardly adequate for a penetrating analysis.

II. Ethology of *Ascia monuste*

(1) Geographical Distribution

Ascia monuste is a pieride butterfly with an essentially tropical distribution. The northern limits of its distribution in Florida vary with meteorological conditions from year to year (fig. 2). The

Fig. 1. *Ascia monuste* feeding on *Bidens*. Slightly dark female on top, male at bottom.

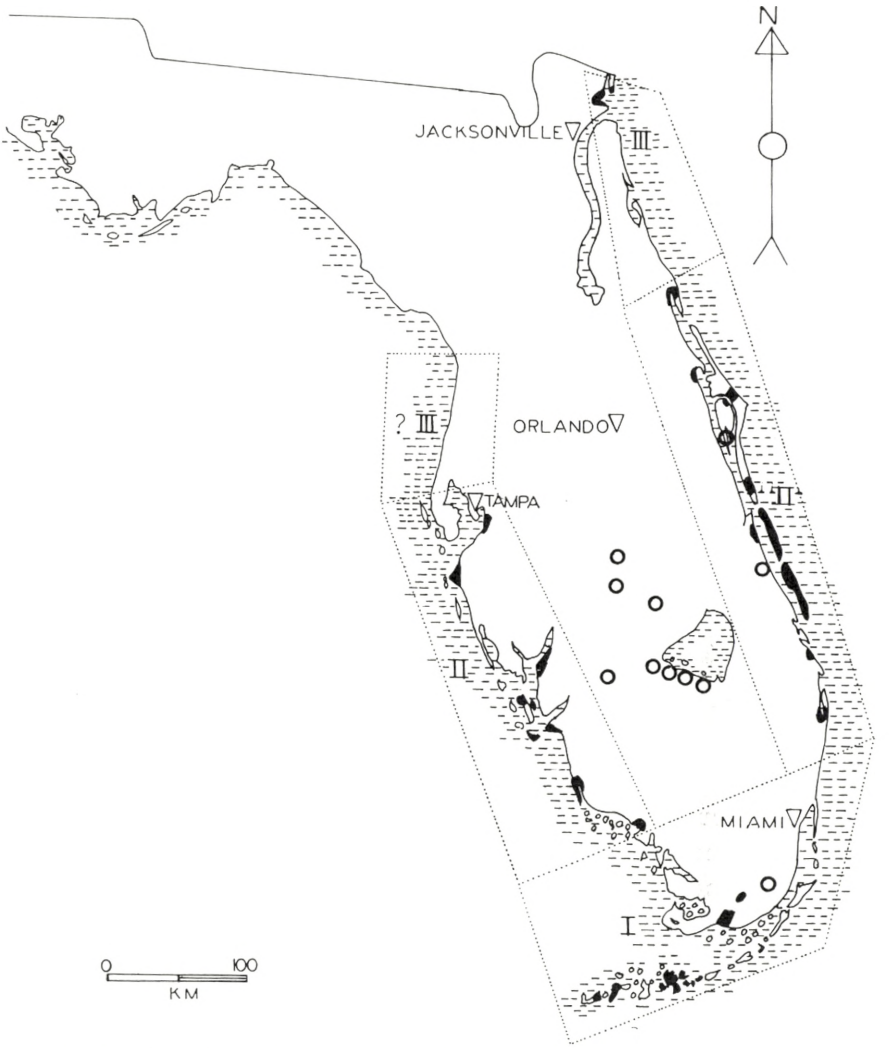

Fig. 2. Sketch map of Florida. The black spots indicate coastal breeding areas; the circles inland populations. "I" is the region of permanent breeding, "II" that of normal breeding, and "III" is the area in which breeding takes place only after a number of favorable years.

average limit approximately follows the line indicating the normal southern limit of damaging frost from Tampa on the west coast running southward to the interior of the peninsula, then following the east coast northward to about St. Augustine. In favorable years populations may be found north of this line; in 1954 breeding occurred on St. Marco Island on the east coast at the border of

Georgia; yet, after a cold winter, very few individuals were found north of the latitude of Miami. The larger, typical populations are always restricted to the coastal area but cross-country migrations following large outbreaks will deposit small, isolated inland populations; a cold winter will wipe out all these small colonies.

Migrations from the permanent breeding areas at the southern tip of Florida eventually re-establish the populations further and further north; the process takes about three or four years before "normal" conditions again prevail. *Ascia monuste* has always been found to breed in well-defined colonies. The formation of more or less isolated populations is caused by the presence of the plants on which the larvae feed. Along the coast, where all the large colonies were found, the larval food plant was *Batis maritima* (Family *Batidaceae*), which grows 30–50 cm tall. The stems tend to sprawl and the succulent leaves have a salty, spicy taste, and a characteristic odour. Usually the plants form a dense cover over vast areas of tidal marshes and under black mangrove. Even where *Batis* covers scores of acres, its occurrence is limited to parts of the coast and alternates with other types of vegetation on which *Ascia* larvae do not feed.

The second most important food plant is peppergrass (*Lepidium virginicum*), a small wayside weed of the family *Cruciferae*. Occasional occurrence on *Tropaeolum* in gardens, *Cleome*, *Cakile*, and (only in one case) cabbage, complete the list of host plants (Nielsen and Nielsen, 1950). Usually these populations are very small; during several months we followed a colony based on a patch of peppergrass where the normal number of imagines was about six.

In the following part the composition of the colonies will be discussed in more detail, but it is very essential to the understanding of the behavior of *Ascia*, and especially of the migrations, to keep in mind that these animals are distributed in discrete units based on the limitations of the breeding areas.

(2) The Egg

The egg and the egglaying was previously described in some detail (Nielsen and Nielsen, 1950). It was observed that the eggs were laid singly or a few together on the commonly used foodplants (*Batis* and *Lepidium*), while the were deposited in

clusters of up to 50 on the broad-leaved plants such as *Tropaeolum* and *Cleome*. In 1954, during the very heavy outbreaks on the east coast in 1954, eggs were found on *Batis* in large numbers, sometimes 30–40 on a single plant. Such eggs were, however, not arranged in neat clusters but scattered in different directions. They were undoubtedly laid on different visits, probably by different females. Females were observed not to lay more than a few eggs before moving to another plant.

The tendency, previously noticed (NIELSEN and NIELSEN, 1950, to deposit the eggs on small specimens of the food plant was also confirmed. In the salt marshes the eggs were usually found on smaller *Batis* plants, 15–30 cm high, growing under the cover of taller ones. When the eggs were laid on taller plants it was usually where there was shade from higher vegetation such as black mangrove. They were of course easier to see here, and when inspecting such a locality for eggs we would first examine *Batis* plants under a mangrove tree. The eggs were often situated so low that occasionally they must be reached by tides. In the laboratory we immersed eggs from time to time, and also kept them constantly moistened with salt water; in neither case was the hatchability of the eggs impaired.

In an orange grove on Lotus Island between Georgiana and Honeymoon Lake, a dense stand of tall *Lepidium* grew in the open spaces between the rows of orange trees, but neither eggs nor larvae were found here. Under the trees, however, grew a dense stand of various weeds and under this vegetation were tiny peppergrass plants. Eggs and larvae were found on these peppergrass plants and pupae on the weed growth above them.

The duration of the egg stage was observed in several cases, both with eggs laid in the laboratory and those we have observed being laid in nature and brought into the laboratory. There was considerable individual variation in the duration of the egg stage under uniform conditions, and this variation or dispersion seemed proportional to the duration, or even relatively greater the longer the duration. At 27° the duration was 84 hours and the dispersion from the first to the last hatching was two hours or about $2^1/_2$ % of the duration; at 19° the eggs hatched at an age of 180 hours and the dispersion was found to be eight hours or $4^1/_2$ % of the duration.

The results of the experiments, in which 274 eggs were used,

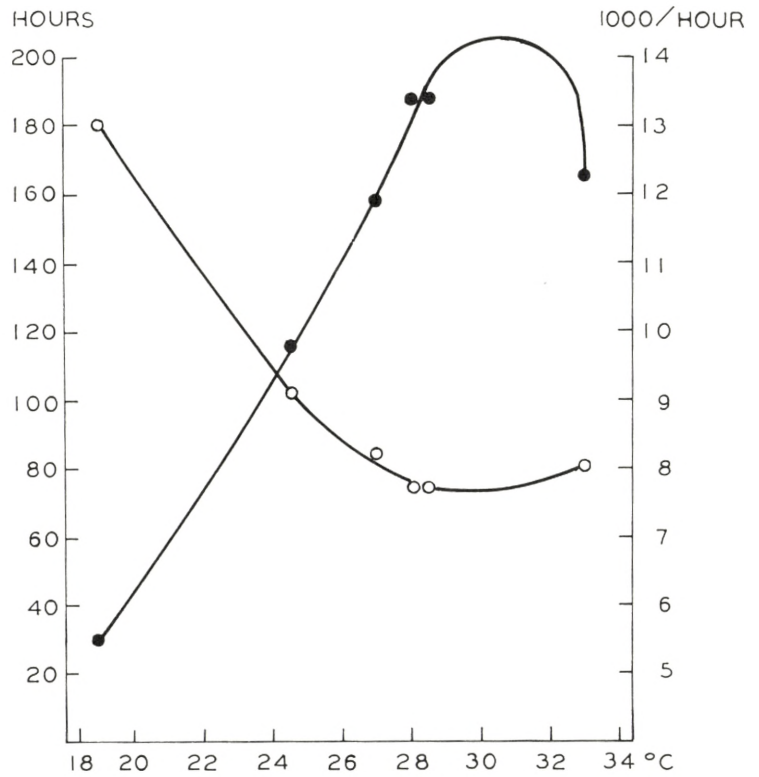

Fig. 3. Duration and velocity of the egg development of *Ascia monuste*.

are shown in fig. 3. The relation between temperature (t) and velocity of development (a) was probably of the usual form: $v = a + b \cdot c^t$ (NIELSEN and EVANS, 1960) but the material hardly permits a detailed analysis. Temperatures above 30° are unfavorable, in good agreement with the observation that shaded places were preferred for the deposit of eggs.

In one experiment at 28.5°, 15 eggs were kept at 90 % relative humidity and ten eggs from the same batch at 60 % r.h. The mean durations were 75 and 74 hours respectively, and variation within this range of relative humidity seemed thus not to have any influence on the egg stage.

(3) The Larva

The hatching of the eggs was preceded by changes in the embryo visible from the outside. From 10 to 20 hours before the hatching (depending on the temperature) it was possible to discern the eyes of the larvae and during the last four to eight hours a number of hairs were seen. It usually took the larva one to two hours to gnaw an opening in the shell.

In the first report (NIELSEN and NIELSEN, 1950) it was stated that there were at least four larval instars; the actual number is five. The head capsules of five larvae from the same batch were measured twice a day. Assuming that I, II, etc. indicates the head widths of the five instars, the ratio II/I was 1.67, III/II was 1.65, IV/III was 1.58, and V/IV was 1.52.

For easier inspection these larvae were kept in the laboratory at room temperature which varied between 26° and 30° with a mean of 28°. It was therefore not possible to determine the duration of the single stages with any degree of accuracy. The first three instars were of about the same duration, 10–15 % of the total each; the fourth instar was about 20 %; and the last instar, about 40 % of the total duration.

DETHIER recently (1959 a, 1959 b) discussed the importance of the availability and distribution of food plants for a butterfly population. This is hardly a problem in the marshes where there is a practically inexhaustible and uniform supply of *Batis*, but it could easily be important for the small inland populations.

It is well known that before pupation the larvae make large roving excursions; similar activity takes place on a smaller scale before each moult, and often—or perhaps even usually—they end up on a new food plant.

The total duration of the larval life varied considerably. All our experiments were made at 24–29° and it is impossible from our data to show the influence of temperature. Larvae from eggs laid within the same eight hours pupated from an age of nine to more than 14-days old (fig. 4). They were kept at a fairly constant temperature of about 24°, had the same food and were rotated on the shelves to compensate for local differences in temperature. In the duration of larval life no differences were found between the sexes. Crowding had no effect on the duration of larval life.

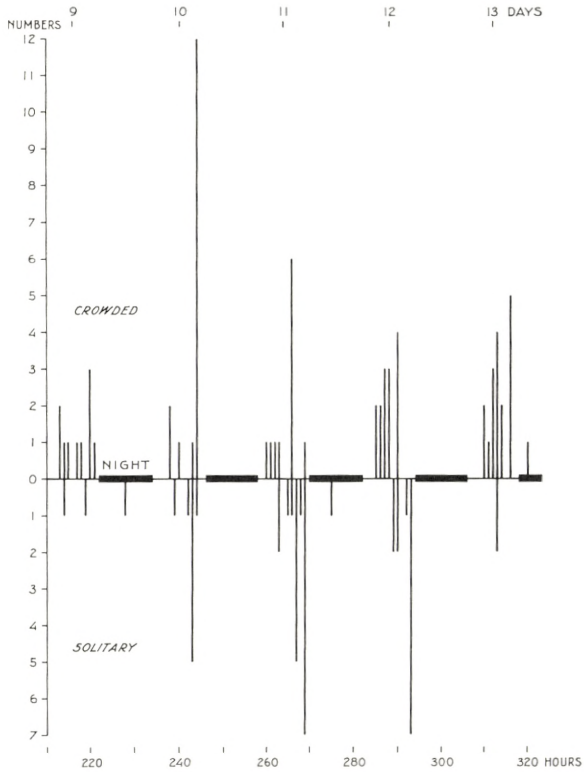

Fig. 4. Duration of the larval stage in *Ascia monuste*. The abscissa is the age at which the pupation took place, given as days (above) and hours (below). The ordinates are the number of pupations of crowded larvae (upper part) and solitary larvae (lower part).

(4) The Pupa

Fig. 4 and also fig. 5 show that pupation is almost entirely restricted to daytime with a very pronounced maximum towards the end of the day.

One, or sometimes two, days before pupation, the larva stops eating and moves around all day. In the field this activity will usually bring the larva to a fairly high point above ground usually more than one meter, sometimes 3–4 meters, e. g. on a twig or a leaf. Finally the larva fixes itself to a certain place to be used as a base, often vertical; if sloping the underside is usually preferred.

The shoe-like support for the abdomen is first spun, after

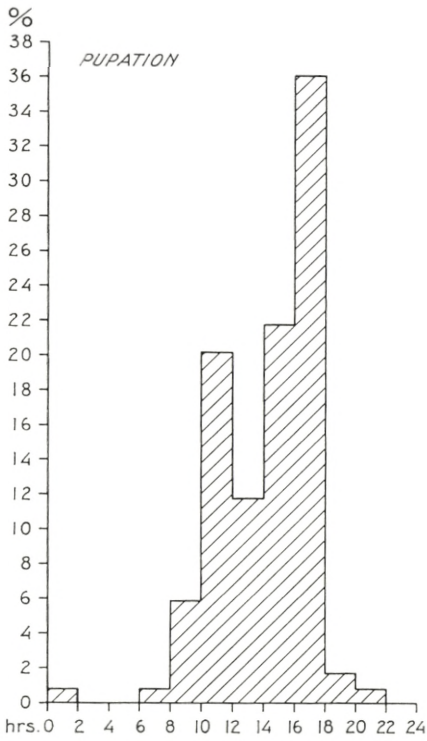

Fig. 5. Distribution of pupations of *Ascia* over the 24 hours.

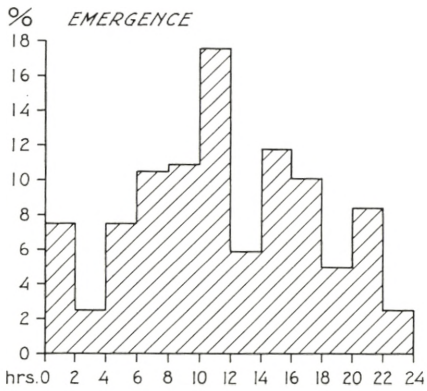

Fig. 6. Similar distribution of emergences of imagines.

which the animal turns around and fixes the tip of the abdomen into the shoe. The thread over the thorax is then made. With the front part of the body curved back nearly 180°, it fastens a thread on one side of the body. While continuously spinning, it moves the head to the opposite side and fixes the thread there. This is repeated until the thread is strong enough. During this process the larval body already begins to shrink in length and increase in width; after a couple of hours it has almost the normal shape of a pupa. There might still remain a long period—often overnight—before the larval skin is shed. For a few hours the coloration remains larval, after which it gradually changes to that of the typical pupa. The color of the pupa depends on the background (Nielsen and Nielsen, 1950).

The duration of the pupal stage is 6–7 days at 28°, and 7–8 days at 25°. No differences were found in the duration of the pupal stage relating to the sex of the adult or crowding of the larva.

(5) Emergence

Males and females emerged in approximately the same number in the breeding experiments; of 210 cases 100 were females and 110 males. There was no evidence either in the field or in the laboratory that one sex emerged before the other.

When pupae were kept in daylight there was a maximum of emergences during the daytime between 09^h and 17^h. Of 31 individuals, 7–9 % emerged during each of these hours against 2–4 % during the remaining 19 hours of the diel.

In another series of experiments the larvae were kept in daylight and the pupae were in a temperature controlled room with artificial light which was on most of the time. Under these conditions the emergences were more equally distributed although most of them occurred in the daytime (fig. 6).

(6) Sexual Development of the Female

As it was found that migrant females normally have immature ovaries, dissections were made to find at what age the eggs became mature. ZAHER and LONG (1959) found that crowding affects the duration of the preoviposition period, and the animals were therefore kept either crowded (C) or solitary (S). In the following these letters are used to indicated the condition of either larva (first letter) or imago (last letter): CC, CS, SC, and SS.

In a preliminary experiment with 12 females it was found that no development took place until the females were about 24-hours old; and in the second experiment, the females were killed at an age of 23 to 46 hours.

The results are given as fig. 7. There seemed to be a slight delay in the development of the females which were kept crowded as larvae but the dispersion was too large to permit any definite conclusions to be drawn. Some of the females of all four groups remained immature after more than 40 hours.

In the virgin female, bursa copulatrix has a peculiar dented shape. The spermatophore is round with a pointed stem; it swells the bursa to a ball shape. Later, the spermatophore empties and bursa deflates again to the dented form. Sometimes two spermatophores are found in bursa; in one case even three.

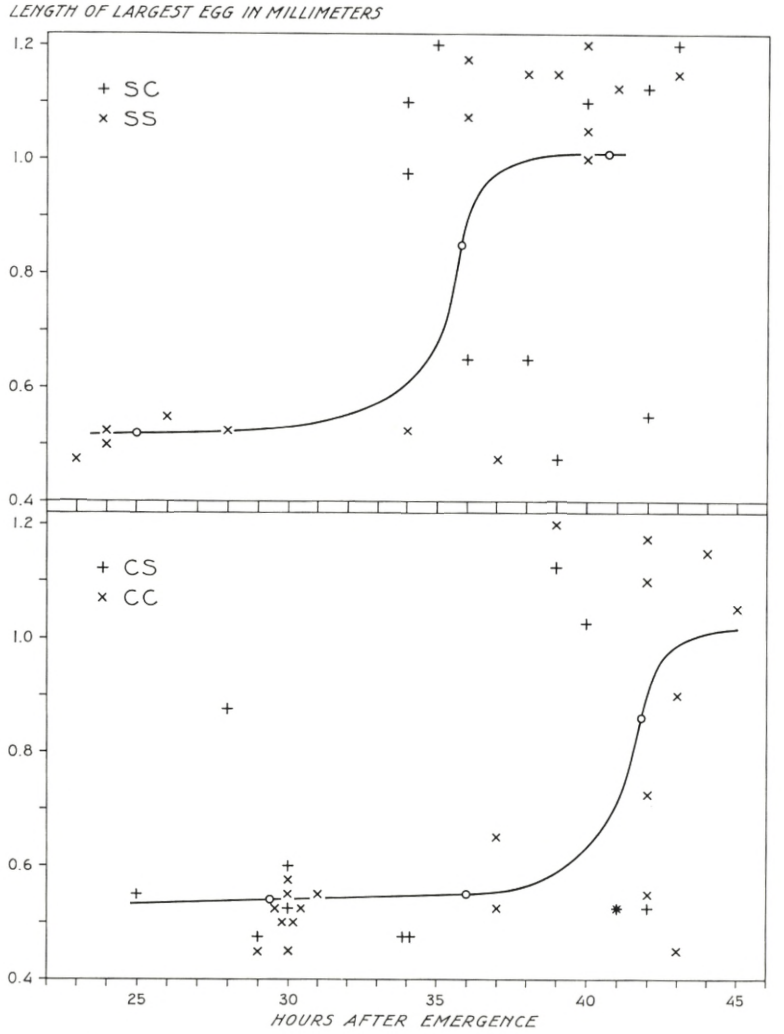

Fig. 7. Ovarian development in crowded and solitary *Ascia*.

SS: Solitary all life
SC: Solitary as larva, crowded as imago
CC: Crowded all life
CS: Crowded as larva, solitary as imago

(7) Copulation

In nature copulation is usually preceded by a series of complicated maneuvers. In the first stage male and female fly close together for some time, the flight becoming faster and faster until

a special whirling flight is adopted. The two animals encircle each other and at the same time each seems to try to rise above the other with the result that the participants are propelled higher and higher in the air. This stage ends in the female alighting, often on a leaf up in a tree or on a palm frond, sometimes after a sudden drop to the ground on a low weed or a bush, or even on the ground.

Contrary to the normal position the female now keeps her wings spread horizontally. The abdomen is exposed and raised and lowered in a sort of waggling movement. With very fast wing beats the male hovers behind and a little above the female, now and then darting towards her until he finally succeeds in seizing her. Very often the female flies away before that happens and the whole procedure may be repeated several times.

Copulating *Ascia* fly around as if they were single individuals, the female often visiting flowers. Usually the copulating pair disappear from view before they separate, but it is not unusual to see the same two animals united for ten minutes or more. Actual coitus has not been observed.

When not feeding on flowers, the males mostly fly around in the breeding area among the food plants, apparently trying to find females. When many males are present, as during the large outbreaks, males frequently copulate with females just beside the empty pupa-skin from which they emerged, the female's wings still soft and unable to carry them in flight. In such cases there cannot have been any introductory flight.

(8) General Activity

Limitation in the freedom of *Ascia* caused a general inhibition of most of their normal behavior. At the Archbold Biological Station a huge open-air insectarium was built to house a colony of them; a screened cage 150 cm enclosed a 3×7 m flower bed with Tropaeolum, a favorite food plant, with eggs and larvae. When the butterflies emerged their only reaction was to try to escape; all day long they fluttered against the screened ceiling, disregarding the birds which soon picked them out through the screen. In smaller cages even the escape reaction disappeared and the animals were usually rather apathetic. Our efforts to

measure the spontaneous muscular activities by means of an actograph were therefore unsuccessful.

Ascia do not always eat voluntarily in captivity. There was considerably individual difference in the "docility" of the experimental animals. They were fed each morning and sometimes also in the afternoon. Some of them stopped eating after a little, others had to be removed before they had eaten too much. After a meal during which one of the butterflies was left to eat as it pleased, its weight had increased from 83 mg to 122 mg, and the abdomen was dilated to an extent never seen in nature.

(9) Longevity

The longevity of the adult *Ascia* is very important to an understanding of its behavior, and especially of the migratory behavior of butterflies. In the laboratory females normally live seven to ten days; there were two exceptions, one lived 12 days and the other 16. The male has a normal lifespan of six days and none lived more than eight days. Individuals caught in nature usually died after a couple of days; very fine specimens of females with perfect scales and wings would live up to a maximum of seven days; comparable males five days. There is no reason to believe that the conditions of captivity caused the butterflies to die prematurely. In captivity they do not waste energy on the stresses of normal life and are protected against enemies. In nature many individuals succumb to predators: ambush-bugs (*Phymata erosa*), lizards, birds, and dragonflies, in decreasing order of importance. In the many cases, where the animals feed along highways, a considerable number are also killed by cars. In the following (p. 32–33) shall be mentioned that the longevity in the field is the same as found in the laboratory.

(10) Variability

Of the several varieties of *Ascia monuste* we observed only the form *phileta* in which the female is dark in the summer instead of white; the male is white in all seasons.

During the first part of this work we saw only dark females migrate and for some time considered it as a "migratory phase". With our permission, Dr. Alexander B. Klots used this infor-

mation in his book "Field Guide to the Butterflies" (1951). Later on it was found that the white females also migrate. I deeply regret misinforming Dr. KLOTS, and I alone am to be blamed for having brought this error into his excellent book.

From November to February all females are white. In 1950 the first dark ones were observed in the last part of February during an outbreak at Boynton Beach. At Fort Pierce the first dark females were seen on March 16, but it was not until April 19 that it was possible to distinguish males from females by color without catching them. Mr. and Mrs. KARL HODGES reported the first dark females in Indialantic, near Melbourne, on March 24. A northbound migration from Titusville, March 28–29, had several dark females, while a simultaneous southbound migration from New Smyrna Beach had only very few. The return to the typical form was not followed closely, but in 1949 it took place during October.

It is remarkable that the change to *phileta* came later in the northern part of the east coast than in the southern. Along this stretch of the coast, about 300 km, there is a considerable difference in temperature; the mean temperature for January is 20° at Boynton Beach, 18°5 at Fort Pierce, and 15°5 at New Smyrna. Eggs laid in December and January and developed at 28°–29° resulted in females as white as their parents; and in August a brood developed to dark females at 20°. Early in February 1960, eggs were taken into the laboratory and developed at 24–26°. The butterflies appeared the first days of March; most of the females were tinted, and about half of them were dark. In the field the same brood was retarded by cool weather and appeared first on April 3. The females were in the same transition stage of coloration as those reared in the laboratory. HOWANITZ (1948) studied the differences in the ecology of several varieties of *Colias*, but that variation was probably of another kind than the dimorphism in *Ascia monuste*.

(11) Preferred Temperature

To obtain a better insight into the effect of temperature on these butterflies a number of determinations of the preferred temperature were made. (NIELSEN and NIELSEN, 1959).

It was found that there were variations in the preferred temperature according to season, sex, conditions of feeding, and possibly to time of the day.

III. Populations of *Ascia Monuste* L. on the East Coast of Florida

(1) Description of the Observed Populations

The Atlantic coast of Florida is characterized by a long row of narrow islands, a sort of offshore bar, often simply called "the Beach" (figs. 8 and 9). It is separated from the mainland by a lagoon which in different sections had different names, e. g. Mosquito Lagoon, Indian River, Lake Worth. A typical cross-section of the coast is given in figure 4. Further details of topography, soils, vegetation, etc., may be found in the book by KURZ (1942).

Both in the mangrove and in the open salt marshes on the west side of the bar *Batis maritima* is a typical plant; it also occurs on the mainland. Most of the flowers on which the adult butterflies feed are found in the region behind the dunes and along the roadsides. Among the more important are the composite *Bidens* sp. and *Helianthemum arenicola*, also such shrubs as *Lantana involucrata* and *Coccolobis uvifera*, and weeds like *Vigna repens*.

Although the butterflies in the breeding areas may visit the mangrove and the composite bush, *Borrichia frutescens* when in flower, they ordinarily move to the roadside flowers to feed. This differentiation of the territory of *Ascia* into a breeding area and one in which the adults forage is characteristic for the coastal populations. As the feeding areas are usually concentrated along the beach roads these places offer a favorable opportunity for inspections and counts of the populations. From the spring of 1950 we concentrated on the middle east coast of Florida as the most convenient area for the work.

Following is an annotated list of what we believe to have been all the important populations between New Smyrna Beach (29°00′ N.) and Boynton Beach (26°30′); which is to say, along 300 km of Florida's east coast (fig. 8).

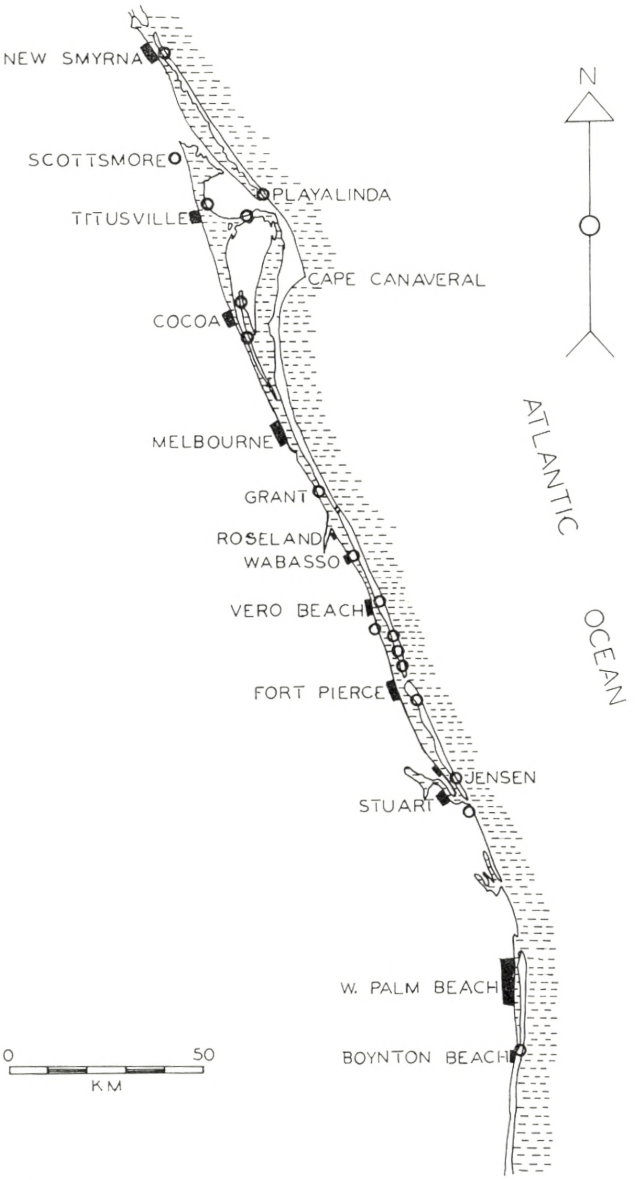

Fig. 8. Sketch map of part of the East coast of Florida. Circles indicate breeding areas. Black squares are cities.

