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REGENERATION IN PLANARIANS INVESTIGATED WITH A NEW TRANSPLANTATION TECHNIQUE

BY

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I. Does the Head of the fully differentiated, adult Planaria act as an Organiser? Experiments on *Planaria lugubris* O. Schm.

It is a question of considerable importance whether the fully differentiated, adult head of a planaria can set up new gradient-fields in the grown-up body when implanted into different parts of an adult planaria; that is to say, whether the head contains agents which are able to overthrow the already stabilised polarity of the fully differentiated body.

If this should prove to be the case, the planarian head would be comparable to the amphibian organiser, and the tissues in the body to the ventral epidermis in the gastrula-stage. The whole system of the grown-up planaria would then in a sense be embryonic in character.

The question has been dealt with, or at least touched upon, by RAND and BROWN (1926), SANTOS (1926), STEINMANN (1933), OKADA and SUGINO (1934), SPIRITO (1935). The results have been rather contradictory. The possible causes of the discrepancy will be discussed after the exposition af my own results.

It is quite clear that transplantations on the highly regenerative planarians may be expected to yield valuable information about fundamental questions in the whole problem of regeneration. Many authors have therefore developed trans-

plantation techniques, thus, besides the above-mentioned, T. H. Morgan (1900), L. V. Morgan (1906), Goetsch (1921), Li (1928).

The techniques may be grouped under two headings. In the one kind the pieces are held together cut face to cut face by glass, collodium, paper and the like. In the other, small pieces are inserted into holes made in the body. The method of Okada and Sugino is unfortunately not stated in their paper. The other methods seem to be rather troublesome. It was therefore to be expected that a technique which would be able to yield convenient and expeditious work would prove to be of great advantage for the solving of many questions in the problems of regeneration in planarians.

The following principles were applied in the development of a suitable technique.

1. Spontaneous muscle-contractions during, and a suitable time after, the transplantation must be prevented. A convenient narcotic had therefore to be found. Chloretone, which is in general use for planarians, proved useless, because the muscles are often in chloretone-narcosis in such a state of tension that the form of the animal is somewhat contorted, and this then prevents a systematical transplantation, inasmuch as the cut surfaces will not fit one another. It is true that in a heavier narcosis the muscles will be more relaxed, but in that state the animal is generally not able to live long enough to afford time for the cut surfaces to grow together. The results of experiments with various other narcotics was that nicotine proved to meet the requirement of a suitable relaxation of the muscles together with a non-impairment of the viability during the narcosis.

2. Experience has shown that living pieces of planarians are almost motionless in darkness until an advanced regeneration has taken place. But even if the transplanted pieces were both narcotised and placed in the dark, they did not coalesce well when placed in water. It was therefore necessary to develop a method whereby the pieces would retain their viability without water as the surrounding medium, but with moisture enough to prevent a drying of this rather delicate kind of tissues. The pieces were placed on silk extended over small frames of glass. SCHOTTÉ (1930) devised a method for operating on Anuran eggs. He used small glass-rings with silk extended over them. These Schotté-tables proved to be excellent.

The new technique was developed on *Planaria lugubris*. This species is known to possess a formidable resistance towards operations and a power of regeneration almost unique even among planarians.

After a good many experiments the following rather simple technique was developed.

The animals are taken directly from the aquaria and put into petri dishes with tap-water containing about 0,001% nicotine. This concentration may vary a great deal. It is convenient to have 50 cc water in the dish and pour 5—15 drops of a 1% nicotine-solution into it. After about one hour the operations can be performed. The cutting is done by means of small scalpels sterilised in the gas-flame before every cut. The operations are performed on wax or a mixture of wax and paraffin. It is most convenient to cast the wax in a 5×2 cm petri dish, and cool quickly so that the block may easily be taken out. Before every operation the surface of the block is heated in the flame.

With a pincet the cut pieces are placed on the Schotté-tables. These have previously been sterilised and covered with sterilised silk which is fastened under the collar of the glass-ring with a likewise sterilised caoutchouc ring. The Schotté-tables are placed in petri dishes, e. g. 10×2 cm, in which is placed nicotine-water of the same concentration as that used for the narcotising of the animals. To avoid troublesome air-bubbles beneath the silk, the nicotine-water is pipetted over the silk so that this is moistened and the water oozes down into the dish from under the Schotté-tables. In order that the silk may be easily moistened it has to be carefully washed before use in pure soap, rinsed several times in tap-water, then in distilled water, after which procedure it can be stored in 70 % alcohol. Before use it has of course to be thoroughly rinsed in distilled water.