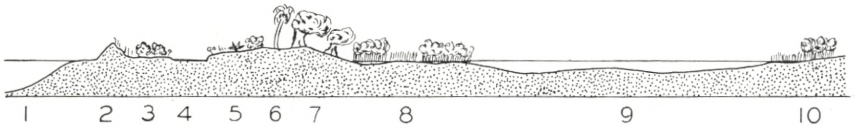

Fig. 9. Diagram of a cross section of the offshore bar and lagoon on the East coast of Florida.

1. The Atlantic Ocean
2. Dunes
3. Weeds and bushes as *Lantana* and *Coccolobis*
4. The road A1A
5. Roadside flowers and scrubs
6. Palms
7. Oak wood
8. Mangrove and salt marsh
9. Lagoon
10. The mainland

The descriptions refer to the situation in 1949–1950; the increasing house building, re-arrangements of roads, impoundments and destruction of the natural vegetation by sand-dredging and other so-called developments are rapidly changing the coastal area to such a degree that several of the points mentioned below no longer can be recognized.

New Smyrna Beach

This was one of the main outbreak centers formerly observed by FERNALD and others. FERNALD (1937 a) had the impression that the butterflies came from the interior of the peninsula, and a little north of New Smyrna on the mainland there was a possible breeding area around Turnbull Bay; the bay penetrates the mainland with areas of mangrove and *Batis* along it.

Only once did we observe an outbreak here, in March 1950, coming from the salt marshes on the islands which here nearly completely fill the lagoon.

Titusville Area (map, fig. 10)

Here there were two smaller populations in open salt marsh, one at the bridge to the mainland and one north of the bridge to Merritt Island. In the salt marshes as Playalinda there were many patches of mangrove, and the enormous breeding area there comprised several types of vegetation with *Batis*.

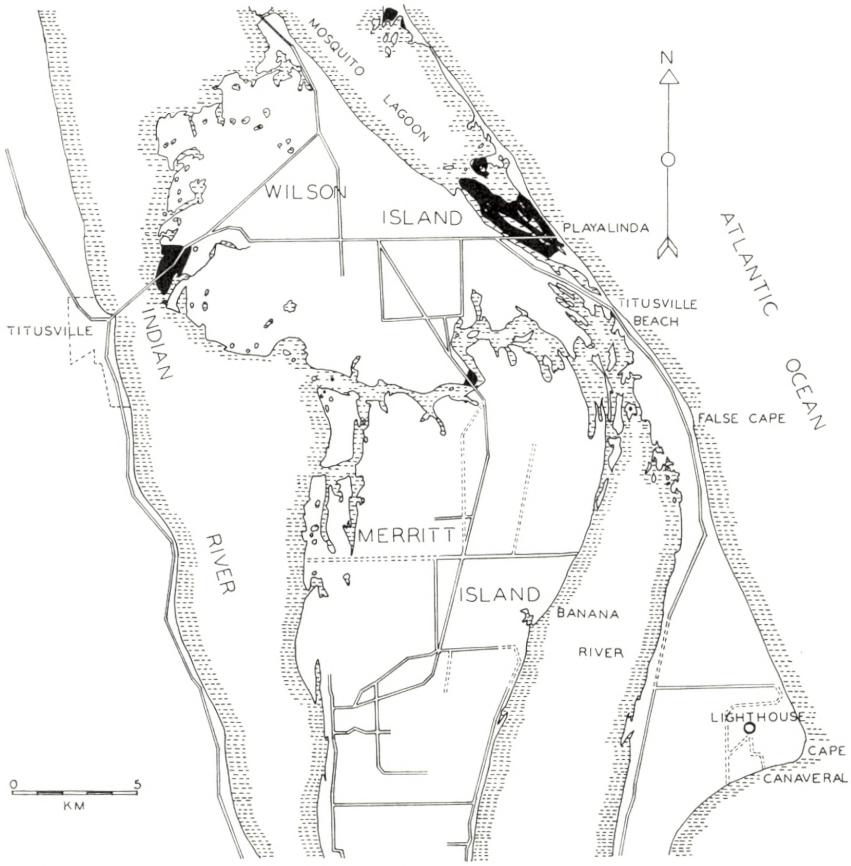

Fig. 10. Sketch map of the Titusville area on the East coast of Florida. Black markings indicate breeding areas.

In 1954 the large *Batis* marsh on the coast of the mainland at Scottsmoor was the center for a very large outbreak.

Observations on migrations indicated a breeding area on Canaveral Island which we crossed only once. At that time it was without roads and we did not find any breeding area. It might have been along Banana River.

Cocoa and Lotus Island (map, fig. 11)

The breeding areas in the bight of New Found Harbor were in the salt marsh, but the one Georgiana and Honeymoon Lake was remarkable because it was in a citrus grove and the food

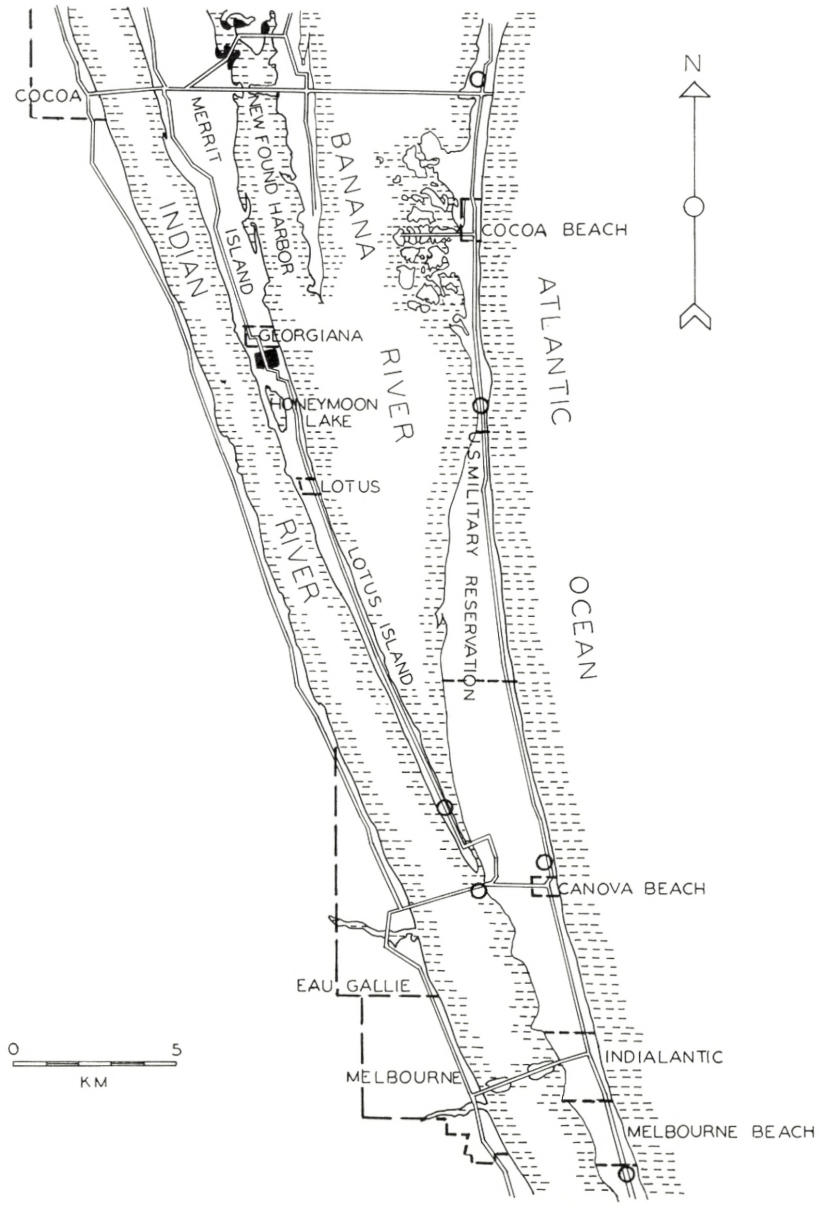

Fig. 11. Sketch map of the East coast of Florida between Cocoa and Melbourne. Black markings indicate breeding areas, circles, the main places for observations of migrants.

plant was *Lepidium*. The population was much larger than in the small inland populations with the same food plant.

Grant and Wabasso

Observations of migrations indicated an outbreak center 15–25 km south of Melbourne. But the seasons was over before it was found that there were potential breeding areas on the islands in Indian River off Grant. Local people were well aware of large numbers of white butterflies which at certain times of the year came to the beach from these islands. This was confirmed by later observations.

Breeding was observed on many of the islands farther south around Wabasso; migrations originating here were observed in 1954 and 1957.

Vero Beach and Fort Pierce (map, fig. 12)

There was a breeding area on the mainland at Crawford Point, North of Oslo Road which was found after observing migrations. This area later became very well known to us after the Entomological Research Center was built on Oslo Road, and the study field belonging to the Center partly included this area.

Our main observation areas were on the Beach between Vero Beach (Rio Mar) and Fort Pierce. Going south from Rio Mar one first passed through about eight kilometers of hammock and oak without breeding areas. Opposite Crawford Point were two coves and there was a large breeding area on the peninsula between them. Regular counts were made at the base of the southernmost of the coves, Floralton (fig. 12, FL). Other points where counts were made are indicated on the map as AI, AII, and AIII, each a feeding area. At DE at small road went down to the mangrove where there was a feeding area; we did not make counts here but secured butterflies for experiments. At a place 3–4 km north of Fort Pierce Inlet, Indian River sent in two long firths nearly up to the road. Between the bight of the southernmost and the ocean is a public picnic area, Pepper Park. The road continues to the inlet, where there was a good observation place for migrants crossing the inlet, but the highway branches off in a T-crossing

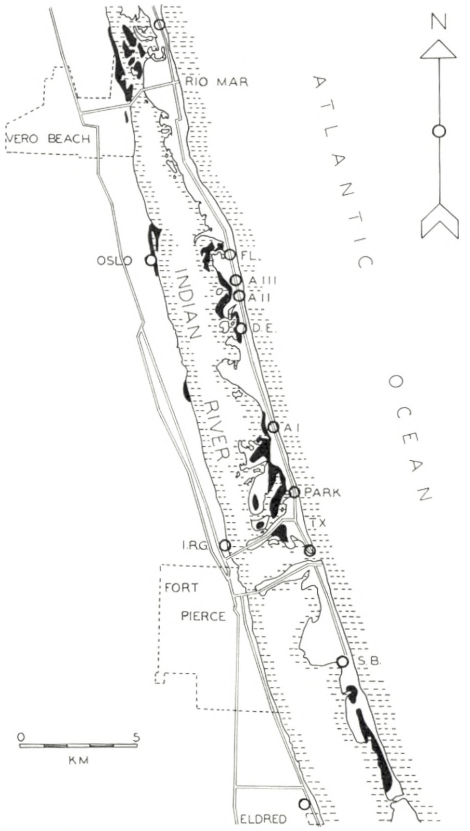

Fig. 12. Sketch map of the East coast of Florida between Vero Beach and Fort Pierce. Black markings indicate breeding areas, circles, the observations places.

(fig. 12, Tx). The road from here to the mainland was much used for observations. There was a breeding area south and east of that part of the road which we called the curve. Nearly all of these breeding areas have now been destroyed.

A little north of the bridge on the mainland we had an observation place for migrants following the coast line, at Indian River Gardens (IRG on map, fig. 12).

On the beach south of the inlet, the road ended 3.6 km from the inlet because a hurricane destroyed the road most of the remaining 21.5 km down to Jensen Beach. We had an observation site at the end of the road. There was a very large breeding area 1–5 km south of this point.

South of Fort Pierce

A small breeding area south of Jensen Beach was mentioned in the first report (1950, p. 5); it was not found again. Another small colony on *Batis* was once seen on State Road 703 north of Palm Beach.

At Boynton, south of West Palm Beach, the lagoon narrows down to a canal bordered by a considerable area of marsh land. *Batis* is almost the only vegetation. In February 1950 we observed an outbreak here.

(2) Estimates of the Size of the Population

At the beginning of this work and with less frequently visited colonies, we contented ourselves with general expressions to indicate the number of butterflies in a given population. Eventually we found that simple counting gave a fairly reliable picture of the size of the population. It was most convenient to confine ourselves to the feeding areas, and what we actually counted was not the population but that fraction of it which at the moment was feeding. The size of this fraction was unknown, even though later it was found possible for us to estimate it (see p. 31). It is certain, however, that this fraction varied with a number of different conditions: the hour of the day, the weather, the flowering of plants, the average age of the population, and probably more factors affect the proportion of a population found at the feeding area at a given moment. But even with all these limitations the method was found useful.

The accuracy of the actual count was tested by two observers (A. T. N. and E. T. N.) simultaneously walking through the area in opposite directions. We tried this five times within three days in the same area. One observer got a mean of 154, the other, 147. The single counts differed from 2 to 26, and treated as double determination, the standard error of a count was found to be 8 %. During the observation period, the counts in this area varied between 8 and 503 in the hours of activity.

In 1954, 21 more counts were made simultaneously by two observers (H. T. N. and E. T. N.). The individual counts varied from 2 to 642. The averages of the counts made by these two

persons were 104 and 94 respectively, and the error on a single count was 14 %.

On the stretch of road between the bridge and TX (se above) the counts were made by driving slowly (about 20 mph) in a car. Simultaneous counts walking and driving showed that the number found by driving was 70 % of the number observed by walking. It is obvious that some are overlooked while driving, but in walking some will also be counted more than once.

(3) Marking: Methods and Experiments

It was clear that a method of marking the butterflies would be very useful. COCKBILL (1942) has summarized a number of methods, all, however, incorporating the disadvantage of having to catch the animals and mark them individually. The simplest of such methods is to print directly on the wing by means of a rubber stamp as proposed by Mr. C. A. ANDERSON of Dallas, Texas. We used this method for individual identification in laboratory experiments.

The ideal method for field work must be (1) fast enough to permit marking a large number of individuals in a short time, (2) gentle enough to have no effect on the behavior of the animals, (3) specific enough to permit recognition of single individuals or groups of individuals, and finally (4) distinct enough to be recognized at some distance. We have not been able to conceive a method which is satisfactory on all points. After a number of trials, the following method was adopted to mark a non-migratory population. A modification for marking migrants was later developed (see below).

A suitable dye (methyl violet, methylene blue, victoria green, or eosin) was dissolved in denatured alcohol to a concentration of 15 grams per gallon (= 3.78 liters, or about 0.5 % by weight). Such a solution was sprayed on feeding butterflies by means of an oil can; we used a "Plews Oiler", which had a capacity of 0.1 gallon (380 ml). With a little training it was possible to hit a butterfly up to a distance of 7–8 m by a few strokes of the trigger.

As to speed, this method is satisfactory. Two persons could easily mark 300 butterflies in 20 minutes, and if there had been

butterflies enough we could have marked more than 1000 per hour. One gallon of solution would mark 1000 butterflies.

How much of a butterfly is colored by this method varies; some will be totally covered, others will get a few spots. Only such markings which could be seen at a distance of 10 m were counted. If more than half the wings were colored, the butterfly could be identified in flight 30–50 m away; a totally covered individual could be recognized at almost any distance at which it was possible to see that it was a butterfly.

The vast majority of the butterflies were not much affected by the coloring. Those which were completely covered were sometimes unable to control their flight. This was especially noticeable when eosin was used. As long as this solution was wet it had a dull yellow color and the butterfly flew irregularly with the wind. After a few seconds the alcohol evaporated, the dye changed to the well-known bright pink, and the flight changed direction and became normal. Totally covered butterflies sometimes dropped to the ground but with few exceptions they recovered and flew away. The exceptions represented less than one per cent of the marked.

Besides a number of preliminary experiments, four experiments with the above-described marking method were carried out in March 1950 in the Fort Pierce area (cf. map, fig. 12).

Experiment 1: In the afternoon of March 7, 100–150 *Ascia* were marked with eosin. Immediately after the marking was over, about one of every four butterflies was marked. A quarter of an hour later most of the marked individuals had disappeared from the area, but during the subsequent half hour marked individuals were seen up to 150 m on either side, feeding along the road. Three days later, three marked individuals were seen feeding on *Lantana* in the marking area. Inspections after four and six days later were negative.

Experiments 2: This experiment was carried out at AII, a 15 m long patch of *Bidens* along the road close to one of the large breeding areas. On March 9, three persons with two oil cans marked with methyl violet every *Ascia monuste* appearing in AII from $07^h00'$ to $11^h30'$ and from $14^h10'$ to $16^h00'$. The first butterfly arrived

at $07^h25'$, and only five came before $08^h00'$. The rest of the morning about 250 were marked per hour, 991 in all. Returning in the afternoon we found hardly any marked one, and in less than two hours 930 were marked, bringing the total for the day up to 1921. The results of inspection on the following days are given as table 1, 2, and 3 and will be discussed below.

Experiment 3: The next marking was made in AIII, about half a mile to the north of AII. Between $07^h30'$ and $12^h00'$ on March 15, about 1200 *Ascia* were marked here with eosin. The same day, however, a number of trees in the surroundings started to bloom and attracted the butterflies to the extent that the usual method of counting along the roadsides became meaningless and at least could not be compared directly with the two previous experiments. The third day after the marking a large number of newly hatch individuals appeared, easily distinguishable because they were the first of the form with dark-winged females.

Experiment 4: It was decided to mark as many of these new butterflies as possible. On March 18 between $09^h00'$ and $11^h00'$ about 800 were marked with victoria green. We were unable to make inspections the following day. On the second day, the population was considerably reduced in number. In the marking area were 177 individuals of which one was green. Another green one was found 800 m to the south, and two more were seen among 166 butterflies in the previously used marking area, AII, half a mile to the south. North of AIII no marked butterflies were seen

TABLE 1.
Total number of *Ascia* marked March 9, 1950.

Hour	Number	Hour	Number
$07^h25'$—$07^h59'$	5	$10^h30'$—$10^h59'$	170
$08^h00'$—$08^h29'$	57	$11^h00'$—$11^h29'$	195
$08^h30'$—$08^h59'$	122	$14^h10'$—$14^h29'$	300
$09^h00'$—$09^h29'$	184	$14^h30'$—$14^h59'$	290
$09^h30'$—$09^h59'$	177	$15^h00'$—$15^h29'$	240
$10^h00'$—$10^h29'$	81	$15^h30'$—$15^h50'$	100
		Total...	1921

among 238 unmarked. It is well worth mentioning that this same day was the first day during the experiments on which we observed migratory activity in this region.

(4) Daily Activity

The activity of *Ascia monuste* is limited to the daytime. Regardless of how many individuals are present during the day, from about two hours before sunset to one hour after sunrise, hardly a single one will be seen in the air. During this period they are resting, some at the feeding places but most of them in the breeding area. In open salt marsh (e. g., Boynton Beach), it was easy to observe the butterflies gathering and disappearing among the *Batis* plants; in the mangrove the resting animals are much more difficult to find.

Their appearance in the morning is rather independent of the temperature. In this respect it is instructive to compare the activity two mornings two days apart when the meteorological conditions were strikingly different. On March 7, 1950, during the marking at AI, the temperature rose from 20.3° at 07^{h}00′ to 23.7° at 09^{h}00′. The relative humidity decreased during the same time from 91 % to 78 %. The wind was a light breeze from the south and there was a cloud cover in the southeast over the ocean, about 3/10 altocumulus. On this perfect morning the first butterfly was seen at 07^{h}18′, but by 08^{h}00′ only a few single individuals had arrived. The number increased then, and from a little after 08^{h}30′ onwards the normal number were feeding in the area. Two days later the corresponding temperatures were 10.7° at 07^{h}00′ and 14.4° at 09^{h}00′ with relative humidities between 64 % and 60 %, 1/10 cirrus, and a fresh wind from NE up to 3.6 m/sec., (8 mph). The shivering observes expected a considerable delay in the appearance of the butterflies. The first one appeared at 07^{h}45′, nearly half an hour later than the preceding day. But several were feeding between 08^{h}00′ and 08^{h}29′ and the normal number was in the area at about the same time as two days before, at 08^{h}45′.

Rain and heavy dew fall delayed the appearance of the butterflies in the morning. They do not seem to become very active until the dew has evaporated.

When, during an outbreak, the number of butterflies rises rapidly from one day to another and many beautiful and apparently newly hatched individuals are seen, both sexes are found in about equal numbers everywhere. But a day or two after the peak of production, it is evident that there is considerable difference in the activity of the sexes. About 80 % of the butterflies arriving at the feeding area the first couple of hours in the morning are females. Well before noon the females return to the breeding area and then spend the afternoon laying eggs. From about $11^h00'$ on, the males dominate the feeding places as much as the females did earlier in the afternoon. It is not known whether the males are just later in starting the day's activity, or if, perhaps more likely, they spend the early activity hours scanning the breeding areas for virgin females.

Not only do the males feed at one time of the day and the females at another, but a certain observation makes it probable that the same individual feeds at the same time each day (see below).

Regardless of sex, the number of butterflies found in a feeding area during a day remains fairly constant. On March 12, 1950, we made seven counts in AII and found that the number varied between 322 and 376 (mean: 345) between $11^h00'$ and $15^h20'$. At $16^h00'$ the number was 207 and at $16^h25'$ feeding butterflies numbered only 66. Three days earlier, when marking took place in this area, there were somewhat fewer, and for the following calculations we shall suppose that a constant number of 300 butterflies fed during the eight hours of that day. During 5 $^1/_2$ hours, 1920 were marked. If as presumed, all visitors were marked, then 1920/5.5, or 350 butterflies arrived every hour, perhaps 2500 during the day. With a constant number of 300 present, the time each individual stayed in the feeding area was $300/350 \times 60 = 51$ minutes.

On March 12 the average of marked indivuals in the marking area AII (see Table 2) was 14 at the inspections made at $11^h00'$, $15^h00'$, and $15^h20'$ whereas at $12^h00'$ and $12^h30'$ it was 7.5. The first three inspections were made at the same time as the markings were made but there was no marking between $11^h29'$ and $14^h10'$. There was no marking after $15^h50'$ either but the total number was lower at this time. To include inspections 6 and 7 (table 2)

TABLE 2.
Marked *Ascia* in all three days after marking.

Inspection No.	Hour	Unmarked	Marked	Marked in % of Total
1.....	$11^h00'$	360	16	4.3
2.....	$12^h00'$	312	10	3.2
3.....	$12^h30'$	339	5	1.5
4.....	$15^h10'$	310	15	4.6
5.....	$15^h20'$	340	16	4.3
6.....	$16^h00'$	206	6	2.8
7.....	$16^h25'$	66	3	4.6

in the calculations it is necessary to use the percentage marked of the total number of butterflies. The average of inspections 1, 4, and 5 is 4.7 % against 2.5 % for the other four inspections. The number of marked butterflies, at times when no marking took place, was thus only half the number at times when marking was going on. This seems to indicate that not only do the females feed in the morning but each individual feeds during a particular time of the day. This observation supports the view that the *Ascia* is governed by a rythm, a view developed from the regularity with which activity begins in the morning and ends in the afternoon when the temperature is still high and the sun is shining brightly.

(5) Longevity

In the laboratory the normal life span for females was 7–10 days and for males 5–6. The observations of marked butterflies in AII (Table 3) corroborate these figures.

The number of butterflies present at any given moment during the day the marking took place was assumed to be 300. Inspections on the subsequent days gave the following numbers of marked butterflies: 102, 76, 62, ?, and 2 (no inspections on the fourth day). The number of marked individuals on March 10 and 11 were probably only half of the true number because these observations were made at a time when no marking took place. Corrected figures for the number of marked individuals on subsequent dates will thus be: 300, 204, 152, 62, 27 (estimated) and 2. Neither in

TABLE 3.

Distribution of marked (m) and unmarked (u) *Ascia* in and around AII subsequent to the marking on March 9, 1950.

Distance from AII (km)	March 10			March 11			March 12			March 14	
	u	m	%*	u	m	%*	u	m	%*	u	m
0	411	49	11.9	295	25	8.5	360	16	4.4	235	0
0 —0.4	605	33	5.6	534	21	3.9	875	21	2.3	1234	1
0.5—0.9	353	12	3.4	902	13	1.4	2265	19	0.9		
1.0—1.4	1019	6	0.5	609	0	0	714	3	0.4	2268	1
1.5—1.9				870	15	1.7	905	3	0.3		
2.0—2.0	426	2	0.5	1801	2	1.1					
Total	2814	102		5111	76		5119	62		3737	2

* As seen from the increase in the total of unmarked butterflies on March 11, a large number of emergences took place. Thus percentages can be used only for comparison within each day and not from day to day.

this experiment nor in any other, with more than 500 observations of marked butterflies, were any *Ascia* seen more than 5 days after the marking. As all these experiments, except the last one, were made at a time when no migration took place in this region, it seems justifiable to conclude that the life duration of *Ascia monuste* in nature is the same as it is in the laboratory or perhaps a little shorter.

(6) Diffusion

Although no migration took place during the first three marking experiments, the inspections showed clearly that butterflies ranged in the surroundings and at neighboring feeding places within a distance of a few kilometers. Information on this point can be obtained by comparing the number of marked individuals found each day following the marking inside and outside the marking area.

In Experiment 2, we know that on the day of marking all the marked butterflies were inside area AII. The next day 49 of the 102 marked ones observed (Table 3), or 48 %, were in the marking area; on the following days the figures were 33% (25 of 76), and 26 % (16 of 62) respectively. The sequence found was thus very close to 1/1, 1/2, 1/3, and 1/4 for the first four days, which,

though most likely a coincidence, at least gives some idea of the process. The only other comparable figure is from Experiment 3 where the day after marking 12 of 29 marked individuals, or 41 %, were in the marking area (AIII).

This slow process of spreading out or mixing up with neighboring populations, which is a very different behavior from migration, had some resemblance to diffusion and it might be useful to borrow this name from Physics to denote the phenomenon.

If the simple fractions found in Experiment 2 should turn out to be at least approximately right, it can be seen that the rate of diffusion decreases during life. Compensating for the death rate, it is seen that the day after marking, half the butterflies had moved away from the area; the next day only one-third of those left had moved away; and the next day, one-fourth. The older the butterfly, the more likely it is that it will return to the usual feeding place.

(7) Fluctuations in Size of Populations

In the first of these reports two outbreaks in the Fort Pierce area with an interval of about 35 days between them were thought to represent two successive generations. Later observations confirmed this interpretation.

In March 1950 regular counts were started and population estimates were made as described earlier in this paper.

At the feeding area west of Tx (cf. map. fig. 12 and fig. 13), there were three rather clear maxima: The first one was small (138) and came during the latter part of March. The last week of April showed a very high population with a peak of 640 on the 27th. The last maximum occurred on June 1, with a population of about the same size as the maximum period in April although the highest count was only 460.

The first maximum in feeding area AI (fig. 13) was not counted but occurred on March 12, at the same time as AII's first maximum. The next population increase, in April, started at about the same time as the one in AII but did not culminate until April 29 with 319 individuals, which was estimated to be a little more than the first maximum. The last maximum in this feeding area was still higher (503) and occurred on the first of June.

At AII (fig. 14) there was a very definite maximum in the

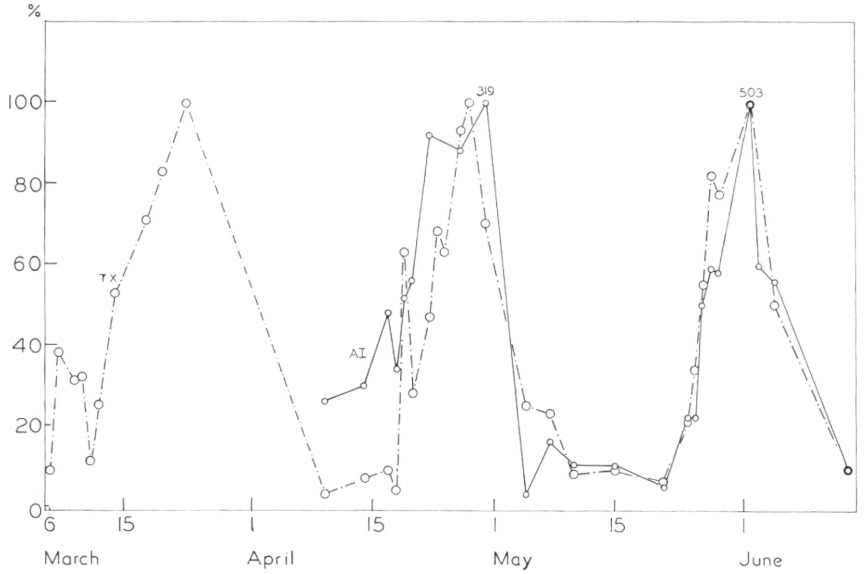

Fig. 13. Variation in the size of the population at AI (————————) and at Tx (-·-·-·-·-·-·-).

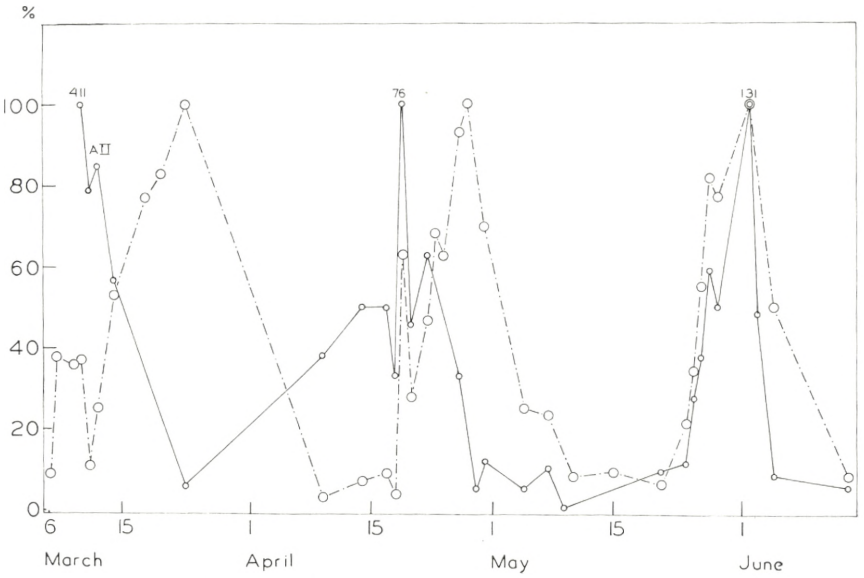

Fig. 14. Variation in the size of the population at AII (————————) and at Tx (-·-·-·-·-·-·-).

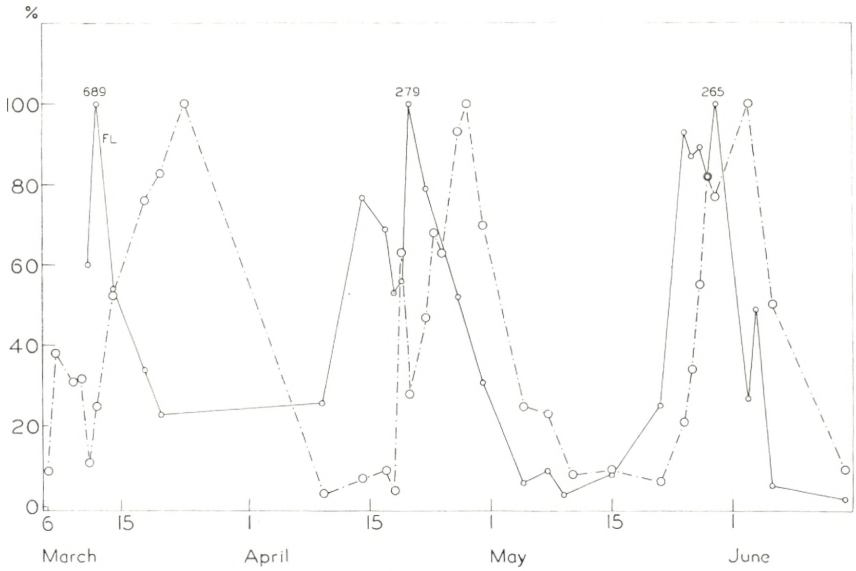

Fig. 15. Variation in the size of the population at FL (————) and at Tx (–·–·–·–·–·–).

middle of March, culminating on March 10 with 411 individuals—this was the second day of marking experiment 2. Later on, the population at this feeding area was rather small. There was an indistinct maximum of 76 in the latter part of April and another, somewhat clearer maximum (131) on the first of June.

At FL (fig. 15) the number increased rapidly during the early part of the month and reached the highest value counted here, 689, on March 12. There were always many butterflies in this area during the ensuing month; the lowest count was 72. On April 14 the number had again risen above 200, and the next maximum occurred on April 20 with 279 butterflies. The subsequent decrease eventually brought the number down to 8 individuals on May 9. Two weeks later it was up to 247 and remained over 200 the rest of May with a maximum of 265 on the 28th. At the last count on June 13, the FL population was down to 4.

It is not possible to present a clear interpretation of these fluctuations but a possible explanation can be advanced, as follows: In March the populations in the three northern areas (FI, AII, AI) had outbreaks in the middle of the month which were the year's highest population levels; later outbreaks were

successively smaller. The population at Tx, however, had its outbreak two weeks later in March and was just then started on its annual upsurge. Migrants from this Tx population (and from the probably simultaneous outbreak on the South Beach) settled down, most of them in AI, fewer in AII, and very few in FI. The descendants of these migrants appeared simultaneously with the generations in Tx and thus delayed the following maxima in the northern areas. In AI the maximum synchronized with Tx as early as April, in AII this happened only in the next generation, and in FL the maximum was also delayed but was a few days short of complete synchrony with Tx (May 28 vs. June 1).

In 1954 the picture was still more complicated; the populations everywhere were at a much higher level, and migrants from distant places as Scottsmoor arrived in such numbers that they obscured the regular fluctuations. It is, however, characteristic that the maximum outbreak at Tx on Fort Pierce's North Beach, which is the best observed area, occurred in 1949 on April 15–23, in 1950 on April 23–26, and in 1954 on April 23–27. In other years, especially 1957, high activity was observed during the last half of April, although the observations were too causal to determine exact dates for the maximum outbreak.

Similar regularity was found in the northern areas around Vero Beach where the activity increased towards the end of the year, and then, through one or two later waves, built up to a maximum in March after which the population began to decrease, one or two months before the population at Tx decreased.

A fact which throws some doubt on the interpretation of these observations is that the time difference between the maxima is nearly the same; at Tx in 1950 the difference between the first and second maximum is 35 days; between the second and third, 33 days. As the temperature difference between the two periods is considerable, a much large difference in duration was to be expected. An average of maximum and minimum temperatures from the U. S. Weather Bureau station in Fort Pierce was the only available meteorological data for the two periods, this average was 20°1 and 24°9, resp. If the duration of the egg stage is used as an index for the effect of temperature on development, the relative duration at 20° and 25° is as 17:10 (fig. 3); in other words, if the first period was 35 days, the second should only have been

20 days; and if the second was 33 days, the first should have been 56 days. Even if this calculation is based on inadequate figures, there can be no doubt that the temperature difference between the two periods makes it difficult to understand the nearly equal periods between the maxima.

Population changes of a different kind were observed some 140 kilometers to the north of the above areas in the Titusville region. That area was inspected for some months when on December 4, 1949, an outbreak was observed. The number of butterflies increased at each following visit: Dec. 8, Dec. 31, Jan. 6, and Jan. 20. On the latter date, the numbers reached a maximum and there were clouds of butterflies not only in the study areas but everywhere on Wilson Island and even on Merritt Island. On subsequent visits, Feb. 4, Feb. 12, Mar. 25–26, Apr. 13, and May 2, the numbers gradually decreased. On the last visit only 12 individuals were seen in the enormous area at Playalinda.

These observations in the Titusville region are hardly consistent with the fluctuations in numbers corresponding to generations so characteristic of the populations in the Fort Pierce area and also in the populations at Oslo, Jensen Beach, and Boynton Beach. It is likely that they also took place in the colonies at Boca Chica and the southern Florida Keys and at Everglades City and Punta Gorda on the lower Gulf of Mexico coast although these places have been observed too little to permit a definite statement. Continuous breeding, or more precisely, breeding without synchronized generations, was not found in any other coastal population than that at Titusville, with the possible exception of Lotus Island. The latter had Lepidium as food plant for the larvae and thus had another point of similarly with inland populations where continuous breeding is normal.

All our observations indicate that the populations have a yearly cycle.

The unusually cold winter, 1950–1951, destroyed all the inland colonies we knew, and the coastal populations were so reduced that the numbers in 1951 and 1952 were much lower than in the preceding years. It was not until 1953 that we again saw clouds af *Ascia* at the old places, as in 1949 and 1950. We were not able to make any real observations during the following years, but what we saw confirmed our conclusions that outbreaks, migrations,

etc. occur every year at practically the same time of the year, albeit at very different intensities. In January and February 1960, Playalinda and Boynton Beach were inspected and the populations were at the same stages as in 1950. The yearly cycle consists of a passive period of about eight months during which very few of these butterflies are seen, followed by an active period of four months with greatly increased breeding occurring either continuously with non-synchronized generations or in outbreaks corresponding to three generations.

Perhaps the greatest difficulty in understanding the fluctuation of *Ascia* populations is the fact that that although the cycle in any given population is 12 months, the active period (with outbreaks) is not limited everywhere to the same season. On the contrary, four of the populations studied in 1949–1950 were so dispersed chronologically that on any given day at least one of them was active: (1) The Titusville population from December to April; (2) The Fort Pierce populations from March to June; (3) The colony at Bradenton was at a maximum in August and ended in September, it had probably started its active period in June; and (4) the Punta Gorda population was active probably in August and in any case was so until November.

The reason for the sudden decrease which marks the end of the active period is another unsolved problem. RICHARDS (1940) found the factors controlling the abundance of *Pieris rapae* to be parasites which kill 75 % of the animals and predators which kill the rest. A chalcid wasp, probably *Pteromalus puparum*, was often found in the pupal cases of *Ascia*, especially towards the end of the activity periods (Titusville in February, Boynton Beach in March). A tachinid fly was also repeatedly found in the pupae. But neither these parasites nor any of the known predators (see p. 17) was the decisive factor. Many times we have marked eggs deposited on small isolated food plants and later were unable to find the newly hatched larvae, especially in the last generation of an outbreak period. They seemed simply to disappear. An unknown predator or parasite was undoubtedly at work here and probably is the most important factor in terminating the active period. It is probably a very specific enemy which after a few generations of mass production of *Ascia* propagates to such an extent that the population is reduced to the passive level from

which it takes eight months or probably six generations to return to the active stages. This hypothesis may be correct, but it still leaves unsolved the problem of why this process should take exactly 12 months.

In June 1954, we found one coccinellid larvae which sucked out the eggs. This may be the suspected predator although we have since spent much time trying to find more of them without success.

IV. The Migrations

(1) Definition of Migration

In order to define the term migration it is useful first to establish the term "home range" to describe the space within which a butterfly is able to consummate all the normal functions necessary to preserving the individual and the species: feeding, mating, resting, etc.

In this paper the term migration is used to indicate such a

Fig. 16. Migratory flight of *Ascia* at Narrow Point (see fig. 23). The view is toward the West across Indian River; the flight is toward the North, from left to right.

Fig. 17. Migratory flight of *Ascia* at Turtle Corner.

Fig. 18. Migrating *Ascia* on the Beach road A1A near the place where the marking took place in 1954 and 1957. A new, much wider road has now been built and only a few rudiments of the old road are left.

particular flight of butterflies by which they actively leave their home range and venture out into places which do not necessarily provide possibilities for the consummation of any of the basic reflexes known to us.

The flight of a migrating butterfly differs so much from other types of flight that even a casual observer is able to identify a migrant. The characteristics of the migratory flight, as it appears to the observer, and some of the conditions prevailing and presumably necessary for the habit of migration of *Ascia*, follow.

(2) Appearance of the Migratory Flight

(a) *General Description*

Some butterflies, e. g. the Monarch (*Danais plexippus* L.), migrate over a wide front; an observer driving from East to West will, mile after mile, cross flights of southbound individuals.

In *Ascia* the migrations occur as narrow streams in which all the individuals follow more or less closely the same track. The streams vary in width from a few meters to 10–15 meters; if the streams are wider they are usually divided into two or more streams.

Within these streams the single individuals usually fly between one and four meters above the virtual surface by which is meant the average height of the tops of dense vegetation if any. Isolated trees or bushes are by-passed by veering around them; a hedge across the flight path will cause the stream to rise over it; there are several good descriptions (Fernald 1937 a, Williams 1930, p. 331) of "climbing" over building and several times this has been observed during the present work. In such instances some individuals will pass less than one meter above the highest point.

At the point where the migration starts, the outbreak center, there takes place what Fernald so aptly described as an enormous milling around. During this, the butterflies often rise high in the air—up to ten or fifteen meters; also, especially in the beginning of the migration, some butterflies will still fly rather high. However, when the migratory flight is definitely adopted, the participants very rarely fly more than 3–4 meters above ground.

The migratory flight is somewhat influenced by the wind (fig.

19). Cross-winds cause the streams to form on the sheltered side of vegetation. Offshore winds cause flight along the ocean beach to be on the sea side, and, if the wind is from the sea, on the inland side behind the dunes. In calm weather the flight is often right over the top of the dunes.

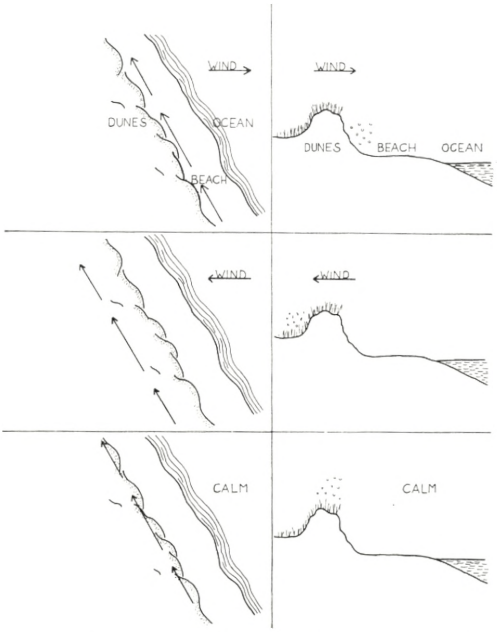

Fig. 19. Displacement of migratory flight of *Ascia* by strong winds. The figures to the left are diagrammatic maps on which the flight is indicated by arrows. To the right is shown a cross section of the same situation as shown to the left, the position of the butterflies being indicated by dots.

Strong wind, especially headwind, will reduce the height of the flight.

(b) *Orientation*

The direction of the flight is typically a straightlined course. Most of the outbreak centers on the east coast of Florida are on the long narrow islands paralleling the coast and the streams usually following the islands. When the migrations come to a wider part of an island they may branch out into several streams, but at narrow places they will unite again. On the lee side some of them may make a short-cut over a cove (fig. 20).

It was mentioned in the first report that streams often follow

such topographical features as roads, etc.; if such a guideline makes a turn, the butterflies usually follow it if the deviation is small.

There used to be a place between Vero Beach and Wabasso where this was clearly demonstrated (fig. 21). Telephone lines on poles ran along the Beach Road and at a certain point the road made at 20° turn to the east while the lines continued the direction.

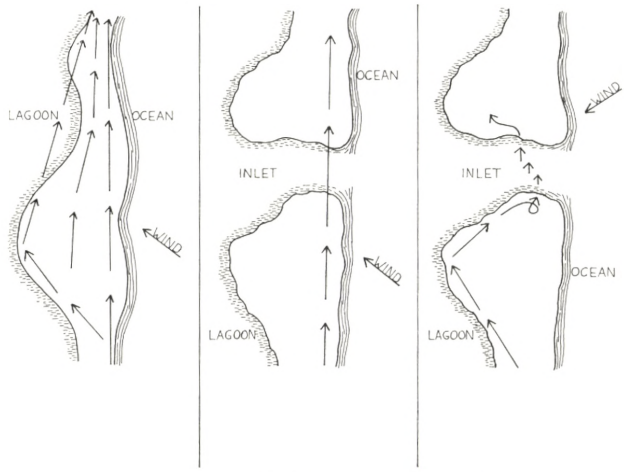

Fig. 20. Effect of strong wind on the flight of *Ascia* as indicated by the arrows. To the left is shown how a stream may be divided into 3 one of which passes over the water on the lee side.
Center figure: Flying with the wind an inlet is crossed without change of direction. Against the wind (figure to the right) the flight is modified.

The vegetation was partly removed around the lines but the cut-out was, of course, less prominent to the human eye than the paved road. The northbound stream of migrating butterflies would divide into two at this point; usually about two-thirds followed the road, one-third kept going along the lines. As a rule there was no indecision in the flight; actually, when the butterflies were 30–50 m from the turning point we could tell which ones were going to make the turn and which would keep the straight course.

The most essential modification of a flight occurs when the track leads the animals over an open place such as water or a flat, sandy area probably because of the influence of increased wind (especially headwinds) in such a place (fig. 20).

As to the orientation when crossing water the following observation is rather enlightening.

A southbound migration took place (April 3, 1950) on the very narrow Lotus Island; at the tip it crossed over to the beach island just north of the old bridge to Eau Gallie. A straight line between this point and the tip of Lotus Island is about 500 m (fig. 22).

The course of a straightlined flight from Lotus Island would be 155° magnetic bearing (SSE), but the butterflies did not arrive

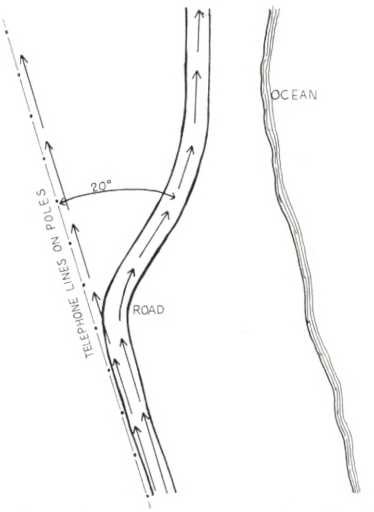

Fig. 21. Effect of topographical features on the direction of the migratory flight of *Ascia*. See text 44.

on this course—they came from the northwest or west; it was possible by means of strong field glasses to follow a single individual from the time it left Lotus Island until it arrived, and it was clearly seen to make a large turn out towards the west before it arrived. The wind was from the east, averaging 2.7 m/sec. This peculiar flight was not understood when the observation was made but a sketch map was drawn on the spot. Later it was found that this sketch corresponded exactly to one of the situations discussed by Acworth (1929) for an animal flying over open water in a cross-wind. There are three possibilities: (1) the animal can take account of the drift, and set the course so that the track becomes a straight line between the point of departure and the goal; (2) it can fly on a fixed course, without the ability to correct for the drift, in which case the track will be a straight line diverging from the course; or (3) finally, if the animal steadily heads towards

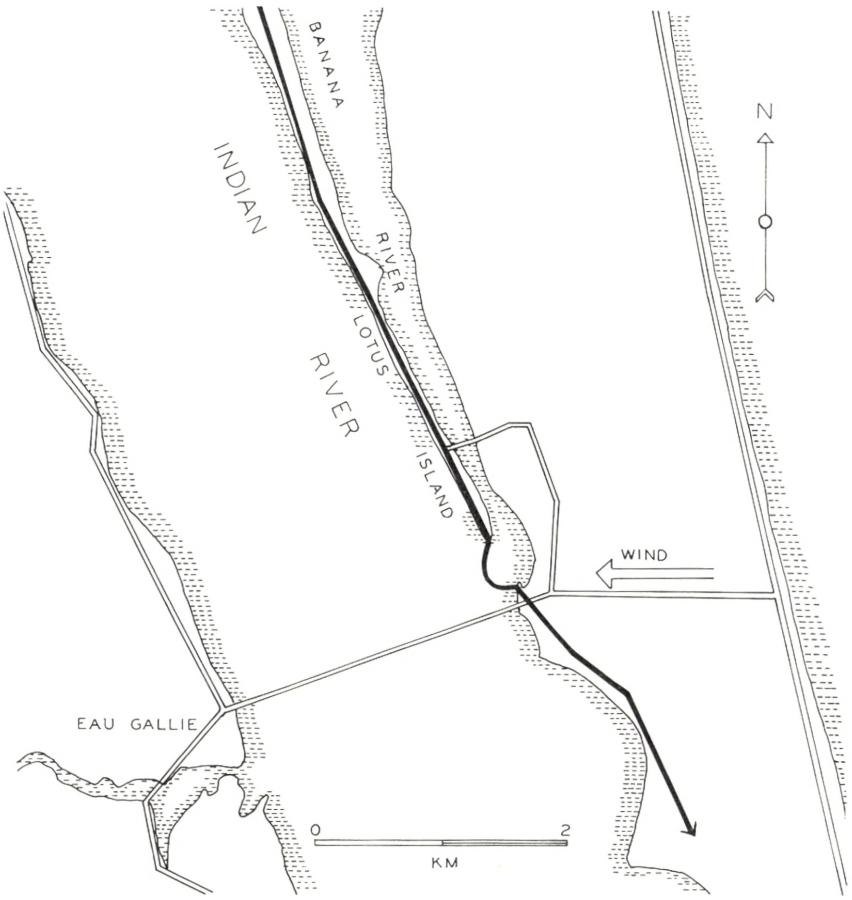

Fig. 22. Flight over open water in crosswind (see text).

the goal, but cannot correct for the drift, the result will be a curved track, exactly as sketched during the observation.

The observations on which this report is based were mostly made along the coast where the most frequent courses follow topographical characteristics; in the first report (Nielsen and Nielsen, 1950) examples of the occurrence of flight with fixed courses, especially in cross-country flights, were given. At that time it was unknown how the butterflies could keep their course by sun compass reaction when the sun changes position; however, after it was shown (Kramer, 1950) that birds and insects are able to correct for the change of the position of the sun, there is no problem of understanding that the same may be the case for *Ascia*.

Nevertheless, it is remarkable that the butterflies are able to resume the same course after a night's rest as observations seem to indicate (NIELSEN and NIELSEN, 1950, p. 15–16).

Although it is obvious that guide lines and visible points are important for orientation during the migratory flight, it must be remembered that they are used only if they are close to the fixed course; in other words, the flight direction is primarily fixed but landmarks are used to follow the course. How the course originally becomes fixed will be discussed on page 68.

(c) *Speed*

In the first part of the work (NIELSEN and NIELSEN, 1950) some consideration was given to the speed of the flight. The speed of a flying animal may be determined either in relation to the surrounding air masses (air speed) or in relation to fixed points on the ground. In estimates of the energy output produced by the butterfly, it is necessary to compute the airspeed, which can be done if the ground speed and the course of the animal is known and the direction and velocity of the wind is measured. As the butterflies have the tendency, mentioned above, to fly in the shelter of irregular objects it is very difficult to measure the velocity of the air through which the butterfly is flying, and it would often be difficult to get a usable estimate of the air speed; observations have shown, however, that the ground speed is fairly constant, and for the description of migrations aimed at here it is hardly necessary to try to calculate the air speed.

In the spring of 1949 the ground speed was found to vary between 11.7 and 16.0 km/h with an average of about 13 km/hr. The larger number of later observations confirmed this, but strong wind may produce more extreme values; against a very strong wind the speed may be as low as 8 km/h; and with a strong tail wind a marked indvidual once flew from a little south of New Smyrna Beach to Playalinda—a distance of 39 km—in one hour and fifty minutes corresponding to 21 km/h. But as said above, these values are exceptional; 12–14 km/h is normal speed.

In the first report (NIELSEN and NIELSEN, 1950) it was strongly emphasized that the migratory flight is very persistent: the individuals do not stop for food, and no sexual activity between migrants was ever seen. This has to be modified: migrants from

the very large outbreaks in 1954 continued the flight much longer (8–10 hours) than those in the earlier observations which lasted only 2–5 hours; those on the long migrations stopped to feed occasionally. These stops were usually very hurried and quite different from the normal flitting around; they occurred after only one or two hours flight, while butterflies on shorter migrations continued the total flight without feeding.

(d) *Dilution*

The participants in a migration seemed to move approximately at the same speed but closer examination showed that some displacements took place.

During the marking experiments in 1954 it was noted that few of the marked migrants were observed even a short distance away from the point of marking, and this was presumed to be caused by displacement.

The experiments in 1957 were meant to throw some light on this problem.

In order to make clear what is here called dilution in migration, let us consider a migratory stream of e. g. 100 individuals per minute. If for 10 minutes every individual passing a certain point were marked we would start out with 1000 marked individuals in a compact section of a stream.

Due to small irregularities in the flight the marked and unmarked individuals will begin to mix with one another. Sometime after the marking, the passage of migrants might require 20 minutes to pass a given point. At this time half the migrants might be marked, half unmarked. Later, the migrants might require one hundred minutes to pass and only 10 % might be marked. The dilution here would be expressed as 50 % and 10 % respectively.

The marking experiments in 1957 were made in order to find a relation between the dilution and the distance covered by the migration. It was decided beforehand to use the duration of the passage as an expression instead of the percentage of marked individuals in the stream; it would be easier for the observers to have to count only the marked ones, and furthermore, migrants from populations passed en route might join the migration. The distribution of the migrants is skew; a large number appear in a relatively short period and a few late stragglers may come hours

later. Therefore, we have not used the total duration of the passage of the passage but the time it takes half the main number of individuals to pass a certain point of the track.

The marking was made by alcoholic solutions of different dyes (see p. 27). The use of methylviolet was discontinued because at a distance the color could not always easily be distinguished from the dark females. Rhodamine and eosin were the most useful colors. Improvement in the technique was achieved when the fluid was dispensed under high pressure through a regular spray nozzle.

A truck with the equipment was parked at a suitable place where previous observations had shown that a concentrated stream would pass. The colored alcohol was poured into a drum which had connections for three ordinary garden hoses. The pressure in the drum was produced by compressions of the motor by means of a valve inserted in place of one of the sparkplugs. Spraying could start when the pressure reached 30 pounds/sq. inch (2 kg/cm^2). For each marking, 40 gallons (150 liters) alcohol was used, and this amount was dispensed in about 10 minutes.

The experiments were carried out on April 24 and 26. The spraying took place behind the dunes about 7.4 km north of Rio Mar. Countings were made at three points, 1.7, 6.0, and 15.0 km, resp., from the place of marking. (Fig. 23). The relatively short distances were chosen in order to obtain figures large enough for calculation. All available personnel were placed at the three counting stations; the total number of marked animals was unknown, but the spraying was limited to exactly 10 minutes and it was estimated that the counts at point 3 represented 60–70 $^0/_0$ of the marked individuals.

Table 4 shows the agreement between the two experiments. The total numbers indicate that only a branch of the migrations was observed at the points 1.7 km and 6.0 km from the marking place. The individuals counted at point 1.7 km flew directly from the marking point, while an inland detour, made by some of the individuals counted at the other points, perhaps is reflected in the slightly lower ground speed at point 6.0 and 15.0 km.

The time it lasts for 50 $^0/_0$ of the marked to pass the observation points appears very nearly to be a straight line function of the

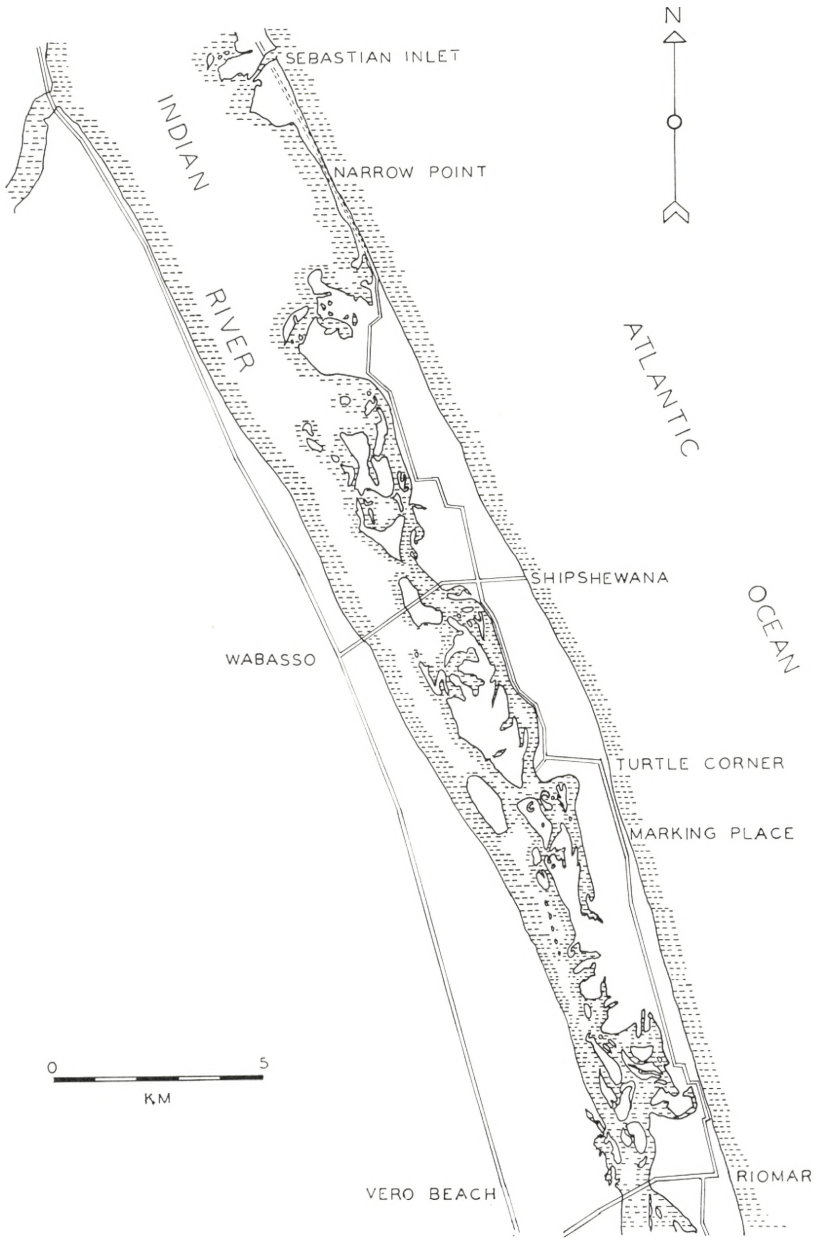

Fig. 23. Marking place and the three points of observation (Turtle Corner, Shipshewana, and Narrow Point) during the marking experiment in 1957.

TABLE 4.

Distance in km from point of marking	Total number observed		Hour when 50 % were marked or had passed by		Speed of flight (km/hour)	
	I	II	I	II	I	II
0.....	—	—	$10^h06^1/_2'$	$10^h05'$	—	—
1.7.....	83	112	$10^h15'$	$10^h13'$	12.0	12.8
6.0.....	42	76	$10^h40'$	$10^h36'$	10.8	11.6
15.0.....	225	394	$11^h27'$	$11^h25'$	11.2	11.3

I and II refer to the two experiments April 24 and 26 resp.

distance from the marking point (fig. 24); it has approximately the parameters

$$y = 1.00 + 0.25\,x$$

and the dilution $(1/y)$ is therefore a hyperbolic function

$$x\left(\frac{1}{y-100}\right) = 4.00\,.$$

The correctness of the straight line function is amazingly good which probably is incidental and should only be considered as an example of how the dilution works. We have tried to extrapolate the curve by applying it to one of the long range experiments from April 27, 1954.

About 3000 individuals were marked during 50 minutes. The main observation points were 24, 67 and 140 km from the marking point which was the same as used in 1957.

It was calculated that there should be an intensity of 9 individuals per minute at the first observation point; 112 marked individuals were observed during ten minutes which is a fairly good agreement. But at the two distant stations there were observed only 0.25 i/m instead of 3.4 i/m 67 km from the marking point; and at a distance of 140 km, 0.20 i/m were found instead of the calculated 1.7 i/m. Some of the migrants had either dropped out of the migration and stayed at one of the breeding areas passed by the flight, or a part of the migration had been diverted to another course.

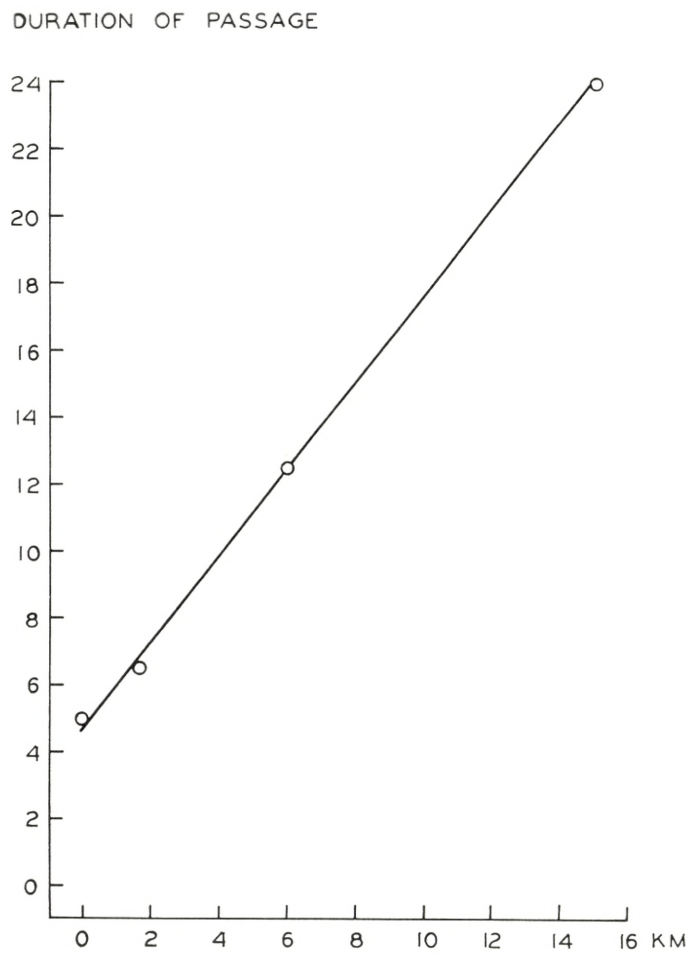

Fig. 24. Duration of the passage of the first half of a group of marked migrants as a function of the distance from the point where the marking took place.

(e) *Numbers*

The most correct expression for the number of migrants is the number per kilometer of the stream (S), which can be calculated by counting the number passing the observer per minute (s) and measuring the ground speed (g) in km/hour.

We know then that:

$$S = \frac{60\,s}{g}.$$

As mentioned above (g) is normally fairly constant and no serious error is committed by using (s) instead of (S) if wind conditions are not extreme. During the first part of the work we found (s) varying between 0.2 and 30 individuals per minute (i/m); HODGES (WILLIAMS, 1942) has observed migrations of 300 i/m and in 1954 we saw streams of 5000 i/m—this figure is, of course, only an estimate.

The total number of participants in a migration can be roughly estimated. As will be shown on p. 63 the number of migrants leaving an outbreak area during the first hour is about 20 times greater than during the average of the following hours; for a medium large migration lasting five hours, we might have a morning maximum of 100 i/m leaving during the first hour = 6000 individuals; in the following four hours there will be $4 \times 5 \times 60 =$ 1200 more departing, or 7200 in all.

Of course, the distance from the starting point also has to be taken into account in these calculations because the distance tends to dilute the morning maximum just as the marked individuals were diluted.

As an example a migration through Indian River Gardens originating from Oslo Road shall be mentioned; the distance is about 12 km.

The maximum density at 10^h was 79 i/m; during a couple of hours around the maximum, the average was 40 i/m. If we estimate the dilution to have been 20 %, the original take-off included 12,000 individuals during the first hour and about 2400 more in the hours following. The estimated total was thus 14,400. Countings were continued that day to try to get enough figures for a direct estimate. The result was a total of about 10,000, an agreement which is sufficient considering the errors involved.

By a similar calculation the largest migrations seen, 5000 i/m, represent a total number of between a half and one million butterflies.

The casual observer of butterfly migrations might easily overestimate the number of participants. Before we learned to make a fairly justified estimate of the numbers, the striking appearance of the phenomenon gave a highly exaggerated impression.

On March 29, 1950 the southbound migration from New Smyrna Beach was observed from one of the old look-out towers

in the dunes. There was a strong offshore wind so that the butterflies flew on the lee side of the dunes on the ocean side. It looked like a white-dotted, glittering band slowly drawn along the dunes as far as the eye could see. The sight was very spectacular, and gave the impression of countless millions of migrants. Actually the average density was about 30 i/m (maximum 38) and hardly more than four to six thousand butterflies took part in the migration all day long.

(f) *Time of the Day*

It has been shown above that the activity of *Ascia* normally is limited to eight hours a day, from 08^h to 16^h. In 1950 the migratory activity was found to be still further limited. In the morning most of the animals fed before they took off; the feeding on flowers usually lasted about one hour. The main migrations, therefore, started normally around 09^h. The end of the migratory activity was found to vary a good deal but in 1950 very few migrants were seen after 14^h. The typical period of migration was five hours.

In 1954 these time limits held true for the early migrations. But as outbreaks increased in size and the density of the migratory flights were much higher than ever observed before, it was noted that the duration of the flights became longer. The migrants left the outbreak area soon after they had appeared from the resting places. It is not possible to say what the single migrant actually did, but the situation gave the impression that the urge to migrate was stronger than the urge to eat. It is hardly possible to avoid recalling the observation that migrants taking part in these large flights occasionally stopped for a hasty feeding.

TABLE 5.

Distance in km from point of marking	Duration of passage of 50 % (minutes)				Dilution
	I	II	Average	Calculated	Calculated
0	5	5	5	1.00	1.00
1.7	5	8	$6^1/_2$	1.43	70
6.0	12	13	$12^1/_2$	2.50	40
15.0	19	29	24	4.75	21

Without claiming that the interpretation is justified, it seems as if the butterflies in the very large outbreaks were so eager to migrate that they "forgot" to eat before taking off and later had to make up for it. Another possible explanation is that the tremendous number of animals at the outbreak centers when feeding either exhausted the nectar content of the flowers or disturbed each other so much that many of them had to leave the usual feeding grounds and seek flowers further away. The more animals there were the further they had to go and during the very large outbreaks some individuals began the migration before getting sufficient food for a prolonged flight.

The very large migrations went on until late in the afternoon, stopping first at sunset. Single individuals were even seen flying after sunset, in darkness.

It is here appropriate to mention the observations by HAYWARD (1953) of *Ascia* in Argentina; he found that the very large flights sometimes went on during the night. The differences between his observations and ours shall be discussed on p. 71.

Summarizing it can be said, that no migrations occur from the small population. From the medium-sized outbreak the migrations last from one to five hours; from the large outbreak the migrations last 8–10 hours and were continued the next day.

(g) *Range*

For the observers of insect migrations the question of the range of these flights has always been a point of special interest. We have in this connection first the make clear what we understand by range. First of all we have to realize that insects might be found far beyond the range carried by passive transport, e. g. car or train or a storm. But we have also observations which show that migrants which have adopted a course carrying them over areas where there is no possibility of breeding may extend the flight as is frequently the case with cross-country migrations as already observed in 1949. The normal range of dispersal by migration is the distance normally covered by an essential part of the migrants leaving an outbreak area.

To get an idea of the range of a migration by stationary observers will usually not give satisfactory results. The only two reliable methods are either to mark the individual migrants or to

follow them during the flight. In 1950 when we did not yet have a method for marking migrants, we followed the migrations by direct observation, using a car to proceed from the outbreak to the end of the flight. It was an advantage for the observer that migrations usually develop every day for several successive days, so what has been missed one day may be seen the next.

It is also helpful that the density of the departing migration is much larger during the first hour—we shall return to the reason below (p. 63).

Just outside the outbreak area the maximum passes shortly after 09^h with a density about 20 times that observed later in the day. Farther away it passes at an hour which is determined by (1) the hour of the morning exodus, (2) the speed of the flight, (3) the distance from the starting point. With two of these factors known the third may be determined.

Observations at the North Bridge at Fort Pierce suggested the possibility of using this method. It was observed here that migrations toward the mainland from the breeding areas around Little Jim culminated about 09^h. During the following hour the intensity decreased but at the same time an increasing stream came from the opposite direction. It appeared as if the butterflies were returning from the mainland, and for a short time this possibility was seriously considered. It was found, however, that the first flight to the mainland fanned out in a number of streams with low intensities but the eastbound stream came from a heavy flight following the coast of the mainland from north to south until it reached the bridge where part of it veered to an easterly direction.

In Indian River Gardens, 700 m north of the bridge, we found that the maximum arrived rather regularly about 10^h, and it seemed likely that these migrants came from a point one hour's flight north of IRG. Most of these mornings there was a rather fresh trade wind blowing from the southeast, probably slowing the flight somewhat. Most of the coast is inaccessible but on Oslo Road 12 km north of IRG we found a good place for observation. The vast marshes here seemed a very likely breeding area, as later they were found to be.

On May 3, 1950, simultaneous countings were made on Oslo Road and in IRG (fig. 25). At Oslo the maximum occurred at

$08^h45'$ thus confirming the idea that this point was on the outskirts of the outbreak area. In IRG the maximum was observed to be at $10^h13'$, nearly one and a half hours later, corresponding to a ground speed of 8–9 km/h. The wind was from the ESE and increased from 0.5 m/sec at $08^h00'$ to 5.4 m/sec at $10^h40'$. Most of the way the migrants had a headwind of perhaps 10 km/h.

Beside the main stream there was a smaller stream over the Indian River paralleling the coast for a distance of 30–40 m. These

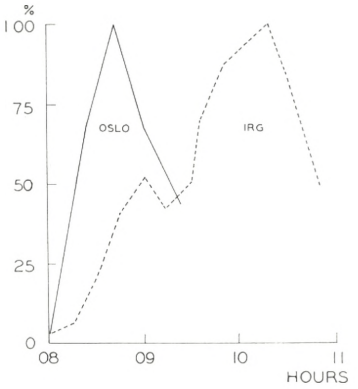

Fig. 25. Relative numbers of individuals in a migratory flight passing two points 12 kilometers apart (Oslo Road and Indian River Gardens). The numbers are given as percentages of the maximum.

butterflies were flying only 10–15 cm above the surface of the water. Eventually, when the wind rose, they came towards the coast and joined the main stream.

Some of the migrants did not turn at the bridge to the North Beach but continued south along the coast. This flight branched out again repeatedly with streams towards the beach, the last of them in the city of Fort Pierce, where they flew towards the breeding areas on the South Beach. On the coastal road going south from Fort Pierce we did not see any migration between the city limits and Eldred, a distance of about 5 km. A little north of Eldred a southbound migration was again observed but these butterflies flew in from the Indian River seemingly coming from the breeding areas around Little Jim. Simultaneous counts showed that when the maximum arrived at IRG at $09^h56'$ it arrived at Eldred at $10^h45'$ against a 5.5 m/sec SE wind. The distance is

about 12 km. The ground speed would thus have been 16 km/h (and the air speed well above 30 km/h) which is very unlikely. Much more likely, and in agreement with the direct observations, is that the maximum at Eldred is the morning exodus from Little Jim started at 09^h and arrived at Eldred at $10^h45'$, with a ground speed of 8 km/h.

The result of these observations is that the southbound migration from the breeding areas north of Oslo Road ended in the breeding areas at Little Jim and on the South Beach. The range was between 16 and 24 km and the duration of the individual flight between $1^1/_2$ and 3 hours.

Similar observations were made on the northbound flight from the area on Fort Pierce South Beach; the southbound flights from this area were not followed closely. We found that this migration ended at AII and at Porpoise Bay about 20 km north of the area and that the duration of the flight was $1^1/_2$–$2^1/_2$ hours.

Mr. and Mrs. KARL HODGES, who for years have cooperated with Dr. C. B. WILLIAMS (WILLIAMS et al. 1942) kindly informed us in Indiatlantic near Melbourne that the southbound migrations in the spring were heaviest around noon. An examination of their notes did not show a distinct maximum but 60 % of the southbound migrants were observed between 11^h and 13^h. For a long time we supposed these migrations to have arrived from the Titusville area but this hypothesis met with three difficulties: (1) the migrations were not simultaneous with outbreaks in that region; (2) the distance—about 65 km—is too large to permit the morning exodus to arrive at Indiatlantic by noon; and (3) counts at the beach north of Indiatlantic showed much lower intensities than south of Melbourne Beach.

Closer examination showed that the migrations which passed through the observation area of the HODGES' came from Lotus Island (map, fig. 11); it was possible to see the migrants cross from the tip of Lotus Island as mentioned above. This flight was then traced back to the breeding areas between Georgiana and Honeymoon Lake, and to the areas ENE of the small town of Merritt Island at New Found Harbour. The distance from these areas to Indiatlantic is 24 and 35 km resp., and with normal ground speed the morning exodus could be expected to arrive at Melbourne Beach between 11^h and 13^h, as observed by the HODGES.

These migrations ended somewhere between Indiatlantic and Sebastian Inlet at Grant where there are islands in the lagoon with breeding grounds. The range of this migration is thus 44–45 km.

The northbound migrations usually observed later in the season by the HODGES probably had their origins in the islands. We were told by Mr. and Mrs. KARL HODGES that they usually arrived earlier in the day, before noon. This is in agreement with the shorter distance (18–20 km) from the islands to Melbourne Beach. The northbound migrations through Indiatlantic usually continued across the Cape Canaveral area to the breeding areas at Playalinda. The range of these flights were therefore probably about 65–70 km.

In spring of 1954 an attempt was made to keep track of all migrations passing the Fort Pierce–Vero Veach area. Eight migrations were clearly recognized as shown in fig. 26. The first one, (A) was small with rather few individuals, from Oslo to Fort Pierce South Beach, similar to the one observed in May 1950; the range was about 20 km. The second, (B) was a very heavy one from the large breeding areas around Scottsmoor. The southbound migration ended on the first day at Vero Beach after a flight of about 130 km; it resumed the next day, adding a distance of 25 km to Fort Pierce South Beach. The third migration, (C) was a rather small one, ranging from Floralton to Wabasso, about 20 km. Two weeks later from nearby areas at Floralton a southbound migration (D) appeared. It passed through Jensen Beach and ended probably in the marshes south of St. Lucie River Inlet at Stuart, a range of 45–50 km. It was not very heavy. The next outbreak occurred a little further south, from McConville's property close to the feeding area AI (E). It was a medium-size migration. It probably ended on the islands at Grant. A few days later followed the largest outbreak we have ever seen (F). It originated from a number of breeding areas between Fort Pierce Inlet to AI and sent equally heavy streams northward and southward. At certain points the northbound stream was estimated at 5000 indivuals per minute. The first day's migration reached nearly to Playalinda, a distance of approximately 135 km. The next morning it continued for a couple of hours and added an additional 20–25 km to the range. The southbound flight against the wind went the 110 km to Boynton Beach; whether there was

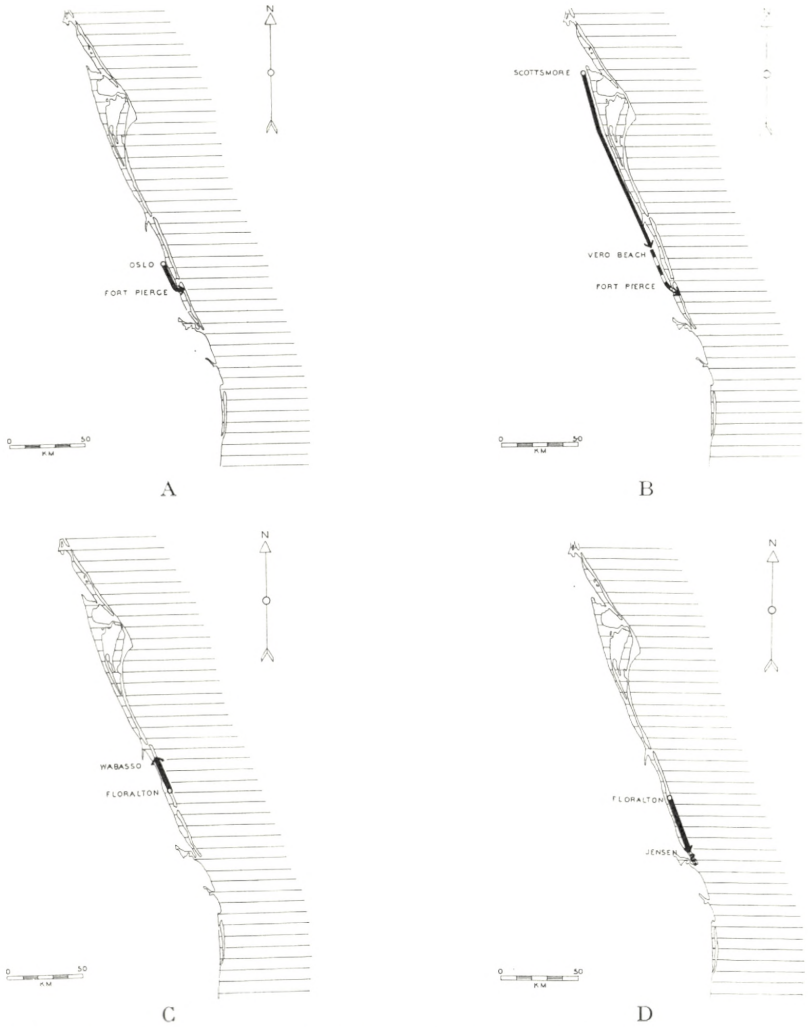

Fig. 26 A–D. Eight migrations on the East coast of Florida during the spring of 1954.

a second day's migration is unknown. The seventh migration (G) observed in 1954 went only south from Fort Pierce; it was observed at Juno Beach about 65 km from its origin but was not followed to the end. The last one (H), in June, came from the islands at Grant and went northward to Playalinda and southward to Fort Pierce South Beach.

Although these observations were rather certain it was a great relief that we were able to mark the migrants in the large north-

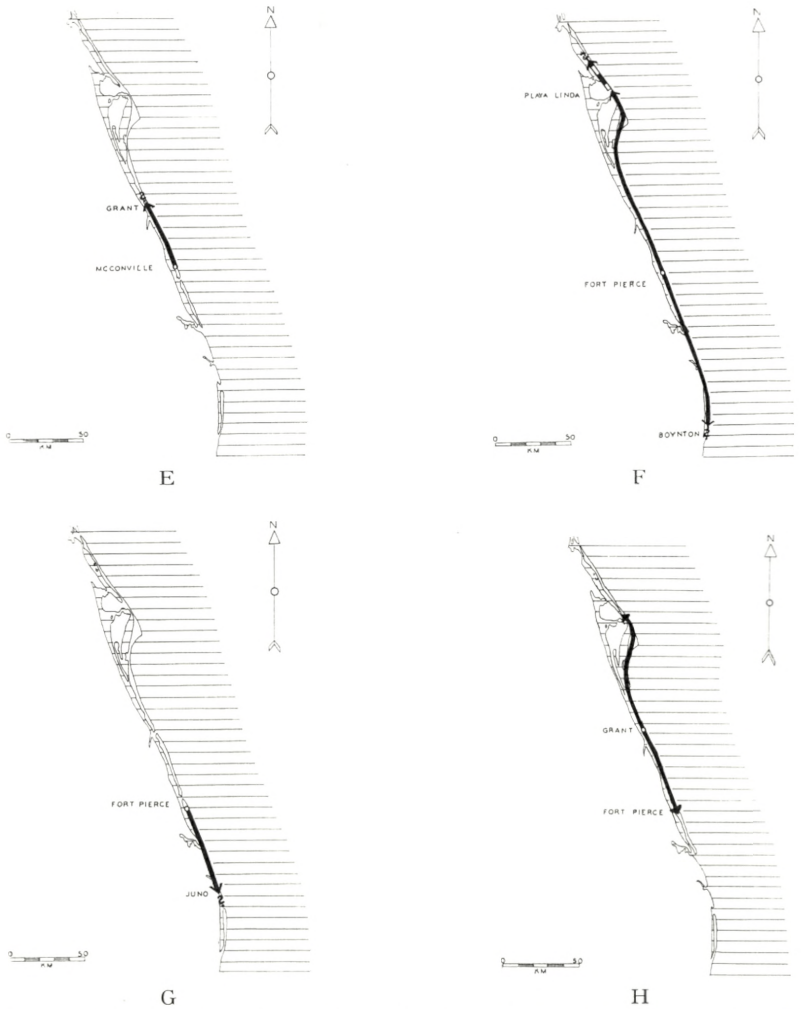

Fig. 26 E–H. Eight migrations on the East coast of Florida during the spring of 1954.

bound flight on April 27, 1954. The marking took place north of Vero Beach (fig. 23). Observations were made at several distances, and the progress followed to Cocoa Beach. The Cape Canaveral area was under military restrictions, and therefore we waited at Playalinda (see map). Calculations showed that although the animals were flying rather fast (14 km/h) with a near-tail wind from the SE, they could not be expected at Playalinda until about 18^h or later. An increase in the number here indicated the

correctness of the calculation, but no marked individuals were seen.

Next morning all observers were deployed over a fairly narrow stretch of the beach about 8 km north of Playalinda. Twenty-three, perhaps 24, marked individuals were observed between 07^h and $09^h42'$ in the northbound migration. The migration decreased suddenly and stopped at 10^h. The observation point was 110 km from the point where the animals were marked which again was 20–25 km north of the outbreak area.

(3) Conditions for the Release of the Migratory Flight

(a) *Internal Factors*

In our present state of ignorance about the nature of migratory flight the best we can do is to describe the conditions under which the migrations start with the presumption that these conditions are necessary for the release of the migratory urge.

We have some reason to believe that this urge first appears at a certain age after emergence.

The migrating *Ascia* are always immaculate individuals, apparently newly emerged, with the wings perfectly scaled. In the sedentary populations badly rubbed specimens were often seen; they were nearly always females. As the males usually live five days and the females 8–10 (see p. 17) it seems justifiable to presume that the females become "worn" when they are older than five days.

As males and females participate in equal numbers in the migration, it seems likely that migrations take place during the first five days after emergence.

Examination of the ovaries of about 90 migrating females showed most of them were in the state of maturing; few of them were completely undeveloped, and none were completely mature. All had copulated, many of them had bursa copulatrix swollen indicating a mating less than 12 hours ago. Their age according to the ovarial development would be estimated to be 20–36 hours old. There are other ways to determine the age at which the migrations start. The following is based on observations made in

1950 when the migrations were small to medium and lasted a maximum of five hours, from 09^h to 14^h.

Let us presume that no butterfly starts the migration outside these hours, and furthermore, that the emergence normally is distributed over the diel as found in the laboratory with 8 % for each hour between 09^h and 14^h, 2–3 % during the remaining 19 hours. By finally assuming different values for the earliest age in which *Ascia* starts migrating, it is possible to calculate the relative number of individuals in each age group, and of migrants leaving the area for each hour of the diel. From these calculations it is possible to find two proportions which may be directly observed:

One is the proportion of migrants to the rest of the population; it has always been observed that some of the butterflies remain in the outbreak areas. This proportion was usually estimated to be between one third and one fifth of the population and the percentage of migrants in a population should be between 60 and 80 %.

The other proportion is made up of those leaving during the first hour in relation to the average of those leaving during the following hours. Whatever the time limits for the migration, during the night there will always be a number of individuals which have attained the age in which they are able to migrate but have to wait until morning to release the activity. In five cases countings were made all day in a short distance from a breeding area and an average found the proportion to be 19:1 = nineteen times more migrants left between 09^h and 10^h than during the average of the following four hours.

The effect of a number of age limits for the period of migratory activity on these two proportions has been calculated; if the limits are 10 and 30 hours, it is found that all butterflies may migrate and the morning migration would be nearly 30 times denser than the rest of the day. By trying a number of other values it is found that if the migratory period is limited to the age between 18–19 hours and 30 hours, we find that 76 % of the population will migrate, and that the morning maximum will be 20.6 times larger than the average density later in the day; both proportions thus in agreement with the observations.

On April 26, 1950, it was noted that in the morning there was

a slight increase in the number of animals (average of 388) feeding at Tx; furthermore that a few dark females appeared for the first time that year. When this place was observed again at $14^h20'$ the number had increased to 597 and many dark females were seen. The next morning at $09^h15'$ the number feeding had increased to 640 but there was still no sign of migration. The migration did not start until $10^h30'$; as the peak of emergence was about 14 the day before, the average age of the migrants at the start of migration probably was 20–21 hours.

The physiological age is probably dependent on temperature and a delay of a few hours can easily be explained by low night-temperature.

The concept of a period of delay before the migration as described here for butterflies was used to explain similar conditions in migratory mosquitoes (HAEGER, 1960; NIELSEN, 1958) and it was found to be a useful hypothesis. For mosquitoes the rotation of the hypopygium which is an indication of the physiological age of the male mosquitoes has been found to vary in duration with temperature (Dr. P. T. LUM, personal communication).

During the preceding discussion it was supposed that the eggs matured at an age of 30 hours and that the urge to migrate would cease with the maturation of the ovary. This would mean, of course, that a migration could not be resumed a second day which would be contrary to observations reported on p. 59 and 62.

This could mean, therefore, that females with eggs are able to continue migration. However, there is another possible explanation, namely the recent finding of ZAHER and LONG (1959) that the preoviposition period in *Pieris* is longer if the larvae have been crowded.

The observations reported here do not permit conclusions to be drawn in this respect, but it seems likely that the age at which eggs mature and migration ends is higher in the crowded conditions of large outbreaks.

We have already reported some observations on the end of migrations; a specially enlightening example follows:

A branch of the migration from Oslo road to Fort Pierce on the mainland followed the railroad track through town to Orange Avenue where it turned 45° to the left, crossed the street in an acute angle, followed the house wall up to the roof, came down

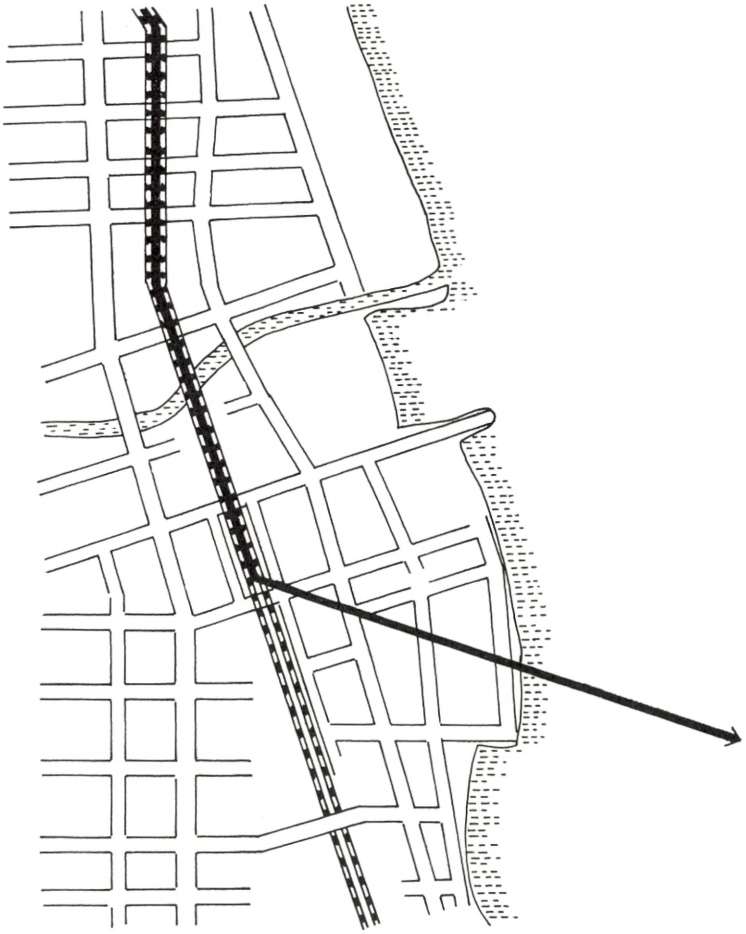

Fig. 27. The end of a migration in downtown Fort Pierce (see text).

on Second Street, and in the same way 'cut' a corner of a building three stories high on the east side of this street (fig. 27). For more than half an hour butterfly followed butterfly through these complicated maneuvers despite intervals often so long that they could not see one another.

The most likely explanation is that the salt marshes with *Batis*, which has a rather characteristic smell even for the weak human senses drew them. The wind during the observation was 5.5 m/sec from the marshes so that after the turn the butterflies flew directly against the wind.

Similar observations were made twice in 1954. In these, the wind was more to the south and the turn occurred correspondingly further north and was not as spectacular.

If the explanation is that the butterflies flew towards the smell of the breeding areas, this would imply that the migratory flight ended at the corner of Orange Avenue and the railroad track, and here became replaced by an appetential flight towards the place for the release of the reproductive behavior.

(b) *External Factors*

The external factors also play a role in the release of the migratory flight.

One of the most essential is undoubtedly the time of the day. We do not know whether this limitation of migration to a certain part of the day is dependent on an internal rhythm or on light change; it seems less likely that the temperature is a decisive factor in this respect since the beginning of the departure occurs at about the same time whenever the temperature is above a certain level.

As reported in the first of these papers (NIELSEN and NIELSEN, 1950) we found no migratory activity below 24° and nor did we observe the typical determined migratory flight at temperatures below 27°. Between 24 and 27° the flight may be uni-directional but less resolute with many stops for feeding on flowers (fig. 28).

This was generally confirmed by later observations although temperature limits were found to be more flexible. Uni-directional flights interrupted by feeding were found to be the normal means of migrating at low temperatures. We propose to call such flights "sub-migrations". The frequent remark in the reports from the HODGES, "Feeding as they fly", is a very good description of the phenomena.

The influence of temperature is clearly seen from our material gathered in 1950. Three times we have observed migratory flights at an air temperature of 23°; they were all submigrations. At 24° five of seven flights were submigrations, and of 13 observations at 25°, five were submigrations. Above 26°, 149 flights were typical migrations.

We found no upper temperature limit for the migratory habit. Nine flights were recorded at 30° and three at 31°.

RAIN will stop migrations and prevent the start as mentioned in the first report (NIELSEN and NIELSEN, 1950). In a few cases, it was observed that a completely overcast sky without rain—a rare phenomenon in Florida—also prevents migration (e. g. March 19, 1954). This indicates strongly the existence of a compass reaction by polarized light from the blue sky as found for bees (VON FRISCH, 1950; CARTHY, 1958).

The relative humidity does not seem to have any influence on migration. Of more importance is the question of wind. KENNEDY

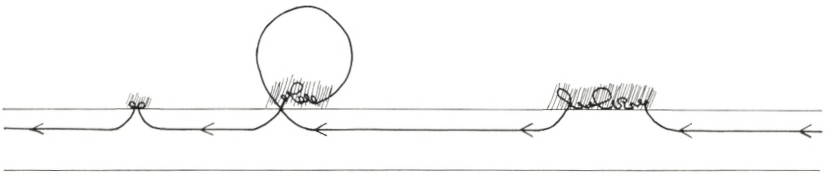

Fig. 28. Typical submigratory flight along a road. Hatched areas indicate roadside flowers.

(1957), WALOFF and RAINEY (RAINEY, 1958) have shown the role played by wind in the migratory flights of locusts. One would expect to find a similar effect on the flights of *Ascia*, but that did not appear to be the case. Except for minor changes in speed, height and unimportant displacements of the flights already reported, the wind lacks influence, particularly in the adoption of the course.

This can be especially clearly seen when neighboring populations during a simultaneous outbreak send out streams in opposite directions in the area between them. This has often been seen during this work; 70 of the 170 cases of migrations observed in 1950 were simultaneous in opposite directions. The participants usually no not pay attention to one another except when two butterflies come very close to one another. Then for a few seconds they might fly around each other as during the introduction to copulation; the flight is the same whatever the sex of the two individuals. After a few seconds they will resume their flight as before.

Although we have only seen a migrant change its flight for a few seconds after such a meeting, it is possible that at the start of a flight direction might be induced by oncoming migrants already in flight. Thus, we had the impression of migrants from

Lotus Island being "pushed" to a southbound migration by the passing of southbound migrants from New Found Harbor.

In 1954 the phenomenon was much less common. Observations of migrations were made almost daily from March 16 to June 20, sometimes at different points but in only 8–10 cases were migrations observed coming from opposite directions. The most spectacular was on April 28 north of Playalinda when a weak trickle of southbound migrants practically had to fight their way over the dunes against a very strong (second day) northbound migration.

Another evidence that wind does not play an important role in adaptating flight directions is that in most cases there are simultaneous flights both to the north and to the south from an outbreak area.

If the migratory activity is limited to an age of 18–30 hours, it is obvious that departures will take place only as long as emergences occur plus one day. In the synchronized broods of the populations in the Fort Pierce area this will occur one to three or four days in a row. In the largest outbreaks the emergences may last more than a week, and in one case it was observed that migrations started in both directions every morning for ten days, April 19–29, 1954.

From previous descriptions of the migrations of *Ascia* one gets the impression that sometimes during the large outbreaks flights proceeded along most of the coast in the same direction. It is possible that in such cases the migrants from one population "pushed" the next one for a considerable distance, thus causing the erroneous impression of very long continued migrations.

This could also explain that the migrations in some cases have been described to go on with the same intensity all day long. If all populations between Wabasso and Fort Pierce started migrations southwards on the same morning, an observer at Fort Pierce would see one maximum after another pass: $09^h00'$–$10^h00'$ the local one; $09^h50'$–$10^h50'$ that from AI; $10^h40'$–$12^h00'$ from AII and Floralton. From Vero Beach the maximum would arrive between $12^h00'$ and $13^h00'$, and from Wabasso between $13^h00'$ and $14^h00'$.

It might be asked how the original course is fixed. The answer is rather obvious to the observer. The butterflies come from their

night-time resting places to the feeding areas about which, at first, they will usually flit casually; soon, however, it will be seen that as they fly from one flower to another they continue in the same direction. The flight gradually becomes a sub-migration, a more or less straight-lined flight with stops for feeding; the stops

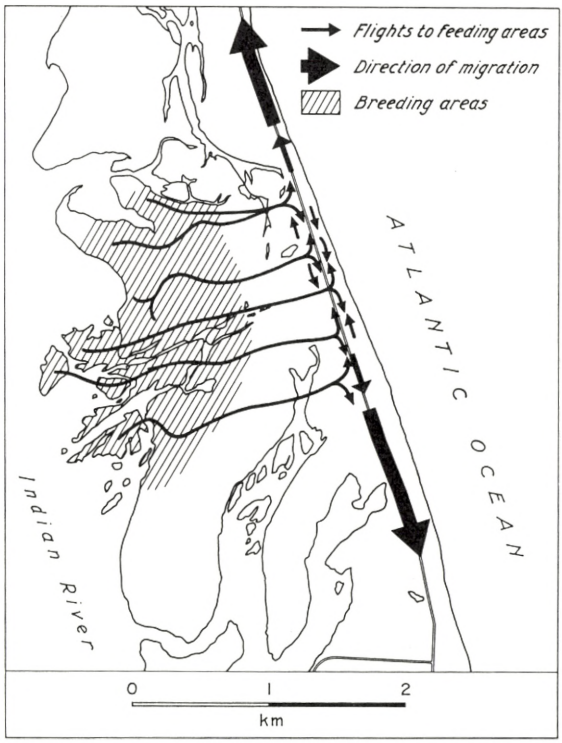

Fig. 29. Flights of *Ascia* on the offshore bar at the beginning of the migration.

for feeding become shorter with longer intervals between them until finally the migratory flight has begun. As the areas on the island where the nectar carrying plants are growing usually are parallel to the coast, the flight follows the coast line (fig. 29).

Where a feeding area has a different direction, as the east-west causeway at the bridge to Fort Pierce North Beach and at Oslo Road, the migrations usually go to the west (as the night resting places are east of the feeding area, the feeding starts in the eastern end and proceeds westward).

This is one, but probably not the only, way in which the course

becomes fixed; inter-individual influence also probably plays some role. During the milling around in the outbreak center it is sometimes seen that a flock of individuals perform flights together like a flock of birds and that they start on the migration simultaneously rather high in the air, and eventually adopt the persistent stride at the usual height. The course in such a case may be determined by a tendency to follow the course of the lower flying individuals which have fixed the course in accordance with the direction of the flight for food.

We have no observations to indicate how the course was adopted from the inland marshes. But most of the irregular flights across the interior of the peninsula can be projected back to inland breeding areas at Scottsmoor or Roseland, or east-west feeding places such as the causeway at Fort Pierce or Oslo Road.

V. Conclusions

It will be unthinkable for a long time to come to discuss any problem of butterfly migration without referring to the work of C. B. Williams. Not only has he stimulated the interest in this problem as nobody else has, but he has elevated it from its former position as a curiosity to its present one as a major element of behavior with far-reaching consequences; he has collected an enormous number of observations and presented them in a long series of publications, three of which are milestones in this research (Williams, 1930; Williams *et al.*, 1942; Williams, 1958). In these larger publications are also found discussions of all possible viewpoints of this amazing habit.

For this work the present author wants to pay homage to Dr. Williams with a grateful acknowledgement of the inspiration he has received from him.

In this work we have tried an approach which in some respects differs considerably from that used in previous work on butterfly migration. It seemed to my wife and me, when we started this work, that to get a better insight into the nature of the migration we had (1) to concentrate on one species, (2) to familiarize ourselves with the general habits of this species, (3) to trace the migrations back to their origin and follow them to the end point,

(4) to mark the animals so that the range of flight of single individuals could be observed, (5) finally to be especially careful in observing the conditions at the outbreak centers which might be of importance for the understanding of the release of the migratory habit—a point which Dr. B. P. UVAROV brought to our attention as I am thankful to remember.

The choice of *Ascia monuste* for this work was based on the observations of Mr. and Mrs. KARL HODGES as reported by C. B. WILLIAMS (1942). The choice was extremely lucky as *Ascia* undoubtedly is one of the easiest species to investigate. The clearly defined populations, the streamlike form of the migrations, in most cases only in two directions, and the road system which usually permits the following of streams over most of their range; all these features make this species especially useful for the study as we intended to do it.

It is unavoidable that our observations in several points are in disagreement with the general concepts of C. B. WILLIAMS and his pupils. As we have concentrated on this one species we have to be careful not to make unjustified general statements; maybe *Ascia* is an exception to the general rules; but we have learned some facts about this butterfly and its migrations and if they are not in agreement with the concepts, it does not mean that the concepts are wrong but that their general validity is limited.

There is actually no reason to expect all butterflies to migrate in the same manner. HAYWARD's observations on *Ascia* in Argentina (HAYWARD, 1953) show clear differences from our observations in several respects: the migrations take place over a wide front and the migrants rise in favorable wind up to 5000 feet above the ground; and the Argentine *Ascia* sometimes continues the migration during the night.

The numbers migrating in Argentina as described by HAYWARD are much larger than those we have seen; whether this is the reason for the differences in behavior or whether the reason lies in the differences between subspecies remain to be shown. The Argentine *Ascia monuste* is the f. *automate*, the subspecies in Florida is f. *phileta*.

With these differences within one species there is all possible reason to be cautious in making general statements about migrations of butterflies from the observations of but a single one.

During this work an intense feeling was acquired of the importance of some knowledge of the general behavior of the animal for study of the migration. Most of the literature (BALL and STONE, 1928; FERNALD, 1936, 1937 a, 1937 b; JOHNSON, 1899, 1929; SCUDDER, 1889; STIRLING, 1923; and the HODGES, WILLIAMS *et al.*, 1942) deal solely with descriptions of the migrations; only FERNALD wondered about the origin of these animals.

Pertinent to the behavior of *Ascia* are only two publications: WATSON and TISSOT, 1942, state that *Ascia monuste* is "by far the most common and troublesome pest on cabbage and collard" in Florida, and give one and a half pages of description on the best way to control this "pest".

It is obvious that if we had been guided by the literature during these observations we would have had a completely erroneous picture of the origin of the flights; although during the first half year we examined every cabbage field we could find, only two larvae were ever found on cabbage.

The other publication is a paper by CHERMOCK (1946), who observed migration south of Miami; he describes how up to 500 imagines fed on an area of half an acre (by comparison, in AII the feeding area was a strip of *Bidens* covering about 30 m^2; the normal count here during the marking experiment was 300 individuals—a density corresponding to 20,000 per half acre).

Based on these observations CHERMOCK advances a complete theory for the migration of *Ascia*: An abundance of food plants for the larvae ("various Cruciferae") combined with favorable "ecological conditions" is postulated at certain times to produce a "population swarm".

In the otherwise so luxuriant subtropical flora of Southern Florida, there is certainly no abundance of cruciferous plants; and the statement seems to indicate that it is not based on observations but is a presumption based on the well-known fact that Pieride larvae in temperate zones predominantly are feeding on Cruciferae. As to the "ecological conditions" it is not specified which conditions the author has in mind; nor is there any indication of how these conditions are favorable to the animal or if they have been found to be present in the field.

CHERMOCK postulated further that when a population swarm (whatever that is) has been produced, the "environment" will

exert a "biological pressure" on the adult butterflies and they will therefore move to a more suitable environment.

Translated from the peculiar idiom of dogmatic ecology to plain English, the meaning is that if there is food enough for the larvae but not for the imagines the latter will move to a place with more food. This is just as utterly wrong as it is unfounded: (1) the migrations always originate in an area with abundant food; (2) dissection of migrants in the beginning of the flight shows that they have usually fed shortly before—anyone who has driven an automobile through a migration will remember that when migrants are killed against the windshield a big splash of more or less digested nectar drips down the glass; (3) Except in special cases of very long flights the migrants do not pay any attention to the occurrence of plants to feed on during the migration.

Any attempt to explain the migrations of *Ascia* as a search for food is wrong.

In his recent book, C. B. WILLIAMS (1958) mentions that the idea of long range migrations often are brought in doubt because of the "widespread misconception, still found in many places today" that the lifespan is too short to permit such flights.

In *Ascia* we have ample evidence that the normal lifespan of the males is about five days and of the females about ten. These figures were first determined in the laboratory and later confirmed by numerous observations in the field. In every case where we watched a population in outbreak, it was found that if no immigration occurred the number of butterflies decreased to a very low level within a couple of weeks after the maximum. The experiments with marked butterflies in a sedentary population was also in full agreement with the laboratory findings.

In *Ascia* the lifespan is short and the period during which there is an urge to migratory flights constitutes only a fraction of the lifespan—in many individuals the flight lasts only a few hours—the maximum found in this work, about one and a half days.

There are reports of butterflies laying eggs in pauses during a migration (WILLIAMS, 1958, p. 182) but they are exceptions. If our findings and conception of the end of the flight of *Ascia* is right, there will be a transition period of "moods" after the eggs have matured during which the animals will continue the migration until the reproduction "mood" is released by an external stimulus.

There are not yet enough observations to consider this a general rule, but to my knowledge there is nothing known to oppose the view. The exceptions mentioned are not quite unlikely to occur during a transition period.

Not much is known about the duration of the life of butterflies in the field; it might be worthwhile here to refer to some marking

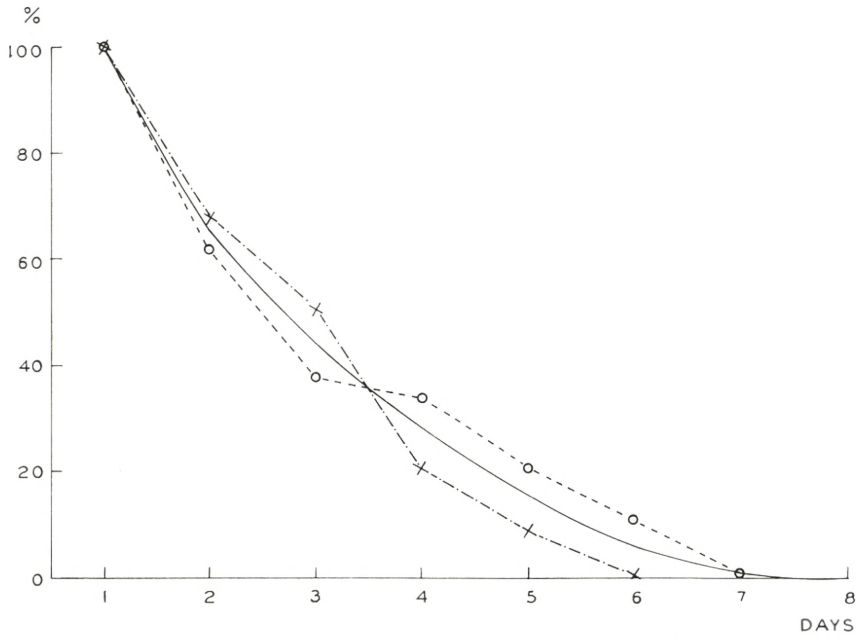

Fig. 30. Rate of disappearance of individuals in a population of British butterflies according to Cogbill (1942) (0), and in a population of *Ascia* (X).

experiments summarized by Cockbill (Williams, 1942, p. 222–226).

During the years 1936–1939 markings were made in England of 394 *Pieridae* (*P. rapae*, and *P. brassicae*) and of 716 *Nymphalidae* of five species (*Vanessa io, V. urticae, V. atalanta, V. cardui*, and *V. C-album*):

To compare their findings with the countings of *Ascia*, the daily recoveries for the first seven days are given as percentages of the number found the first day. The percentages used for *Ascia* are from the figures for marking experiment II during which no migration took place.

The result is given as fig. 30. It will be seen that there is no definite difference. During the first days the English butterflies seemed to decrease faster than *Ascia*; whether this was due to an emigration, as presumed by COCKBILL, is a question, but it is a possibility; the last day the decrease was slower than found in *Ascia* which also could be expected; even if the two groups had the same death rate under identical conditions, the British individuals would undoubtedly have a longer life span in the cool summer in England compared with the March temperature of Florida, even if the difference is not very great.

After these introductory remarks the main problems of the migrations of *Ascia monuste* can be discussed.

The first of these problems (A) *Why do Ascia migrate?* actually consists of two questions:

(A I) What is the Biological Significance of the Migration?

The answer seems obvious: As the populations are very unstable it is an extremely useful habit that in periods of a large outbreak the majority emigrates to other potential breeding areas so that in periods of minimum size the original populations may be repopulated.

This explanation is simple and in agreement with the observations. It is presented here as a mere possibility for those who want a teleological explanation.

The author must confess to be rather uninterested in efforts to establish causality on guesswork; even when they are seemingly very clear they might still be wrong and thus be a serious hindrance in the understanding of habits. And, in any case, they do not explain anything.

(A II) What is it that Makes an *Ascia monuste* a Migrant?

This question has again to be divided into two parts: What produces the urge to migrate? and What releases the migratory flight?

As to the first point there is hardly any doubt that the urge to migrate in *Ascia* is produced by crowding, and it appears only in a certain period of the life of the butterfly. Both factors need further elaboration.

When this study began we had no idea of an age limit for the migrants. It is, of course, well known that locusts stop migrating at the time of oviposition; and the same seems to be true for *Ascia*.

It is actually more difficult to understand the delay in the appearance of the migratory activity. It cannot be explained simply as a waiting for the hardening of the chitin, a process which only lasts an hour or less. In mosquitoes a similar period of inactivity was found (NIELSEN, 1958) lasting 6–8 hours. In some species the extension of the fibrillae on the antennae is a good indication of preparedness for prolonged flight (NIELSEN and NIELSEN, 1958); and the delay of this habit shows that there is no urge to prolonged flight until after the inactive period even when the stimulus to flight is present.

From the consistent result of many observations we know that migrations occur only in large populations of *Ascia*; and the migratory activity is more intense the larger the population is. From this it can only be concluded that crowding is at least one of the factors producing the migratory "mood". "Crowding" as used here means a situation in which an abnormally high number of individuals are concentrated with certain spatial limits.

The importance of crowding in the behavior and phase change of locusts was shown many years ago and a similar relationship between crowding and migration has been found in so many cases that it is reasonable to accept it as a general rule.

How the crowding produces the migratory urge is, of course, a very fundamental question—with no answer available. Pertinent to this question are observations on gregariousness in *Ascia*. The very young larvae from the same batch of eggs will sometimes stay closely together as described in the first report (NIELSEN and NIELSEN, 1950). This tendency to gregariousness is never seen in older larvae, and the pupae are always found far apart.

If the stimulus for the migratory urge is derived from contact between individuals—and the effect cannot very well be understood otherwise—it is most likely that it occurs after the emergence when the butterflies are incessantly meeting other individuals. It is also doubtful whether the density of larvae per unit of area in the small inland populations is less than in the medium-sized outbreaks in the larger colonies at the coast; but the absolute number over a vast area will be so much larger that the adults will be surrounded all the time by other individuals wherever they

move, especially at the places for feeding and at the night resting places. However, in the experiments mentioned (p. 14) to show a delay in sexual development by crowding, the only possible delay was found in crowding the larvae.

If it is true that the stimulation to migrate is produced by contact of the butterflies after emergence it seems not unreasonable to consider the 18-hour period before the urge appears as one during which the effect of the stimulus accumulates a sufficient "motivation" to permit the adequate stimulus to initiate the actual flight.

In mosquitoes the situation is different because the immature stages have a strong tendency to aggregate; maybe this is the reason why the pre-migratory period in mosquitoes is so much smaller than in *Ascia* (1–2 % of the average lifespan (400 hours)) while in butterflies it is about 10 %; in *Ascia* the migration stops after 15 % of the lifespan is over and a similar figure (17 %) is likely to be case for mosquitoes although our knowledge here is definitely incomplete.

The next question (AII b) is what conditions will bring *Ascia* to the consummation of the migratory urge when it is present; in plainer language; under what conditions do the migratory butterflies start the migration?

This has already been discussed (p. 62) and it should be only summarized here that the four conditions are (1) the right time of the day, (2) a suitable temperature, (3) visibility of the blue sky, and (4) absence of heavy rain.

B. How do *Ascia* Migrate?

The immediate answer is: The migration is a persistent flight, essentially following a straight course in relation to magnetic bearing, one to three meters above the ground.

But this answer leads to a series of other questions, most of which already have been discussed (p. 42) and here need only to be mentioned.

BI: How is the Straight Course Adopted?

The migrations are preceded by feeding; at least in some cases the course is a continuation of the main direction of flight during the feeding. Possibly it is also guided by topographical features

such as roads and shorelines. It also seems that some individuals adopt the course by imitation of migrants already on their way.

The narrow streams formed by the migrants are typical for *Ascia*, but not for many other butterflies. The independence of external factors, especially wind, is clearly seen by the facts (1) that south of an outbreak center the migration will go southward; north of it, it will go northward, etc.; (2) that migrations frequently occur in opposite directions between two outbreak areas.

B 2: How does the Migrant Keep the Course?

There does not seem to be any other possible explanation than a sun-compass direction. As the migrations do not stop when the sun is momentarily hidden behind clouds it is most likely that the orientation is based on polarized light. Our observations are in agreement with this concept but they do not prove it.

Besides the compass-based fixing of the course there is also an orientation according to visual stimuli as shown by examples given above. The guide lines are used according to their usefulness in keeping the magnetic course. The goal of flight over open water is determined by the course, and the course is kept towards this point (p. 45). This clearly shows that the orientation during the flight is a combination of a fixed magnetic course and a course toward a visual goal.

C: What is the Range of the Migration?

The problem of the range of migration is not only a question of distance covered by the migratory flight, it is closely connected with the speed and the duration of the flight.

The ground speed is about 12 km/hour but varies with the wind, normally from 10–15 km/h. This variation in ground speed is much smaller than the variation in air speed. If the air speed were 12 km/h, and the wind were 10 km/h against the flight, the ground speed would be 2 km/h; and with a tail wind of the same velocity the ground speed would be 22 km/h; it is therefore, justifiable to consider the ground speed as nearly constant; is shows again that the speed is regulated by visual stimuli.

The other main factor determining the range is the duration of the migratory flight which was found to vary with the size of

the population. From the smallest population emitting migrants the flight lasts only a couple of hours and usually ends in one of the first breeding areas the migrants come to; the largest migrations last ten hours the first day and may go on for several hours the next day. Twice during this work migrations were observed lasting 12–15 hours and covering a distance of up to 160 km. The average range is a good deal shorter probably 30–60 km.

As already mentioned, it is impossible to make general statements about the migrations of butterflies from studies of one single species; yet, it is encouraging that several of the elements found for *Ascia* have also shown to be valid for mosquitoes.

The period is over when the mere recording of directional flights can be considered the central part of the study of migration. Any real progress in this field can be achieved only by an intensive study of the general behavior of the animals, of their development and of their ecological characteristics. The conditions for the production and release of the migratory habit, the longevity and the duration of the migratory urge are the basic elements on which increased insight in the nature of migration rests.

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NOTES ON *HYOLITHELLUS* BILLINGS, 1871, CLASS *POGONOPHORA* JOHANNSON, 1937

BY

VALDEMAR POULSEN

København 1963
kommission hos Ejnar Munksgaard

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Synopsis

The genus *Hyolithellus* BILLINGS, 1871 is discussed, and it is demonstrated that the much disputed "operculum" (*Discinella* HALL, 1871) must be excluded from *Hyolithellus*. The "operculum" has possibly monoplacophoran affinity. Evidence indicates that *Hyolithellus* may well be referred to the class Pogonophora JOHANNSON, 1937. The structure of the tubular skeleton in members of the Pogonophora is strikingly similar to that of *Hyolithellus*. X-ray fluorescence analysis of the tubes apparently also confirms the pogonophoran relationship and the exclusion of *Discinella* and its allies from *Hyolithellus*. Considering the early appearance of representatives of the Hemichordata (Lower Ordovician), it does not seem unreasonable to regard the Lower- and Middle Cambrian *Hyolithellus* as a close relative of the Hemichordata.

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Introduction

Classification of several Cambrian animal groups presents many difficulties. *Hyolithellus* has lately (FISHER, 1962) been referred to an order Hyolithelminthes, the affinities of which supposedly are quite unknown. At present the author is studying a Lower Cambrian fauna from the island of Bornholm containing numerous specimens of *Hyolithellus*. A revision of this genus has long been needed on account of the error made by many authors, in assigning an operculum to it. As will be shown, the "operculum" was correctly established as a molluscan genus by HALL, 1871.

Mr. J. B. KIRKEGAARD, M. Sc., of the Zoological Museum of the University of Copenhagen, called the author's attention to certain hemichordate relatives, class Pogonophora, the members of which mostly belong to the benthonic environment of the deep sea. Their tubular skeleton bears much resemblance to *Hyolithellus* tubes. Mr. KIRKEGAARD kindly placed a specimen of the tube of *Galathealinum bruuni* at the author's disposal for study.

Class ***POGONOPHORA*** JOHANNSON, 1937

Order **HYOLITHELLIDA** SYSSOIEV, 1957, (partim)

Family HYOLITHELLIDAE WALCOTT, 1886, (partim)

Genus Hyolithellus BILLINGS, 1871

Type species: *Hyolithes micans* BILLINGS

The type species is the most widely distributed of the species referred to *Hyolithellus*, and the discussion mainly concerns this species.

The "operculum": BILLINGS (1871), when describing *Hyolithellus*, assigned an operculum to the tubular fossil, both occurring in the Lower Cambrian of Troy, New York. From the same beds HALL described specimens of *Discinella*. His paper should have been published in 1871, but the whole edition was destroyed by fire, and his results were not published until 1873. In any case, HALL as the first differentiated *Discinella* under a special name. He concluded that, on account of the peculiar muscle scars, the fossil could hardly be recognized as a brachiopod, but rather as a gastropod. The general character of the shell, he further concluded, is such as to ally it with the Discinidae, and, accordingly, the name *Discinella* was proposed. WALCOTT (1886, p. 142) called attention to the fact that the supposed operculum of *Hyolithellus* was the same form, as the one called *Discinella* by HALL. Since then, practically all American paleontologists have followed BILLINGS and WALCOTT in regarding *Discinella* as the operculum of *Hyolithellus*. In this connection it must be noted that they usually occur in the same beds. KNIGHT and YOCHELSON (1960, p. 324) rejected *Hyolithellus* and its "operculum" from the Gastropoda and Monoplacophora. FISHER

(1962) regarded *Barella, Discinella,* and *Mobergella* as hyolithelminth opercula.

Moberg (1892) pointed out that in Swedish sediments, in which *Discinella* may cover almost entire bedding planes, no tubular fossils to match the "opercula" are found. At the same time *Discinella* is absent in beds rich in tubular fossils.

Holm (1893), when discussing the Hyolithidae refused to recognize any relationship between *Hyolithes* and *Hyolithellus.* With regard to *Hyothellus* he stated that the tube was difficult to assign to any class, and the "operculum" should be removed from the genus.

Hedström (1923), after including *Discinella* in a new genus, *Mobergella,* referred the univalve fossil to the Gastropoda, as already tentatively suggested by Hall. In 1930 Hedström realized that the name *Discinella* could not be suppressed, as the action was contrary to the rules of international zoological nomenclature. On the other hand, a differential analysis showed that important differences in number and arrangement of the muscle scars occur, and, accordingly, Hedström concluded that the name *Mobergella* might be applied to specimens, which differ clearly from *Discinella.*

C. Poulsen (1932) described *Hyolithellus micans* and *Discinella micans* from the Lower Cambrian of East Greenland. He referred *Discinella* to the family Patellidae. Poulsen's material clearly demonstrates that a disparity exists with regard to the diameter of the specimens of *Discinella* and that of the tubular *Hyolithellus* shells. The diameter of *Discinella* is up to twice the diameter of the tubes. The same disparity is quite evident in specimens figured by Lochman (1956). In the author's opinion an eventual operculum should be expected to fit in with the tubes or to be slightly smaller.

Cobbold, who in several papers described occurrences of *Hyolithellus* in England, never found an operculum, which could be assigned to the genus. In Sweden the "opercula" and the tubes occur at definitely separate levels. The author may add that *Hyolithellus micans* occurs abundantly in a Middle Cambrian clay on the island of Bornholm. In this deposit *Discinella* and *Mobergella* are totally absent. The absence cannot be explained as an effect of separation during transportation, as the hyolithellid

tubes in the clay are associated with thousands of inarticulate brachiopod shells, which from point of view of transportation dynamics are very similar to the shell of *Discinella*.

The author believes that the above mentioned objections clearly demonstrate that the "operculum" of *Hyolithellus* must be excluded from the genus. At the same time the muscle scars seen in *Discinella*, *Mobergella*, and *Barella* in some respects are very similar to those found in the Monoplacophora (text fig. 1 d). In the Lower Cambrian faunas only members of the order Tryblidioidea bear any resemblance to *Discinella* and its allies, which possibly should be regarded as close relatives of the Monoplacophora.

Previous classification of *Hyolithellus*

Since Billings established *Hyolithellus* in 1871, the genus has been assigned to various classes and phyla. Originally *Hyolithellus* was believed to be closely related to *Hyolithes*, and naturally the presence of a hyolithellid operculum was essential in this connection.

As late as in 1959 Syssoiev grouped the order Hyolithellida (comprising *Hyolithellus*, *Coleoloides*, and *Coleolus*) and the order Hyolithida in a superorder Hyolithoidea of the phylum Mollusca.

Also a relationship to some worm phylum like the phoronids has been suggested, and this viewpoint is favoured by several paleontologists. Fisher (1962) established an order Hyolithelminthes, comprising the families Hyolithellidae and Torellellidae. He cautiously regarded class and phylum as uncertain, but the entoproctid or phoronid relationship was suggested on account of the phosphatic shell composition.

As mentioned earlier the author's attention has been called to the class Pogonophora, the tubes of which strikingly resemble that of *Hyolithellus*. The resemblance even included the concentrations in the tubes of certain elements.

Hyolithellus and class Pogonophora Johannson, 1937

The following analysis of structures in *Hyolithellus* and the Pogonophora is based mainly on specimens of *Hyolithellus micans*, obtained by elutriation of a Middle Cambrian clay from the island

of Bornholm, and on a 30 centimetres long tube of the pogonophore *Galathealinum bruuni* KIRKEGAARD, kindly furnished by Mr. J. B. KIRKEGAARD.

Tubular structure: It is a characteristic feature in *Hyolithellus* that the tubular shell is curved and irregular near the apical end but straightening toward the aperture. The growth angle in the adult section varies from one to four degrees. The cross section is circular, and the diameter may rise to about three millimetres.

This description also perfectly covers *Galathealinum*.

Tubes of *Hyolithellus* show characteristic surface markings consisting of an irregular annulation and transverse striation, considered to be growth lines. The annulations and striae are usually diminutive, but irregularly spaced, coarser markings occur, possibly indicating major changes of growth conditions (text fig. 1 b). Interior surface of tube is smooth, devoid of septa or any other structure. The tube is thin-walled at the apical end, but thickens progressively toward the aperture (text fig. 1 c).

Galathealinum has the same type of surface markings (text fig. 1 a), a similar smooth interior surface, and a gradual thickening of the tube wall is also seen. Characteristic in the pogonophore are one to two centimetres long, irregularly spaced sections, in which the annulation is particularly coarse. In the dried specimen of *Galathealinum* the transverse markings on the surface present points of weakness, at which the tube easily breaks. Apart from this, brittle and more soft sections of the tube evidently alternate, at least in some pogonophores. Similar points of weakness are probably also present in *Hyolithellus*, and this may explain, why the fossil fragments rarely exceed five centimetres in length. Fragments of *Hyolithellus* with unusually coarse annulation on the surface have been recorded (WALCOTT, 1890, and LOCHMAN, 1956). WALCOTT thus recognized a variety, *H. micans rugosa*, based on a single tube, which shows coarse transverse ridges and striae, between which are fine longitudinal striae. Similar specimens collected by LOCHMAN show no trace of longitudinal striae, and she was reluctant to refer these specimens to WALCOTT's variety, or to describe them as a new subspecies. The present writer believes that the coarsely annulated hyolithellid fragments may well correspond to the likewise coarsely annulated sections in *Galathealinum*.

Lochman (1956) reported that some thin tubular fragments of *Hyolithellus* show a scattering of oval holes or pores through the lamina of the tube wall. She suggested that this is probably the structure of each individual layer, but superposition of layers in complete tubes gives the appearance normally observed. Despite careful study of the beautifully preserved hyolithellid fragments from Bornholm the author has not been able to find a similar porous structure, and he believes that the pores may possibly be due to imperfect state of preservation, or eventually a result of the method of preparation by etching with acetic acid. Natural pores are not seen in the specimen of *Galathealinum*, but a slightly damaged section of the tube shows mechanically produced holes through the outer lamina.

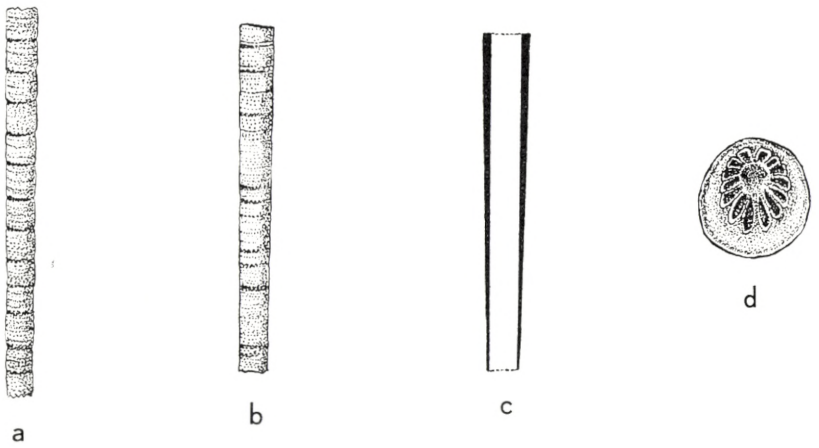

Fig. 1. a: Growth lines on tube-fragment of *Galathealinum bruuni* Kirkegaard (2 ×); b: Growth lines on tube-fragment of *Hyolithellus micans* Billings (2 ×); c: *Hyolithellus*, longitudinal section, showing progressive thickening of the tube wall (2 ×); d: *Mobergella holsti* (Moberg), ventral view, showing muscle scars (4 ×).

The tubular substance: The tube of *Hyolithellus* is composed of many fine laminae, added progressively layer by layer. The thin apical part of the tube thus contains only a few layers. Exactly the same structure is found in *Galathealinum*.

Mr. Ib Sørensen, cand. polyt., of the Geological Institute of the University of Copenhagen, kindly made X-ray fluorescence analysis on specimens of *Hyolithellus* and the tube of *Galathealinum*. Considering the remarkable results (table 1, p. 12), a

similar analysis of specimens of *Mobergella* was imperative. In order to differentiate the influence of diagenesis in the fossils in the deposit, several specimens of the associated brachiopod: *Acrotreta socialis* were analysed.

The specimens of *Hyolithellus* and *Acrotreta* were collected from the unconsolidated Middle Cambrian clay on the island of Bornholm. Evidently, the deposit, apart from precipitation of some pyrite, can only have been imperceptibly affected by diagenetic processes, and, accordingly, much of the original shell structure of the fossils is preserved. The specimens of *Mobergella holsti* originate from a sample of pure, Lower Cambrian sandstone from the island of Öland.

The tubes of *Hyolithellus* consist mainly of an organic substance and some calciumcarbonate. X-ray analysis shows that no phosphorus is present. A control analysis of a minimal amount of pure phosphate indicates that even extremely poor concentrations or traces of phosphorus will show up. The absence of this element is most unexpected. The specimens of *Acrotreta* and internal molds of hyolithellid tubes from the same deposit show a "normal" concentration of phosphorus. These conditions have caused the author to believe that the phosphate found in hyolithellid tubes from deposits elsewhere is mainly secondary, having replaced the calciumcarbonate, and probably to some extent dependant upon the phosphorus originally contained in the soft parts of the animal, and now preserved in the internal molds of the tubes. The replacement of calciumcarbonate by phosphatic material is a common phenomenon in sediments.

Most probably the tube of *Hyolithellus* consists primarily of thin laminae of organic substance, separated by films of calciumcarbonate. Such texture is found in the pogonophore *Galathealinum*. The X-ray fluorescence analysis of the pogonophore shows a relatively high content of calcium, but no phosphorus. According to Ivanov (1960, p. 93), the organic substance of the pogonophore tubes is a polysaccharid (tunicin). It is commonly believed that the organic substance in *Hyolithellus* is chitinous, but this cannot be ascertained.

The distribution of other elements needs some comments. *Galathealinum* contains a small amount of vanadium, iron, and manganese. In the three fossil genera analysed, vanadium is

absent, and they further differ in containing titanium and chromium, which are absent in the pogonophore. These elements originate supposedly from precipitation of mostly iron-rich solutions. In the Middle Cambrian clay from Bornholm, containing *Hyolithellus* and *Acrotreta*, the iron is accompanied by a notable amount of manganese. The sediment is seen to contain some pyrite, which also to some extent has affected the fossils, as some of these show a brownish stain or coating originating from iron.

Cerium, which is absent in *Galathealinum*, accompanied calcium, and partly substituted this element, when subsequently precipitated in the fossils. Cerium may have been present in *Mobergella* and *Acrotreta*, but absent in *Hyolithellus*, when the animals were alive.

After burial, the tubes of *Hyolithellus* and the shells of *Acrotreta* and *Mobergella* were further enriched in potassium and silicium. At the same time the eventual amounts of iodine and sulphur were carried away in solution. Vanadium may, or may not, have been present originally, only to be substituted by chromium and titanium.

Hyolithellus and *Galathealinum* are both distinguished by the rich concentration of zinc, which is only very poorly represented in *Mobergella* and *Acrotreta*. In the last mentioned genera the poor concentration supposedly balances with the average concentration of this element in the sea water, when the animals were alive. The pogonophore is rich in nickel, and also *Hyolithellus* contains a surplus of this element, as compared to *Mobergella* and *Acrotreta*. Finally, the hyolithellid tube and the pogonophore tube are distinguished by the absence of phosphorus, and deficiency, respectively absence of cerium. In the present writer's opinion the chemical similarities, pointed out above, support the proposed pogonophoran affinity of *Hyolithellus*.

The X-ray analysis also serves to show that *Mobergella* and its allies, probably primarily phosphatic, are to be regarded as distinct genera, which cannot be referred to as hyolithellid opercula.

The shell material of *Mobergella* differs from that of *Hyolithellus* in the content of calciumphosphate, the surplus of cerium, the pronounced deficiency of zinc, and the somewhat smaller content of nickel.

TABLE 1. Concentrations of elements in the specimens of *Galathealinum*, *Hyolithellus*, *Mobergella*, and *Acrotreta* as registered by X-ray fluorescence analysis.

	Galathealinum	*Hyolithellus*	*Mobergella*	*Acrotreta*
CALCIUM	+++	++++	+++	++++
CERIUM	÷	+	++	+++
CHROMIUM	÷	++	++	++
IODINE	++	÷	÷	÷
IRON	++	++++	++	++++
MANGANESE ..	+	+++	+	++++
NICKEL	++++	+++	++	++
PHOSPHORUS ..	÷	÷	++	++
POTASSIUM ...	÷	+	+	++
SILICIUM	÷	+	+	+
SULPHUR	++	÷	÷	÷
TITANIUM	÷	++	++	++
VANADIUM	++	÷	÷	÷
ZINC	++++	+++	+	+

Relative concentrations indicated by following symbols: ÷ = absent, + = traces or very poor, ++ = poor, +++ = moderate, ++++ = rich. The symbols are only valid for intergeneric comparison. No quantitative distinction between the individual elements is implied.

Summary of conclusions: The occurrence on the island of Bornholm of numerous specimens of *Hyolithellus* in a deposit devoid of discinelloid forms, and the Scanian occurrence of *Mobergella* at stratigraphical levels, definitely separate from levels containing *Hyolithellus* and *Torellella*, indicates that *Mobergella* and its allies cannot be regarded as hyolithellid opercula. Also the disparity with regard to the diameters of tubes and "opercula", and differences in concentrations of elements in the shells and tubes support a separation.

The genera *Discinella*, *Mobergella*, and *Barella* with regard to pattern of muscle scars show affinities to the Monoplacophora.

In *Hyolithellus* the entire tubular structure is identical to that of recent members of class Pogonophora, which is closely related to the hemichordates. Also the texture of the hyolithellid tube is identical to that of the pogonophore tube. The similarity is further

accentuated by the concentrations of zinc and nickel. In both groups the tubes are believed to consist mainly of organic substance in thin laminae separated by films of calciumcarbonate. The available evidence thus favours the reference of *Hyolithellus* to the Pogonophora.

The class Pogonophora is divided into two orders: Athecanephria Ivanov, 1955 and Thecanephria Ivanov, 1955. The orders are classified by differences in the anatomy of the soft parts, and, accordingly, the position of the hyolithellids within the class is open to discussion. The present writer tentatively suggests that a third order: Hyolithellida, comprising the families Hyolithellidae Walcott, 1886 (partim) and Torellellidae Holm, 1893, may be added to the class.

Biology of the Hyolithellida

It is indeed interesting that the class Pogonophora originated in the Lower Cambrian and has been able to persist up to the present day. Several paleontologists believe that fauna elements living in the deep sea or on the abyssal part of the continental slope may obtain a geologically long life owing to the supposed stable ecology of these habitats. If representatives of animal groups only known as fossils are to be found, they will most likely be inhabitants of the abyssal regions, and evidence at hand shows that these regions do contain relicts. During the Danish Galathea Deep Sea Expedition 1950–1952 the monoplacophore *Neopilina* Lemche was found, and this genus belongs to the order Tryblidioidea, which was formerly regarded as a Paleozoic group (Cambrian — Devonian). Also the Pterobranchia (Ordovician-Recent) may be mentioned. The author regards the class Pogonophora as another example. Possibly more relicts are waiting for discovery, and in future work special attention should be directed to the abyssal part of the continental slope.

The majority of the pogonophore species are found between 1500 and 10000 metres of depth, but species of *Siboglinum* are known to range into littoral waters. As the pogonophores are obtained by dredging, mostly at abyssal depths, not too much is known of their habits. They are assuredly benthonic, leading a sedentary life in the bottom ooze, never leaving their tubes,

which apparently have an upright position, with the irregularly curved apical end buried in the ooze. The larval development is known only in a few species. In these the eggs are hatched in the female tube in the section close to the aperture, and also the larval development takes place in the tubes. The larva probably only leads a brief free existence, before starting the building of the tube.

The hyolithellids occur in practically all types of sediments, but are not too common in sandstones or siltstones, unless they are argillaceous, and they are very rare in dolomites and graywackes. Like the pogonophores the hyolithellid tubes were most likely anchored in an upright position with the apical end buried in the soft bottom. The uncommonly wide distribution of *Hyolithellus* has been explained by the wide tolerance as to facies, and furthermore a planktonic larval development has been suggested. The present writer is of the opinion that a tolerance also of depth may be assumed, and pathways of migration may have crossed depths, which would present unsurmountable obstacles to the common shallow water fauna.

The hyolithellids may be regarded as the scarce shallow water representatives of the Cambrian pogonophores, predominantly occupying the abyssal regions, which are not represented in the sedimentary record. As stated by LOCHMAN, the nature of *Hyolithellus* is such that several species may be present, but a differentiation has not yet been possible.

Considering the fact that other groups with hemichordate affinity (Pterobranchia) appeared in the early Ordovician, the occurrence of Pogonophora in the Cambrian must be regarded as very probable.

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CTENOPYGE (CTENOPYGE) PECTEN TENUIS N. SUBSP. FROM THE UPPER CAMBRIAN OF BORNHOLM

BY

VALDEMAR POULSEN

København 1963
Kommissionær: Ejnar Munksgaard

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Synopsis

Ctenopyge (*Ctenopyge*) *pecten tenuis n. subsp.* was originally (C. POULSEN, 1923) assigned to *Ctenopyge pecten* SALTER. The original material of cranidia, pygidia, and thoracic parts is redescribed and discussed.

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Introduction

A few years ago, Dr. A. H. WESTERGÅRD in a letter called Dr. C. POULSEN's attention to the specimens, which C. POULSEN had previously referred to *Ctenopyge pecten* SALTER. The specimens from Læsaa, Bornholm, clearly differed from *Ctenopyge pecten* in being narrower.

C. POULSEN kindly placed the material at the present writer's disposal for study and publication.

Several trilobite families, including the Olenidae, contain narrow and wide forms, which in other respects may be almost identical. The significance of the difference in width is unknown, and in the literature such forms are usually differentiated either at the specific or at the subspecific level. For reasons given below, the present writer prefers to differentiate the Læsaa specimens of *Ctenopyge pecten* at the subspecific level.

Family **Olenidae** BURMEISTER, 1843

Subfamily Leptoplastinae ANGELIN, 1854

Genus ***Ctenopyge*** LINNARSSON, 1880

Type species: *Olenus* (*Sphaerophthalmus*) *pecten* SALTER, 1864; designated by VOGDES, 1890.

Ctenopyge (*Ctenopyge*) *pecten tenuis n. subsp.*
Text fig. 1 a—c

1922. *Ctenopyge pecten* (SALTER) *[partim]* — WESTERGÅRD: Sveriges Geol. Undersökning, ser. Ca, no. 18, pl. 12, figs. 26, 27, 29. (Figs. of cranidia).

1923. *Ctenopyge pecten* SALTER — C. POULSEN: Danm. Geol. Undersøgelse, ser. 2, no. 40., pp. 42—44, pl. 2, figs. 1—3. (Descr. and figs. of cranidium and thorax with pygidium attached).

1957. *Ctenopyge (Ct.) pecten* (SALTER) *[partim]* — HENNINGSMOEN: Norske Vidensk.-Akad., Oslo, I Kl., no. 1, pp. 208—09. (C. POULSEN's reference cited).

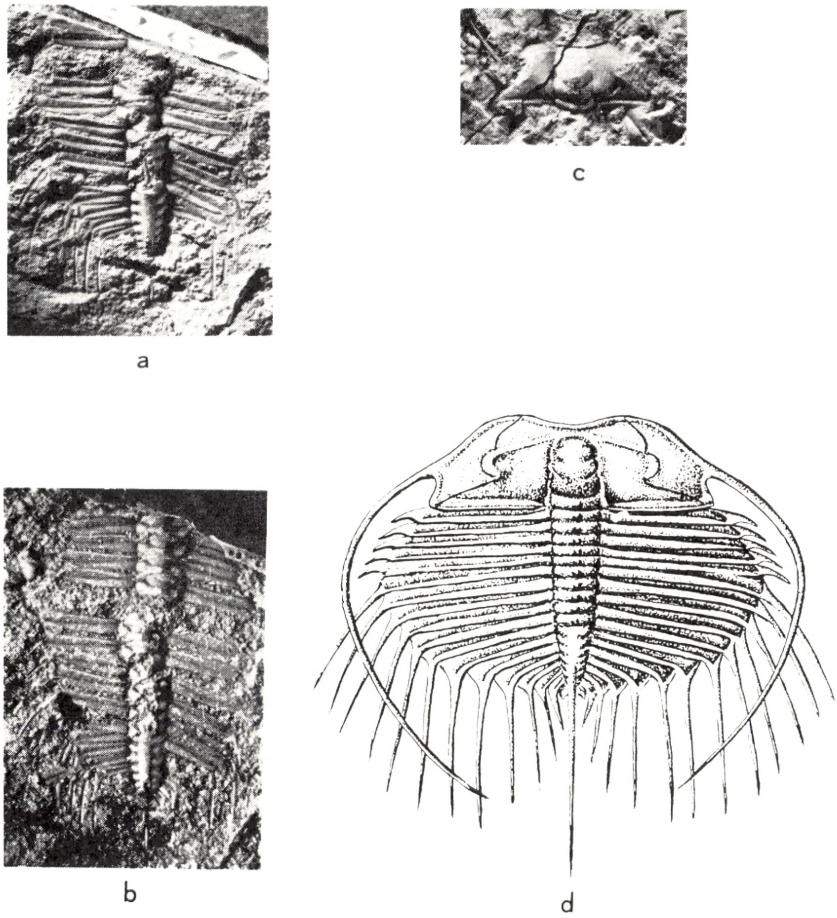

Fig. 1. Subspecies of *Ctenopyge* (*Ctenopyge*) pecten (SALTER, 1864).
a-c: *Ctenopyge* (*Ct.*) *pecten tenuis n. subsp.*
a. Incomplete thorax with pygidium attached (MMH no. 1973), holotype, X 3.
b. Cast of natural mold (MMH no. 1972) of holotype (MMH no. 1973), holotype, X 3.
c. Cranidium (MMH no. 1971), X 3.
d: *Ctenopyge* (*Ct.*) *pecten pecten* (SALTER, 1864), X 3. After C. POULSEN, 1959.

Holotype: Incomplete thorax with pygidium attached (MMH no. 1973) and natural mold of the same specimen (MMH no. 1972); C. POULSEN, 1923: Pl. 2, figs. 2—3. Also text-fig. 1 a—b, herein.

Material: A number of cranidia, a dorsal exoskeleton with slightly dislocated segments and with the cephalon missing, thorax fragments, a few fragmentary detached pygidia. The material is preserved in anthraconite. The figured specimens are new photographs of C. POULSEN's specimens (MMH nos. 1971—1973). These specimens are preserved in the collections of the Mineralogical and Geological Museum of the University of Copenhagen.

Redescription: The cranidium is subtrapezoidal in outline. The almost parallel-sided glabella is only slightly tapering forward, flatly rounded anteriorly, moderately convex transversely, strongly convex sagittally, profile highest slightly behind midpoint. There are three pairs of lateral glabellar furrows; anterior and second pair are short and indistinct, at right angles to glabella; posterior pair curving inward-backward, united into a transglabellar furrow, which is laterally well-impressed, mesially shallow. Occipital ring moderately long and wide, mesially slightly expanded, provided with a node; the occipital furrow is wide and well-impressed throughout, laterally further accentuated by oblong pits, which are parallel to the occipital furrow. The axial furrows are shallowing up opposite the preoccipital glabellar lobes, otherwise well-impressed; the preglabellar furrow is indistinct, coincident with the anterior border furrow.

Frontal area is extremely narrow, only consisting of the narrow and upturned anterior border, which is delimited by an ill-defined and narrow border furrow; anterior margin of cranidium is strongly concave, almost reaching glabella.

Anterior area of fixigenae wide, moderately convex, strongly inclined anteriorly, moderately inclined laterally; anterior border is narrow (sag.) and upturned; border furrow narrow and ill-defined; faint genal caeca are seen just in front of the eye ridges. Palpebral area of fixigenae of approximately the same width as adjacent portion of glabella, essentially flat, up-sloping, making

the cranidium appear concave, as seen from above; the palpebral lobes (missing in the figured specimen) are small, strongly arcuate, situated slightly in front of midline across glabella, anteriorly continuing into prominent, slightly curved and oblique eye ridges, which join glabella at the antero-lateral corners; the palpebral furrows are wide and shallow; faint genal caeca are situated close to the eye ridges. Posterior area of fixigenae moderately long and wide, somewhat downsloping postero-laterally; posterior border furrow moderately wide, well-impressed, terminating a little short of the axial furrows; posterior border almost straight, moderately convex.

Anterior sections of facial suture curving inward-forward, cutting anterior margin of cranidium not far out at sides; posterior sections of facial suture sinuous, diverging backward at an angle of 50—60° to the sagittal axis, at posterior border furrow curving back, cutting posterior margin moderately out at sides.

The hypostoma and librigena are not known.

Thorax consisting of 10 segments, attaining its maximum width at the middle segments. The axis is about two-thirds the width of the pleural regions (excluding spines), slightly tapering backward. Axial furrows are well-impressed; the axial rings are short mesially, provided with a simple node, laterally expanding, assuming a globular shape. The pleural regions are flat, consisting of moderately long (tr.) pleurae, which are laterally drawn into flat, long and arcuate spines that are more strongly backward-directed in the posterior part of the thorax; the oblique pleural furrows are well-impressed.

The pygidium consists of 7 or 8 segments. The axis is slightly tapering, with rounded terminal axial piece; the axial rings are fused, and traces of segmentation and nodes are lost mesially, whereas ring furrows and the abaxial globular structure of the axial rings are preserved laterally. Axial rings 5—7 provide the base for a mesial ridge, which is posteriorly drawn into a straight, horizontal, long and slender spine (broken off in the figured specimen). The pleural regions are tapering rapidly backward; the pleurae are apparently free and in most respects identical to those of the thorax; the pleural spines are almost parallel to sagittal axis, posteriorly becoming progressively shorter and

simultaneously turning inward-backward; the terminal plate is crescent-shaped.

Dimensions of figured cranidium:

Length	about	3.5 mm.
Width at posterior margin		7.0 –
Width between the eyes	estimated	5.0 –
Length of glabella		2.3 –
Width of glabella at base		1.7 –

Dimensions of holotype thorax with pygidium:

Length of thorax	estimated	5.0 mm.
Width of thorax at middle segments (excluding spines)		8.5 –
Width of axis at middle segments		2.0 –
Length of pygidium (excluding spines)		3.3 –
Width of pygidium at anterior margin	about	6.0 –

Occurrence: Denmark: *Peltura scarabaeoides* zone (*Ctenopyge linnarssoni* and *Parabolina lobata* subzones); C. POULSEN's loc. no. 6 at the stream Læsaa, Bornholm.

Sweden: *Peltura scarabaeoides* zone (*Ctenopyge linnarssoni* subzone = Upper part of WESTERGÅRD's *Peltura scarabaeoides* subzone); Scania, Västergötland.

Discussion: The new subspecies in many respects resembles *Ctenopyge pecten pecten*, and the only notable differences pertain to the width of the dorsal exoskeleton. The difference is very conspicuous, when complete exoskeletons are at hand (see text-fig. 1), but also detached cranidia can readily be differentiated. In *Ctenopyge pecten tenuis n. subsp.* the posterior sections of facial suture are diverging backward at an angle of 50—60° to the sagittal axis. This feature is demonstrated by all the Læsaa specimens, and it also quite apparent in several specimens of *Ctenopyge pecten* figured by WESTERGÅRD (1922, pl. 12, figs. 26, 27, 29). In cranidia of *Ctenopyge pecten pecten* the corresponding angle of the suture is about 80°. The thoracic axis is about two-thirds the width of the pleural regions (excluding spines) in the new subspecies and about one-fourth the width of the pleural

regions in *Ct. pecten pecten*. The posterior part of the thorax of *Ctenopyge pecten pecten* is more rapidly tapering than in the new subspecies, and, accordingly, the pygidia will be of practically equal dimensions in the two subspecies.

Ctenopyge pecten pecten is apparently restricted to the *Ctenopyge linnarssoni* subzone, but the new subspecies also occurs in the *Parabolina lobata* subzone.

The significance of the occurrence of wide and narrow forms of otherwise identical trilobites has not been satisfactorily explained. In the present case sexual dimorphism must be left out of consideration, as all the Læsaa specimens are narrow, and as they range into the *Parabolina lobata* subzone, from which *Ctenopyge pecten pecten* is unknown.

It is possible that extremely stagnant conditions may have caused the narrowing of the dorsal shields (KAUFMANN, 1933), but the available data do not allow of any definite conclusions.

As it may be impossible to refer detached pygidia and imperfectly preserved cranidia to either the wide or narrow form of *Ctenopyge pecten*, the present writer prefers a differentiation at the subspecific level. He further believes this taxonomical procedure warranted, as long as the significance of the variation in width is unknown.

Geological Institute of the University of Copenhagen

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THE LOWER MIDDLE CAMBRIAN KALBY-LER (KALBY CLAY) ON THE ISLAND OF BORNHOLM

BY

VALDEMAR POULSEN

København 1963
Kommissionær: Ejnar Munksgaard

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Synopsis

The Kalby-ler (Kalby clay) formation is a new name for the deposit previously believed to be weathered *Exsulans* limestone, which belongs to the *Triplagnostus gibbus* zone. The emendation is necessitated by evidence, which shows that the clay belongs to the *Tomagnostus fissus* — *Ptychagnostus atavus* zone. The evidence is discussed, and the lower Middle Cambrian stratigraphy is revised. A tentative account of the Lower and lower Middle Cambrian history and paleogeography is presented.

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Introduction

In 1942 C. POULSEN described several new fossils from the Middle Cambrian clay at Læsaa. The fossils were obtained by elutriation of a limited quantity of clay, and therefore the present writer a few years ago collected more material hoping to obtain additions to the fauna. Apart from the species reported by C. POULSEN the present writer thus found several new species of *Stenothecopsis*, *Stenotheca?*, spicules of *Protospongia?*, eocystid plates, and problematica. The non-trilobite fauna elements, practically all of which presumably are of Lower Cambrian age, will be described in a subsequent paper. The fossils in the clay comprise Lower and Middle Cambrian elements. In the following mainly the Middle Cambrian trilobite material will be discussed.

The material is in the collections of the Mineralogical and Geological Museum of the University of Copenhagen.

Summary of previous work

GRÖNWALL (1902a) described the Middle Cambrian sections and the fauna from the streams Øleaa and Læsaa on the island of Bornholm. At Øleaa the Lower Cambrian Rispebjerg sandstone is overlain by 25 centimetres of gray *Exsulans* limestone. The limestone is rich in grains of glauconite and pyrite, especially in the lower part. Equally conspicuous is the content of irregular, somewhat rounded pebbles of phosphoritic sandstone. The lithology of these pebbles corresponds to the upper 40 centimetres of the underlying Rispebjerg sandstone. Fossils, predominantly trilobites, occur abundantly in the *Exsulans* limestone, especially in the upper part. In the section at the stream Læsaa the Rispebjerg sandstone is succeeded by 15 centimetres of a gray clay, which is

not too well exposed. GRÖNWALL reported that most of the clay was unconsolidated and soft, but contained harder lumps, which showed that the clay was a residual deposit formed by weathering of a fossiliferous limestone. The carbonate supposedly was washed away. Determinable fragments of fossils were not found. The clay was found to be rich in glauconite and pyrite, pebbles or lumps of phosphoritic sandstone, and well-rounded quartz grains identical to those of the Lower Cambrian Rispebjerg sandstone.

Considering the absence of determinable fossils, the agreement in lithology, the thickness, and the position in the sequence it is understandable that GRÖNWALL (1902a, pp. 20 and 32) correlated the clay at Læsaa with the *Exsulans* limestone at Øleaa. The deposits were referred to the lowermost *Ctenocephalus exsulans* subzone of the *Paradoxides tessini* stage. When discussing the Scandinavian *Paradoxides* beds GRÖNWALL (1902b) maintained the correlation as mentioned above.

KAJ HANSEN (1937) made a comparison between the Lower Cambrian deposits in Scania and on Bornholm. He called attention to a pyritic layer immediately above the clay at Læsaa. The pyritic layer, previously described by GRÖNWALL, is a 5 centimetres thick conglomerate with pebbles of phosphoritic sandstone, irregular bodies of phosphorite, and fragments of weathered sediments with traces of fossils. The pebbles of phosphoritic sandstone are identical to those found in the underlying clay. Some of the rock fragments could be identified as belonging to the Lower Cambrian siltstone (»Green shales«). The pyrite forms the matrix of the conglomerate. KAJ HANSEN correlated the pyritic conglomerate with a somewhat similar layer occurring at one locality at Øleaa. Here, at HANSEN's locality no. 6, the somewhat calcareous and glauconitic Rispebjerg sandstone is followed by a conglomerate layer 10 centimetres in thickness. The lower part of this layer has a matrix of fine-grained phosphoritic and glauconitic sandstone. The matrix surrounds rounded quartz grains, phosphoritic sandstone, and elongate lumps of pyrite. The upper part predominantly consists of coarse quartz grains embedded in a matrix of phosphorite and pyrite. The pyritic layer at Øleaa is followed by the *Exsulans* limestone, and, consequently, the clay at Læsaa, underlying the supposedly equivalent pyritic conglomerate,

would be older than the *Exsulans* limestone. Thus K. HANSEN referred the clay at Læsaa to the Lower Cambrian as an equivalent to the somewhat calcareous and glauconitic Rispebjerg sandstone at locality no. 6. He concluded that the principal difference between the beds at Øleaa and Læsaa was that the clay at Læsaa was unconsolidated. He further stated that if the clay indeed was the residue of weathered *Exsulans* limestone, one would expect the layer of clay to be much thinner, than it actually is.

By elutriation of samples of the clay K. HANSEN obtained some brachiopod specimens, which were referred by C. POULSEN to *Acrotreta sagittalis* (SALTER) and *Acrotreta cf. eggegrundensis* WIMAN. K. HANSEN then, after comparing these species with the known Scandinavian occurrences of the same species and their associated fossils, referred the clay at Læsaa and the calcareous part of the Rispebjerg sandstone to the Lower Cambrian *Holmia kjerulfi* zone or *Strenuella linnarssoni* zone. GRÖNWALL (1902) was of the opinion that the Bornholm section contained a hiatus comprising these two late Lower Cambrian zones.

C. POULSEN (1942) stated that the vertical range of *Acrotreta eggegrundensis* was unknown, and as *Acrotreta sagittalis* is widely distributed in the Middle Cambrian, the two species were accordingly of no value for the correlation. Attempting to obtain species, which might be of stratigraphical relevance, C. POULSEN had excavated additional material of the clay for elutriation of fossils. He made a distinction between worn (rolled) and well-preserved shell specimens. The well-preserved specimens were supposed to be normal members of the fauna of the clay, and worn specimens supposedly had been washed out of older deposits to be embedded in the clay and in the *Exsulans* limestone. However, the present writer in his considerably larger fossil material has observed worn as well as well-preserved specimens of *Hyolithellus* and other genera, and, consequently, the state of preservation is of little or no value with regard to the determination of the relative age of the fauna elements. A comparison of the non-trilobite fauna of the clay with similar faunas elsewhere has caused the present writer to believe that the majority of the species must be of Lower Cambrian age.

When excavating the clay C. POULSEN succeeded in finding

fragments of a relatively fresh and hard limestone containing *Jincella parva*, *Ctenocephalus exsulans*, and *Holocephalina linnarssoni*. As these species are well-known from the *Exsulans* limestone, he followed GRÖNWALL in correlating this with the clay at Læsaa. The present writer may add that by etching samples of the *Exsulans* limestone he has obtained species listed from the clay. Thus the beds are in good agreement faunistically and lithologically.

C. POULSEN concluded that the Rispebjerg sandstone, deposited at the time of the *Holmia torelli* zone, was followed by presumably thin deposits belonging to the *Holmia kjerulfi* zone or the *Strenuella linnarssoni* zone. Both zones might have been developed, but the beds most likely were thin and consequently readily removed by the erosion in the time interval corresponding to the *Eccaparadoxides oelandicus* stage. The numerous worn shells of *Acrothele* (*Redlichella*) *granulata* supposedly indicated that a deposit corresponding to the *Acrothele granulata* conglomerate, which is the lowermost bed in the *Paradoxides paradoxissimus* stage, once existed in the Bornholm area. C. POULSEN pointed out that the specimens of *Acrothele* were quite free of adhering rock particles. Probably the deposit only existed a very short time and was eroded, before lithification could take place. He finally concluded that in the time interval between the deposition of the Rispebjerg sandstone and the *Exsulans* limestone the Bornholm region was affected by two transgressions, the first of which occurred in the late Lower Cambrian, the second in the Middle Cambrian.

The age of the Kalby-ler (Kalby clay)

When restudying C. POULSEN's trilobite material from the clay, the present writer noticed that all the specimens were molds consisting of a fine-grained, phosphoritic and argillaceous sandstone. This type of preservation does not occur in the *Exsulans* limestone, where only carbonate exoskeletons are present. Also the present writer's elutriated material contains molds of fragmentary cranidia. Especially interesting is a considerably worn agnostid cephalon (text-fig. 1).

Fig. 1. Fragmentary cephalon of *Ptychagnostus atavus* (MMH no. 9296), X 15. From the Kalby clay at Læsaa.

Even if the border and part of the posterior lobe of glabella are missing in the specimen (MMH no. 9296), it may safely be referred to *Ptychagnostus atavus* (TULLBERG). A comparison with Swedish species listed by WESTERGÅRD (1946) from the *Triplagnostus gibbus* zone (B 1), to which the *Exsulans* limestone belongs, shows that the above mentioned specimen differs from the species of *Peronopsis* in the shape of glabella and in the genal region being corrugated. The specimen differs from *Triplagnostus praecurrens* and *T. gibbus* in possessing a corrugated genal region. It shows some resemblance to *Tomagnostus fissus* and *T. cf. corrugatus*, but the anterior glabellar lobe in these species is subquadrate, cleft in front by a short sagittal furrow, which for a short distance continues into the preglabellar field. The Bornholm specimen has an anterior glabellar lobe tapering forward, and a distinct sagittal furrow crossing the preglabellar field, presumably reaching anterior border.

None af the Swedish species from the *Eccaparadoxides oelandicus* stage show any resemblance to the specimen from the clay. The lower alum shale overlying the clay and the *Exsulans* limestone contains *Ptychagnostus atavus* and *Hypagnostus parvifrons* and thus belongs to the *Tomagnostus fissus — Ptychagnostus atavus* zone (B 2). Accordingly, the worn specimen of necessity must

belong to the lower part of this zone, among the species of which only the index fossil *Ptychagnostus atavus* bears any resemblance. A comparison shows that the fragmentary cephalon in all preserved details, including the pattern of genal furrows, is identical to this species.

The *Exsulans* limestone then must be older than the clay, which the present writer prefers to regard as a separate formation, the Kalby-ler formation. See also text-fig. 2.

The pyritic conglomerate overlying the clay must belong to the same zone as the clay. The pyrite in the matrix most likely is of later, epigenetic origin, the precipitation being dependant upon the presence of a considerable amount of detrital pyrite grains. Determinable fossils have not been found in this conglomerate.

The Kalby-ler formation

Type locality: Section at the western bank of the stream Læsaa, Bornholm. The section is situated 240 metres southeast of the farm Kalbygård. This so far is the only known occurrence of the Kalby clay. The thickness of the formation amounts to 15 centimetres. It is delimited vertically by the Lower Cambrian Rispebjerg sandstone and the Middle Cambrian pyritic conglomerate immediately below the lower alum shale. Due to the dip of the strata the outcrop is very small, situated close to the water-level of the stream.

Stratigraphical position: The *Tomagnostus fissus — Ptychagnostus atavus* zone (B2).

Lithology: The gray Kalby clay is extremely heterogeneous with regard to composition of components and grain sizes. The material may be identified as debris from Lower and Middle Cambrian deposits and comprises fragments of siltstone ("Green shales"), rounded quartz grains and phosphoritic pebbles from the Rispebjerg sandstone, glauconite, pyrite, muscovite, Middle Cambrian fine-grained sandstone or siltstone, brachiopod fragments and tubular fossils. According to K. Hansen (1937) 60 % of the particles are below 0,2 mm. in diameter, and this fraction contains 7,5 % calciumcarbonate, possibly originating from the *Ex-*

sulans limestone. Most of the pyrite undoubtedly is of later origin.

There is no evidence that the Kalby clay should be regarded as the residue of a decomposed limestone. The deposit probably never was subjected to lithification. As mentioned earlier, the solid, fossiliferous samples collected by C. POULSEN consist of silt, practically devoid of calciumcarbonate. The present writer unsuccessfully attempted to obtain fresh or only partly decomposed samples of a possible original limestone. Furthermore, the presence of well-preserved eocystid plates consisting of calciumcarbonate indicates that the sediment can only have been influenced by solution to a very small extent.

The source rocks must predominantly have been non-calcareous sediments. The Lower Cambrian fossils, usually well-preserved and free of adhering rock particles most likely came from the same unconsolidated sands, which contributed to the *Exsulans* limestone.

Probably due to an insufficient amount of calciumcarbonate cementation did not take place. It has been suggested that carbonate cementation in sandstones may be based on carbonate introduced from without, but in most cases the carbonate will be the product of the solution of shells and other calcareous material buried in the sand. By slight migration and reprecipitation the local cement is formed. In the Bornholm region at the time of the deposition of the Kalby clay the only carbonate source available was the *Exsulans* limestone, the upper part of which must have been developed as a siltstone corresponding to the samples from the clay containing *Jincella parva* and *Ctenocephalus exsulans*. The time interval between deposition of the *Exsulans* limestone and the Kalby clay was supposedly of short duration, only allowing the erosion to remove the upper, non-calcareous part of the *Exsulans* sequence. The conclusion must be that the supply of calciumcarbonate was very limited, and this possibly accounts for the missing lithification at the time of or shortly after the sedimentation. The reason why lithification has not taken place up to the present day is not known.

Discussion: The type locality of the *Exsulans* limestone is Kiviks Esperöd in Scania. The limestone, even if not properly named according to modern principles of stratigraphical classi-

fication, ranks as a formation. The name is well-established in the literature, and for this reason possibly should not be rejected.

With regard to Bornholm the present writer considered dividing the *Exsulans* limestone as a formation into two members: The Borregård limestone member (the limestone proper) and the Kalby clay member. The two members then in many respects would form a unit, and they are only separated by a minor break in sedimentation. On the other hand the *Exsulans* limestone is widely recognized as a unit belonging to the *Triplagnostus gibbus* zone. Then, by establishing the Kalby clay as a formation the *Exsulans* limestone is maintained in the customary sense.

On the island of Öland the *Exsulans* limestone is overlain by gray, more or less calcareous, thin-bedded limestone with interstratified laminae and thin strata of shale. This sequence, although none of the index fossils from zone B 2 have been found, is correlated with that zone (Westergård, 1946, p. 14). The basal part of the sequence may be equivalent to the Kalby clay. In Scania the *Tomagnostus fissus* — *Ptychagnostus atavus* zone sequence consists solely of alum shale, as does the part of this zone overlying the Kalby clay.

Early Middle Cambrian history and paleogeography

After deposition of the Rispebjerg sandstone at the time of the Lower Cambrian *Holmia torelli* — *Kjerulfia lundgreni* zone (see text-fig. 2) a regression set in, and phosphorite impregnated the upper part of the sandstone. The following hiatus, apart from a minor transgression, corresponds to the rest of the Lower Cambrian and the basal part of the Middle Cambrian. The presence of beds from the *Eccaparadoxides oelandicus* stage in the South Baltic has not been ascertained. A thin sequence of shales and limestones without any distinctive fossils found at some Scanian localities may arbitrarily be referred to this stage or to the *Paradoxides paradoxissimus* stage.

As indicated by the non-trilobite material in the Kalby clay the Bornholm area was submerged for a short while in the late Lower Cambrian. The present writer has not yet finished his studies of the non-trilobite fossils, but he believes that all of the

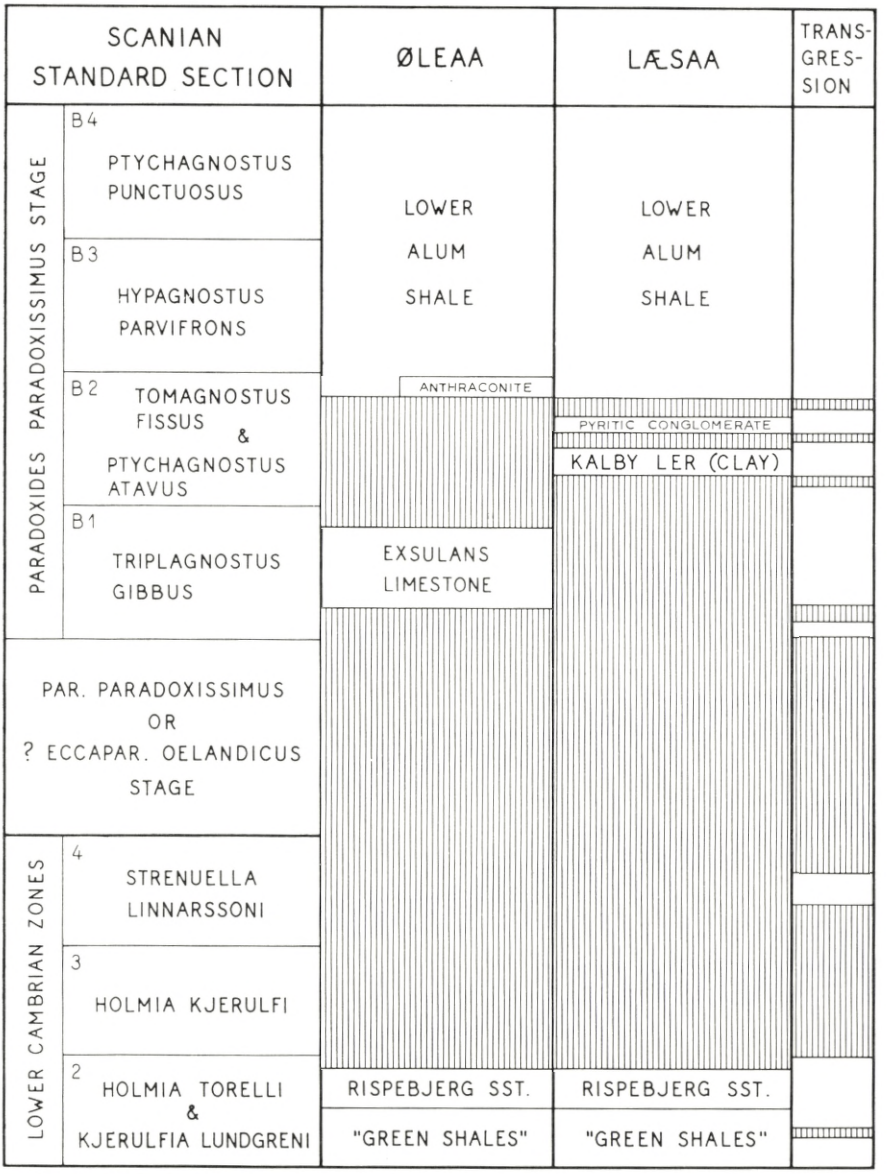

Fig. 2. The Lower and early Middle Cambrian sections at Øleaa and Læsaa, Bornholm.

Lower Cambrian species contained in the clay and in the *Exsulans* limestone most likely originated from the *Strenuella linnarssoni* zone. The sea covering Bornholm at that time was supposedly shallow, leaving a thin deposit of sand poor in carbonates. The deposit was not affected by lithification. This appears from the beautifully preserved fossils, free of adhering sediment, found in the Kalby clay.

The next transgression occurred at the time of the initiation of the *Triplagnostus gibbus* zone. The occurrence of numerous specimens of *Acrothele* (*Redlichella*) *granulata* in the Kalby clay indicates the former presence of a bed similar to the *Acrothele granulata* conglomerate, as clearly demonstrated by C. POULSEN (1942). The species is known from the *Eccaparadoxides oelandicus* stage, but as all the Læsaa specimens are strongly worn and rolled, they most likely originated from a conglomerate like the basal layer of the *Triplagnostus gibbus* zone (B1). During this transgression part of the Lower Cambrian unconsolidated deposits was reworked, and the resulting bed, of a lithology similar to that of the Kalby clay, contained a mixture of Middle Cambrian brachiopods and Lower Cambrian brachiopods and tubular fossils.

After deposition of the basal conglomerate the sea withdrew for a short while. The sea returned, and the *Exsulans* limestone was deposited. Material from the unconsolidated *Acrothele granulata* conglomerate including rounded quartz grains and phosphoritic pebbles from the Rispebjerg sandstone were incorporated. Also previously undisturbed deposits from the *Strenuella linnarssoni* zone were reworked, and Lower Cambrian fossils were embedded in the limestone.

Towards the end of the time of the *Triplagnostus gibbus* zone the basin shallowed up, and fine sand was deposited. A regression followed, and phosphorite impregnated the sandy upper part of the *Exsulans* limestone. Possibly the sedimentation continued practically uninterrupted slightly across the boundary between the *Triplagnostus gibbus* zone and the *Tomagnostus fissus* — *Ptychagnostus atavus* zone. This possibility is indicated by the specimen of *Ptychagnostus atavus* showing the same phosphoritic state of preservation as the *Exsulans* siltstone trilobites found in the clay.

At early *Tomagnostus fissus — Ptychagnostus atavus* zone time the sea withdrew completely for a short while, and the erosion removed the sandy upper part of the *Exsulans* limestone. Still at the time of this zone the sea returned, and the Kalby clay was deposited probably over an area of the same extent as that of the *Exsulans* limestone. In the clay the elements of the four previous transgressions may be identified, as demonstrated above. The Lower Cambrian fossils may be divided into a group of well-preserved specimens and a group of badly preserved (worn) specimens. Some of the species are found in both groups. Probably all of the species originated from the *Strenuella linnarssoni* zone. The badly preserved group is supposed to have been redeposited two or three times, in the first place in the *Acrothele granulata* conglomerate, secondly in the *Exsulans* limestone, and finally in the Kalby clay. The well-preserved specimens are supposed to have been washed out from original *Strenuella linnarssoni* zone beds, undisturbed by the previous transgressions.

The transgression responsible for the deposition of the Kalby clay was of short duration. Following a short break in the sedimentation the pyritic conglomerate was deposited on top of the Kalby clay. Another hiatus followed, and during this interruption the erosion removed the remaining undisturbed parts of the *Strenuella linnarssoni* zone beds, the rests, if any, of the *Acrothele granulata* conglomerate, and in most places the Kalby clay and the overlying conglomerate.

When the subsequent transgression set in at the time of the upper part of the *Tomagnostus fissus — Ptychagnostus atavus* zone the environment had changed, and abruptly, without any transition beds, alum shales were deposited. This phase was initiated at the close of zone B 2, as the basal part of the shale sequence at Øleaa contains the index fossil *Ptychagnostus atavus*. The stable conditions persisted throughout the *Hypagnostus parvifrons* zone and the *Ptychagnostus punctuosus* zone of the *Paradoxides paradoxissimus* stage.

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EARLY CAMBRIAN DISTACODONTID CONODONTS FROM BORNHOLM

BY

VALDEMAR POULSEN

København 1966
Kommissionær: Munksgaard

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Synopsis

Two early Cambrian distacodontid conodonts are described from the island of Bornholm. *Hertzina? danica* n. sp. is an unusually large species from the late Lower Cambrian. The species is at the present time hesitantly included in *Hertzina* MÜLLER, 1959 which is not considered to be a synonym of *Coelocerodontus* ETHINGTON, 1959. *Hertzina? bisulcata* MÜLLER, 1959 is recorded from the Middle Cambrian *Triplagnostus lundgreni – Goniagnostus nathorsti* Zone and the *Jincella brachymetopa* Zone.

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Introduction

The Middle Cambrian sequence on the island of Bornholm includes an interesting deposit of unconsolidated clay. The clay only contains a few Middle Cambrian fossils, whereas Lower Cambrian non-trilobite fossils are common and easily obtained by elutriation of the clay. Among these are about fifty specimens of a large distacodontid conodont which is described in the present paper.

A previously known species is represented by a few specimens from the Andrarum Limestone (*Jincella brachymetopa* Zone) and the subjacent anthraconite (*Triplagnostus gibbus* – *Goniagnostus nathorsti* Zone).

Longitudinal section of the new species was kindly made by H. J. Hansen M. Sc. who also made the photographs. Drawings were made by the present writer.

The material is in the collections of the Mineralogical and Geological Museum of the University of Copenhagen.

Descriptions

Order **Conodontophorida** Eichenberg, 1930

Family Distacodontidae Bassler, 1925

Genus ***Hertzina*** Müller, 1959

Type species: *Hertzina americana* Müller, 1959

The type species is of Franconian age belonging to the *Elvinia* Zone in the Eureka District, Nevada. Two additional species have been recorded from the Middle and Upper Cambrian of Sweden and Northern Germany (erratics).

Lindström (1964, p. 139) regards *Hertzina* as a synonym of *Coelocerodontus* Ethington, 1959, but the present writer is of the

opinion that *Hertzina* is a distinct genus which differs from *Coelocerodontus* in possessing only two costae, as the anterior side is gently rounded.

The type species of *Coelocerodontus* – *C. trigonius* – has a regular triangular cross section and the three edges of the cusp are keeled. *Coelocerodontus tetragonius* has a quadrate cross section and the cusp accordingly has four keeled edges. *Hertzina* has a rounded, semicircular anterior side which is not in any way delimited from the lateral sides, and only the two posterior side edges are keeled.

Hertzina? danica n. sp

Pl. 1, figs. 1–8. Text-figs. 1–2.

Derivation of name. – Latin *danicus* = Danish.

Holotype (here selected). – Imperfectly preserved cusp (MMH no. 9970), pl. 1, figs. 1–2; text-figs. 1–2.

Other material. – About fifty cusps, most of which are represented by phosphoritic internal moulds of the basal cavity. The cusps are of a beige to greyish-white colour and appear to be somewhat abraded; they consist of calcium phosphate.

Horizon. – Presumably Lower Cambrian, *Strenuella linnarssoni* Zone.

A discussion on the origin and age of the material is appropriate, as *Hertzina? danica* n. sp. at the present time seems to be the oldest known conodont.

The Middle Cambrian clay – the Kalby Clay – containing the conodonts belongs to the *Tomagnostus fissus* – *Ptychagnostus atavus* Zone of the *Paradoxides paradoxissimus* Stage (V. Poulsen, 1963). As a number of reworked Lower Cambrian faunal elements is found in the clay, no definite statement with regard to the age of the conodonts can be given. However, the conodonts appear to be reworked and have a beige to greyish-white colour very much like the associated specimens of *Stenothecopsis* and hyolithellid tubes. These tubes are commonly represented by lustrous black, fine-grained phosphoritic internal moulds, and most of the conodonts are only preserved as internal moulds of an identical type.

Phosphatic material like that described above is unknown

from the slightly older Exsulans Limestone (*Triplagnostus gibbus* Zone), and the present writer is of the opinion that *Stenothecopsis*, the hyolithellid tubes, and the conodonts most likely originated from the Lower Cambrian *Strenuella linnarssoni* Zone. Beds from this zone are no longer present in the sequence on Bornholm, but it has been suggested (C. POULSEN, 1942 and V. POULSEN, 1963) that thin, unconsolidated beds of this age were deposited and later reworked into the Middle Cambrian beds.

Fig. 1. *Hertzina? danica* n.sp. ×25. Imperfectly preserved cusp (holotype, MMH no. 9970); oblique posterior, lateral, and oblique anterior view. Kalbygård at Læså, Bornholm.

Locality. – Kalbygård at Læså, Bornholm.

Diagnosis. – Cusp large and simple, symmetrical, gently curved. Lateral walls thin. Basal cavity extending to the tip of the cusp. Basal portion simple, not flared. Posterior side distinctly concave throughout, edges forming well-defined costae outlined by wide, aborally well-impressed furrows on lateral sides.

Description. – The slender, undenticulated cusp is large, symmetrical, gently but evenly curved towards the posterior side. The angle between the anterior and posterior side at the tip is about 4–6°. Basal portion is simple and no tendency to flare is observed; the base itself is not preserved in any of the specimens and possibly no proper base was developed. The lateral walls are thin and surround a basal cavity which extends all the way to the tip of the cusp.

The anterior side is rounded, almost semicircular in cross section, and smoothly continues into the lateral sides which cannot be delimited anteriorly. The lateral sides are rounded anteriorly and concave posteriorly due to the presence of a wide furrow running from base to the tip of the cusp. The posterior

side is strongly or at least distinctly concave throughout, and the edges form well-defined costae which are outlined by the furrows on the lateral sides. The costae attain their maximum width at the base where the lateral side furrows are deepest; the furrows are shallowing up in direction of the tip, and the costae accordingly become narrower. The tip itself is simple due to the gradual reduction of costae and furrows.

Cross sections are perfectly symmetrical at all levels; the anterior side together with the lateral sides roughly form from half to three-fourths of a circle which is cut off by the concave, keeled posterior side.

Longitudinal section shows that the walls are of uniform thickness throughout. The material has clearly been affected by diagenetic changes and the eventual laminated structures are destroyed.

Dimensions. – The length of the cusps vary from about 0.7 mm to about 8.0 mm (estimated from large fragments).

Affinities. – Species of a simple form like *Hertzina* will of necessity have a majority of features in common, and distinction of new genera of this type requires a large material and a better knowledge of early Cambrian conodont faunas. The present writer is of the opinion that the *Hertzina* group may eventually be divided further, but at the present stage tentative assignments to *Hertzina* are preferable.

Hertzina? danica n. sp. differs from the type species – *H. americana* – in having a slender basal portion, in being perfectly symmetrical, and in the posterior side being evenly concave throughout.

The new species resembles *Hertzina elongata* MÜLLER, 1959 in many respects, but *H. elongata* has a flat or even convex posterior side, and is also distinctly asymmetrical.

Hertzina? bisulcata MÜLLER, 1959 has a general shape similar to that of *Hertzina? danica* n. sp., but MÜLLER's species has a gently convex posterior side, the costae are less pronounced, and the longitudinal furrows on the lateral sides are generally more shallow and more distant from the posterior edges.

Hertzina? danica n. sp. appears to be more primitive than the other species of *Hertzina*, and particularly the perfect symmetri shown by the cusps is of interest.

Remarks. – The known natural assemblages of conodonts are from the late Palaeozoic, and it is an open question if the Cambrian discrete conodonts were combined into an "apparatus". MÜLLER (1956, p. 1327) suggested a statistical approach to solve the question, but so far no positive answers have been obtained. On the other hand the knowledge of Cambrian conodonts is still very limited.

If the Cambrian conodont-bearing animals did have the conodonts grouped together in an "apparatus", it is only reason-

Fig. 2. *Hertzina? danica* n. sp. ×25. Cross sections of two phosphatic internal moulds and one shelly specimen with mould in place (holotype, MMH no. 9970). The sections are from the middle portion of the cusps and show the range of variation.

able to expect a very simple structure strongly different from the Carboniferous (Mississippian – Pennsylvanian) natural assemblages.

LINDSTRÖM (1964) stated that the structure of the assemblages suggested that the conodonts might have been supporting a frilled, lophophore-like organ which had a function as a filter. By assuming current directions, which would pass the conodont-supported frills in the most favourable way, the circulation pattern of the water was reconstructed.

The present writer is of the opinion that an eventual lophophore-like organ in the Cambrian forms might have been supported by only a few conodonts and possibly even of the same type. During the later development the compound blade-like, bar-like, and plate-like conodonts evolved, the distacodontids disappeared, and at the same time a decrease in size is evident. These trends leading to the highly complex assemblages most likely resulted in an increased capacity of the "apparatus".

In the known natural assemblages the conodonts are arranged with their cusps and denticles more or less at right angles to the sagittal axis of the animal. If the early Cambrian distacodontid conodonts are placed in a similar position, it becomes evident that the "apparatus" might be up to about 20 mm in width, whereas the known natural assemblages are 2–3 mm wide and

about 9 mm long. The present writer believes that the Cambrian distacodontids more likely had an orientation roughly parallel to the sagittal axis of the animal, with the tips in anterior direction and the flat or concave posterior sides facing the sagittal axis.

Hertzina? bisulcata MÜLLER, 1959

Pl. 1, fig. 9. Text-fig. 3

1959 *Hertzina? bisulcata* n. sp. MÜLLER: Kambrische Conodonten, p. 456, pl. 13, figs. 22–24, 27. (Description and figs. of four cusps)

Fig. 3. *Hertzina? bisulcata* MÜLLER, 1959. ×25. Lateral view of slightly flattened cusp (MMH no. 9977). Southeast of Kalbygård at Læså, Bornholm.

Material. – One almost complete and three somewhat fragmentary cusps. The material is frail, and most of the damage was probably done during the etching and later handling of the residue. They do not possess internal moulds of phosphatic material like in *Hertzina? danica* n. sp.

Horizon. – Middle Cambrian, Andrarum Limestone (*Jincella brachymetopa* Zone) and subjacent anthraconite (*Triplagnostus lundgreni – Goniagnostus nathorsti* Zone). The cusps were obtained by etching with acetic acid of only one hand-sized sample of the limestone and the anthraconite. Thus the species may possibly be common at this level.

Locality. – Southeast of Kalbygård at Læså, Bornholm.

Description. – The cusp is long and slender, gently curved towards the posterior side, and slightly asymmetrical, as the tip portion is slightly twisted. The basal cavity, which extends all the

way to the tip, is surrounded by extremely thin walls. The base is not preserved in any of the specimens.

The anterior side is rounded, semicircular in cross section, smoothly continues into the lateral sides which are not well-delimited from the anterior and posterior sides. The lateral sides are distinguished by a wide and shallow longitudinal furrow running from the base to the tip of the cusp. The posterior side is gently convex to flat, the edges are gently rounded, and thus no real costae are developed.

Cross section is oval with constrictions corresponding to the longitudinal furrows on lateral sides.

Dimensions. – The almost complete cusp is about 2 mm long.

Remarks. – *Hertzina? bisulcata* is undoubtedly closely related to *Hertzina? danica* n. sp., but differs in having a wider basal portion, the posterior side is generally convex, the cusp is asymmetrical, and the cross sections are distinctly different. The wider basal portion in MÜLLER's species problably indicates the progressive development of the flared base which distinguishes the majority of the Upper Cambrian conodonts.

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PLATE

Explanation to plate I

Fig. 1–8. *Hertzina? danica* n. sp. 4

1–2: Incomplete cusp (MMH no. 9970); oblique posterior and oblique lateral view. Holotype. Kalby Clay; Kalbygård, Læså. ×35.

3: Worn cusp (MMH no. 9971); lateral view. Kalby Clay; Kalbygård, Læså. ×35.

4: Incomplete cusp (MMH no. 9972); oblique posterior view. Kalby Clay; Kalbygård, Læså. ×35.

5: Incomplete cusp (MMH no. 9973); oblique lateral view. Kalby Clay; Kalbygård, Læså. ×35.

6: Phosphatic internal mould of basal cavity (MMH no. 9974); lateral view. Kalby Clay, Kalbygård, Læså. ×30.

7: Incomplete phosphatic mould of basal cavity (MMH no. 9975); lateral view. Kalby Clay; Kalbygård, Læså. ×30.

8: Incomplete phosphatic mould of basal cavity (MMH no. 9976); oblique lateral view. Kalby Clay; Kalbygård, Læså. ×30.

Fig. 9. *Hertzina? bisulcata* MÜLLER, 1959 8

Almost complete, slightly flattened cusp (MMH no. 9977); oblique lateral view. Andrarum Limestone; southeast of Kalbygård, Læså. ×30.


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DESCRIPTION OF SEVEN TYPE SPECIMENS OF FORAMINIFERA DESIGNATED BY D'ORBIGNY, 1826

BY

HANS JØRGEN HANSEN

København 1967
Kommissionær: Munksgaard

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Synopsis

Seven type specimens of foraminifera designated by D'ORBIGNY in 1826 were recently discovered in the collections of the Mineralogisk Museum, Copenhagen. They are described and illustrated. (*Textularia gibbosa; Fasciolites oblonga; Gyroidina orbicularis; Pararotalia bisaculeata; Asterigerina rosacea; Anomalina elegans; Nummulina discoidalis.*) The genus *Gyroidina* is emended.

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Since D'ORBIGNY in 1826 published his work on foraminifera and illustrated some of the species by plaster models, much confusion has existed as to the proper content of the species named by him as no descriptions were given. PARKER, JONES & BRADY (1865) figured the models but no actual type specimen was described and pictured.

Recently a collection of D'ORBIGNY plaster models in the collections of the Mineralogisk Museum, Copenhagen was studied and besides 100 models 19 specimens of actual foraminifera were present giving further illustration of some of the species.

Some of the specimens were glued to small glass plates by aid of gummi arabicum and protected by another glass plate resting upon 4 small pieces of cardboard along the edges. The cardboard was labelled with a number corresponding to a number of a model. The whole arrangement was kept in place by a thin twisted copper wire.

The larger specimens were glued to pieces of cardboard without any cover.

Due to the mounting technique used by D'ORBIGNY many of the specimens were fractured. This was caused by the shrinkage of the gummi arabicum. When a fractured specimen was moistened with water in order to dissolve the glue, it was found that the fractions fell apart and the test was destroied. When, however, the test was moistened with collodium dissolved in amylacetate and dried before the glue was dissolved in water, the fragments were kept in place. The thin film of collodium on the surface of the test was afterwards removed with a needle by carefull preparation.

Dr. Y. LE CALVEZ kindly informed me that 7 species of the 19 represented in Copenhagen are missing in the D'ORBIGNY collection in Paris.

Due to the drawing technique used at the time when the d'Orbigny models were made they are all inverted.

It has not been possible to trace the exact year when the collection arrived in the Mineralogisk Museum as it carries no registration number. The registration books of the museum do not cover the period before 1829. On one of the wooden boxes which contain the models can be read: "*Commissaires Messieurs Geoffray StHilaire, Duméril et Latreilles* (7 *Novembre* 1825)." This represents as far as it has been brought to my knowledge a firm in Paris trading with fossils, minerals etc. The collection may accordingly have arrived in the Mineralogisk Museum after the 7th of November 1825 but before 1829.

The letters "MMH" and a number used in the systematic part of this paper refer to the registration code of the Mineralogical Museum of the University of Copenhagen where the material is kept.

I am greatly indebted to Dr. A. Nørvang for specimens of *Gyroidina orbicularis* from Rimini and to Dr. Y. le Calvez for information concerning the d'Orbigny collection in Paris. Mr. Christian Rasmussen prepared the illustrations with his usual skill and care.

Order **Foraminiferida** Eichwald, 1830

Suborder **Textulariina** Delage and Hérouard, 1896

Superfamily **Lituolacea** de Blainville, 1825

Family **Textulariidae** Ehrenberg, 1838

Subfamily **Textulariinae** Ehrenberg, 1838

Genus ***Textularia*** Defrance, 1824

Textularia gibbosa d'Orbigny, 1826

Pl. 1. Figs. 1a, b.

1826. *Textularia gibbosa* d'Orbigny: p. 262, modèles no. 28, 2me livraison.

Description:

Test biserial throughout. Consisting of 8 faintly inflated chambers. Outline smooth. Initial end rounded. Cross section

broadly elliptical. Sutures faintly depressed. The angle between the sutures and the axial line is about 70°. The aperture is a low, broad slit at the base of the final chamber. The basal part of the apertural face at the ends of the aperture is somewhat projecting. Test wall apparently composed of calcareous material containing some agglutinated quartz grains with an average diameter of 10 microns. The surface of the test is rather smooth. No adventive chamber has been found.

Length: 782 microns. Width: 630 microns. Thickness: 540 microns.

Type level according to D'ORBIGNY: *Recent et fossile.*

Type locality according to D'ORBIGNY: *La mer Adriatique; Castel-Arquato.*

Remarks:

The syntype in the collection of the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

Lectotype: Pl. 1. Figs. 1a, b. MMH No. 10.313.

Suborder **Miliolina** DELAGE and HÉROUARD, 1896

Superfamily **Miliolacea** EHRENBERG, 1839

Family **Alveolinidae** EHRENBERG, 1839

Genus ***Fasciolites*** PARKINSON, 1811

Fasciolites oblonga (D'ORBIGNY, 1826)

Pl. 1. Figs. 2a, b, c.

1826. *Alveolina oblonga* D'ORBIGNY: p. 306.

Description:

Test involut, ellipsoidal. 17 chambers in the final whorl. Septulae alternating in adjacent chambers with pro- and postseptal passages. Apertural character unknown. Basal thickening developed in the oldest chambers. Initial part regularly enrolled.

Length: 5,61 mm. Thickness: 2,54 mm.

Type level according to D'ORBIGNY: *Fossile.*

Type locality according to D'ORBIGNY: *Les environs de Soissons.*

Remarks:

The syntype found in the collection of the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

Three specimens of the species are present in our collection. Two of these have been ground for preparation of thin sections.

Lectotype: Pl. 1. Fig. 2a. MMH No. 10.317.

Figured syntype (axial section): Pl. 1. Fig. 2c. MMH No. 10.320.

Figured syntype (equatorial section): Pl. 1. Fig. 2b. MMH No. 10.321.

Superfamily **Cassidulinacea** d'Orbigny, 1839

Family **Anomalinidae** Cushman, 1927

Subfamily **Anomalininae** Cushman, 1927

Genus ***Gyroidina*** d'Orbigny, 1826

Type species: *Gyroidina orbicularis* d'Orbigny, 1826
subsequent designation by Cushman, 1927

Original diagnoses:

"Test libre, trochoïde, régulier; spire tronquée, très-aplatie; côté oppose très-convexe; ouverture en fente longitudinale contre l'avant-dernier tour, mais n'occupant que la partie médiane de la loge."

Emended diagnoses:

Test free, trochospiral. Umbilical side involut, spiral side evolut. Umbilicus closed. Umbilical side strongly convex, spiral side less convex. Aperture a low arch placed interio-marginally on the umbilical side neither extending to the periphery nor to the umbilicus, at the base of the final chamber. Wall calcitic, perforate and granular in structure. Bilammellar septa.

Differential diagnoses:

The genus *Gyroidina* differs from the genus *Heterolepa* in having the aperture restricted to the umbilical side, while it extends onto the spiral side in *Heterolepa*.

Gyroidina orbicularis D'ORBIGNY, 1826

Pl. 2. Figs. 1a, b, c; 2a, b, c.

1826. *Gyroidina orbicularis* D'ORBIGNY: p. 278, modèles no. 13, 1re livraison.

Description:

Test free, trochospiral. All chambers visible on the spiral side which is somewhat convex. Umbilical side involut and strongly convex. 7 chambers in the final whorl. Sutures flush with the surface, gently curved. Periphery bluntly angled. General outline smooth. The central part of the spiral side is thickened by deposition of secundary calcite layers.

The aperture is a low arch at the base of the last formed chamber. It is confined to the lower part but not extending across the periphery or to the umbilicus. Test wall composed of granular calcite. The septa are bilamellar. The wall is perforate with an average pore diameter of 10 microns. The pores on the spiral side are slightly larger than those on the umbilical side.

Diameter: 730 microns. Thickness: 513 microns.

Type level according to D'ORBIGNY: Recent.

Type locality according to D'ORBIGNY: *La mer Adriatique, pres de Rimini.*

(Additional specimens from Rimini were kindly placed at my disposal by Dr. A. NØRVANG.)

Remarks:

According to the morphology of *Gyroidina orbicularis* described above, the assignment of the genus *Gyroidina* to the family *Alabaminidae* must be considered incorrect.

The genus *Gyroidina* should instead be placed within the family *Anomalinidae* and close to the genus Heterolepa.

The syntype of *Gyroidina orbicularis* in the collection of the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

Lectotype: Pl. 2. Figs. 1a, b, c. MMH No. 10.319.

Figured specimen: Pl. 2. Figs. 2a, b, c. MMH No. 10.318.

Superfamily **Rotaliacea** EHRENBERG, 1839

Family **Rotaliidae** EHRENBERG, 1839

Subfamily **Rotaliinae** EHRENBERG, 1839

Genus ***Pararotalia*** LE CALVEZ, 1949

Pararotalia bisaculeata (D'ORBIGNY, 1826)

Pl. 2. Figs. 3a, b, c.

1826. *Rotalia* (*Rotalie*) *bisaculeata* D'ORBIGNY: p. 273, modèles no. 15, 1re livraison.

Description:

Test umbilicoconvex. Spiral side slightly convex. Umbilical side strongly convex. Periphery angled with a serrate keel carrying on each chambers one blunt spine. All chambers visible on the spiral side. Chambers on the umbilical side inflated. Chambers on the spiral side not inflated. Sutures on the umbilical side hardly depressed near periphery, becoming more depressed towards umbilicus, radial. Sutures on the spiral side with a backward curve, hardly depressed. General outline lobate. Aperture umbilical-extraumbilical. Older apertures open into umbilicus below overhangning lips. Central part of umbilicus occupied by a rounded plug. Test wall composed of perforate, radiate calcite. Lamellar character unknown.

Diameter: 280 microns. Thickness: 63 microns.

Type level and locality unknown. According to D'ORBIGNY: *Un sable de délestage.*

Remarks:

The syntype in the Mineralogisk Museum is here chosen as lectotype.

Lectotype: Pl. 2. Figs. 3a, b, c. MMH No. 10.312.

Suborder **Rotaliina** DELAGE and HÉROUARD, 1896

Superfamily **Discorbacea** EHRENBERG, 1838

Family **Asterigerinidae** D'ORBIGNY, 1839

Genus ***Asterigerina*** D'ORBIGNY, 1839

Asterigerina rosacea (D'ORBIGNY, 1826)

Pl. 3. Figs. 1a, b, c.

1826. *Rotalia* (*Rotalie*) *rosacea* D'ORBIGNY: p. 273, modèles no. 39, 2me livraison.

Description:

Test trochospiral, biconvex. Spiral side more convex than umbilical side. 6 chambers in the final whorl. Periphery angled with a smooth keel of clear calcite. Secundary chambers showing a rosette-like pattern on the central part of the umbilical side. Test smooth except for the area in front of the aperture which is tuberculated. The youngest foramen has an elliptical form. It is placed midway between the periphery and the umbilicus on the umbilical side. The sutures are oblique and curving on the spiral side. They are in level with the surface except for the central part of the umbilical side where they are somewhat elevated and provided with small tubercles. Lamellar character unknown. Wall calcitic, perforate and radiate in structure.

Diameter: 432 microns. Thickness: 180 microns.

Type level according to D'ORBIGNY: *Fossile.*

Type locality according to D'ORBIGNY: *Les environs de Bordeaux.*

Remarks:

The syntype found in the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

The specimen is not well preserved. The final chamber and a small area near umbilicus are missing. In spite of this there can, however, be no doubt as to the assignment of the species to the genus *Asterigerina* because of the rosette-like arrangement of the secundary chambers, the wall structure and general morphology.

Lectotype: Pl. 3. Figs. 1 a, b, c. MMH No. 10.314.

"*Anomalina*" *elegans* D'ORBIGNY, 1826

Pl. 3. Figs. 2 a, b, c.

1826. *Anomalina elegans* D'ORBIGNY: p. 282, modèles no. 42, 2me livraison.

Description:

Test free, trochospiral. Involut on both sides, biconvex. Periphery bluntly angled. Outline smooth. Only the eight chambers of the final whorl visible. Strongest convex on the aboral side. Sutures slightly depressed between the last three chambers on

the aboral side else they are filled with clear calcite and flush with the surface which is unornamented. Sutures are gently curving on both sides. The sutures on the oral side are thickened and raised towards the central depression. The aperture is interiomarginal and continues on the least convex side below all chambers and opens into the central depression where it is covered by overhanging serrate lips. The apertural face is bluntly angled against the aboral side and imperforate in the area near the aperture. Wall calcitic, radiate and perforate. Lamellar character unknown.

Diameter: 460 microns. Thickness: 190 microns.

Type level according to D'ORBIGNY: *Fossile.*

Type locality according to D'ORBIGNY: *Aux environs de Bordeaux.*

Remarks:

The syntype in the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

As only one specimen is present no thin section has been made and accordingly the lamellar character is unknown.

Due to the radial arrangement of the calcite of the wall of "*Anomalina*" *elegans* it can not be referred to the genus *Anomalina* or even to the superfamily *Cassidulinacea*. The radial wall and missing information as to the lamellar character make an assignment of this species even to superfamily impossible. According to this fact, the genus name *Anomalina* is written in quotation marks.

Lectotype: Pl. 3. Figs. 2a, b, c. MMH No. 10.315.

"*Nummulina*" *discoidalis* D'ORBIGNY, 1826

Pl. 3. Figs. 3a, b.

1826. *Nummulina* (*Assiline*) *discoidalis* D'ORBIGNY: p. 296, modèles no. 88, 4me livraison.

Description:

Test involut, becoming evolut in the final half of the final volution. 21 chambers in the final whorl. Periphery with a broadly rounded keel. On both sides is found a rounded, elevated

and smooth umbonal disc. Sutures curving gently backwards and provided with a row of tubercles of clear calcite. The tuberculation becomes coarser on the older part of the test. Aperture unknown. Interiomarginal foramen forming a low arch with a median notch. Wall calcareous, laminated, fibrouse and radiate.

Diameter: 2062 microns. Thickness: 1062 microns.

Type level according to D'ORBIGNY: *Recent.*

Type locality according to D'ORBIGNY: *La mer du Sud, à l'ile de Rawack, Nouvelle-Hollande.*

Remarks:

The syntype in the Mineralogisk Museum, Copenhagen is here chosen as lectotype.

The final half of the final volution is missing on the specimen. The wall structure was studied on a grind section made on a very small fragment of the test. According to missing information concerning essential characters a safe determination to genus can not be carried out and accordingly the genus name is written in quotation marks.

Lectotype: Pl. 3. Fig. 3a, b. MMH No. 10.316.

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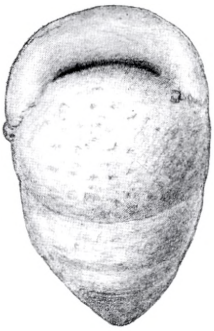
Færdig fra trykkeriet den 16. februar 1967.

PLATES

Plate I

Fig. 1a, b. *Textularia gibbosa* d'Orbigny. Lectotype (MMH No. 10.313). × 54.
Fig. 2. *Fasciolites oblonga* (d'Orbigny).
a. Lectotype (MMH No. 10.317). × 12,5.
b. Syntype (equatorial section) (MMH No. 10.321). × 17.
c. Syntype (axial section) (MMH No. 10.320). × 17.



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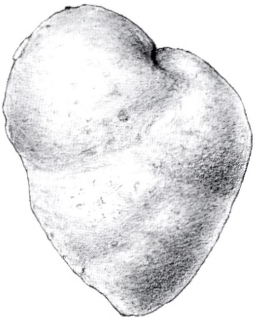

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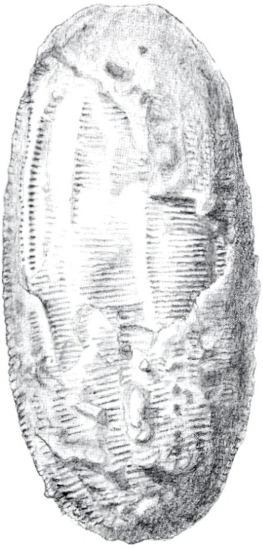

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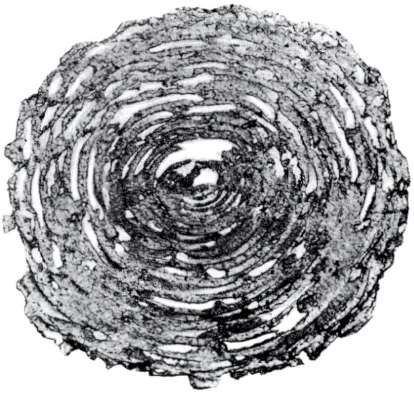

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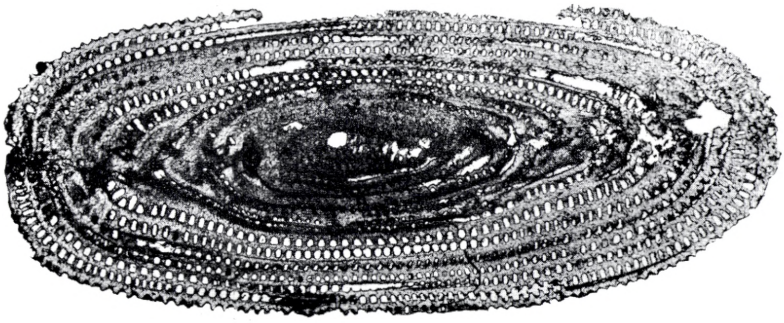


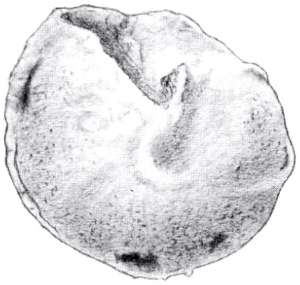
2c

Plate II

Fig. 1. *Gyroidina orbicularis* d'Orbigny. Lectotype (MMH No. 10.319). × 54.
a. Umbilical side.
b. Apertural view.
c. Spiral side.

Fig. 2. *Gyroidina orbicularis* d'Orbigny. (MMH No. 10.318). × 54.
a. Umbilical side.
b. Apertural view.
c. Spiral side.

Fig. 3. *Pararotalia bisaculeata* (d'Orbigny). Lectotype (MMH No. 10.312). × 128.
a. Umbilical side.
b. Apertural view.
c. Spiral side.



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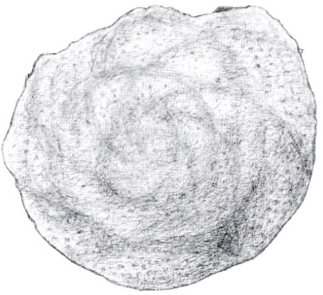

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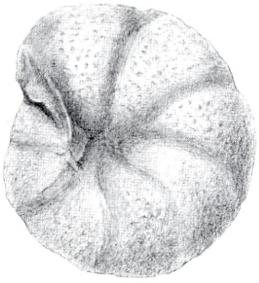

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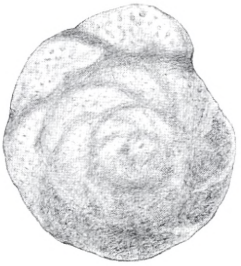

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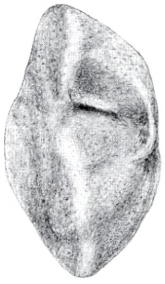

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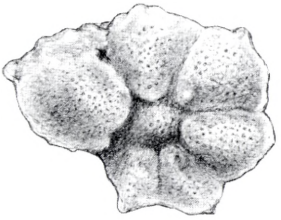

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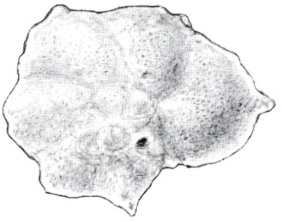

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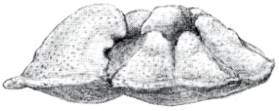


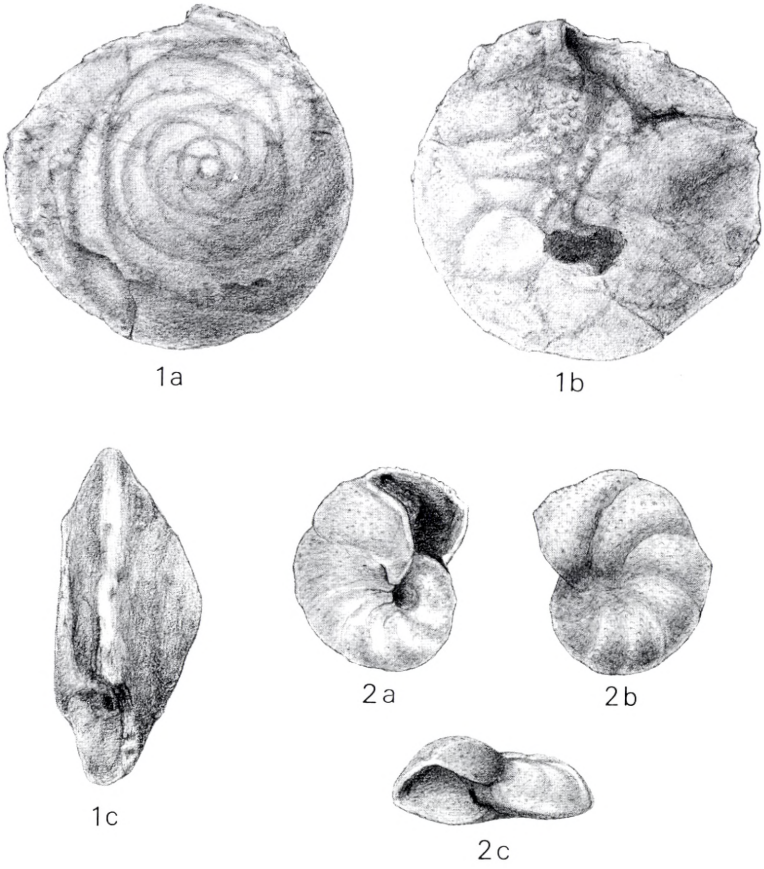
3b

PLATE III

Fig. 1. *Asterigerina rosacea* (D'ORBIGNY). Lectotype (MMH No. 10.314). × 104.
a. Spiral side.
b. Umbilical side.
c. Apertural view.

Fig. 2. "*Anomalina*" *elegans* D'ORBIGNY. Lectotype (MMH No. 10.315). × 58.
a. Oral side.
b. Aboral side.
c. Apertural view.

Fig. 3. "*Nummulina*" *discoidalis* D'ORBIGNY. Lectotype (MMH No. 10.316). × 27.
a. Side view.
b. Edge view.



1a

1b

1c

2a

2b

2c

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3b

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