When the cut pieces of the planarians are put into their proper place on the silk, a few drops of the nicotine-water are pipetted over them in order to clear away loose cells and the like. With a flamed pincet and a glass-rod with a globe (according to Spemann) the pieces are now pushed close together. The cover of the petri dish is put on in order to prevent evaporation, and a cover of pasteboard is placed over the dish in order to make the moist chamber dark. This must be done after every transplantation. A few pieces can be placed on every Scotté-table, and several tables in every dish. When the desired transplantations have been made, the dish should be put in a dark and cool room. A room or ice-box of 5—10° C. has proved most useful. If some of the pieces have moved apart during transport they may be replaced.

If healthy animals are used, especially if they have only

been in the laboratory for a few days after capture (they are not to be fed), up to 100% of the transplantations may give useful results.

After 24 hours the pieces of the animals will adhere so closely that they can be put into pure tap-water. This is best done in the following way: the Schotté-tables are put in a petri dish with tap-water reaching half way up to the silk with the animals. Then more water is very cautiously poured into the dish so that it will slowly cover the silk itself. In most cases the animals will separate from the silk, and the table can be removed without touching them. If not, they can be removed with the glass-rod.

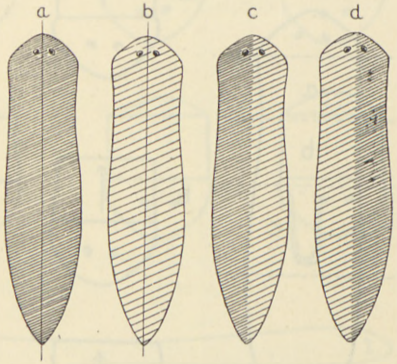


Fig. 1. *Planaria lugubris*. a, dark- coloured, b, light-coloured sample. The longitudinal lines indicate the plane of cutting. c and d, light and dark-coloured halves from a and b grafted together to form chimaeras.

After this procedure the dish should again be placed in the dark, but it is not necessary to put it in the ice-box. After another 24 hours, the pieces have grown so closely together that they may be handled without further caution.

The effectiveness of the method will be seen from fig. 1, where the longitudinal half of a dark animal has been transplanted to a light-coloured one.

With this method the first question put in this paper can be investigated: can the fully developed head of a planarian set up new gradient-fields in the grown up body when implanted elsewhere?

These experiments were also made upon *Planaria lugubris.*

In order to avoid the formation of regeneration-blastemes which would only obscure the results, the cuts were made

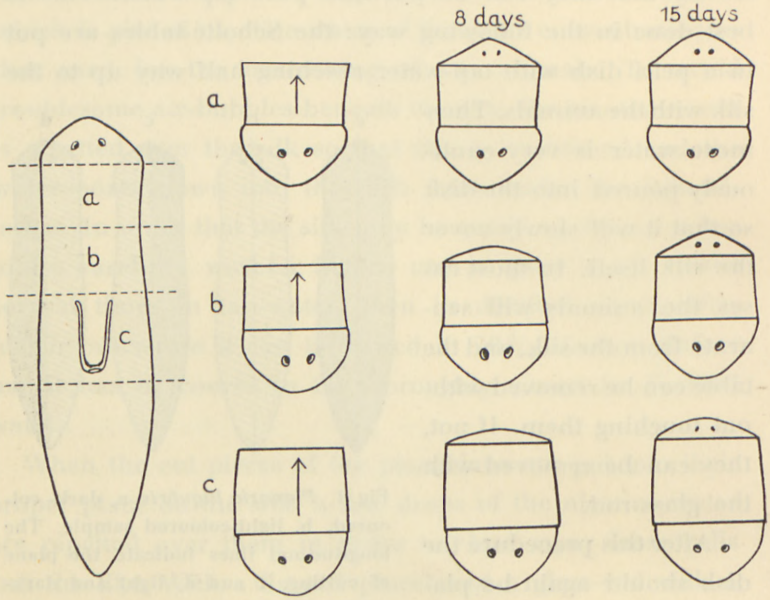


Fig. 2. *Planaria lugubris.* Exper. I. The dotted lines indicate the cuts made. The arrows indicate the polarity of the segment. The transplanted heads are pointing downward.

in such a way that the faces which were to be placed against one another were of the same size. All the transplantations were auto-transplantations.

Experiment I. This was conducted in three series (fig. 2), in order to test the question at different levels of the body. 5 transplantations were made in each series. After 8 days the body segments in the a-series had built blastemes with eyes in accordance with its own polarity. The same

situation only occured after 15 days in the b- and c-series. After this time several animals had died, only 4, 3 and 1 chimaeras remaining in the series a, b, c respectively.

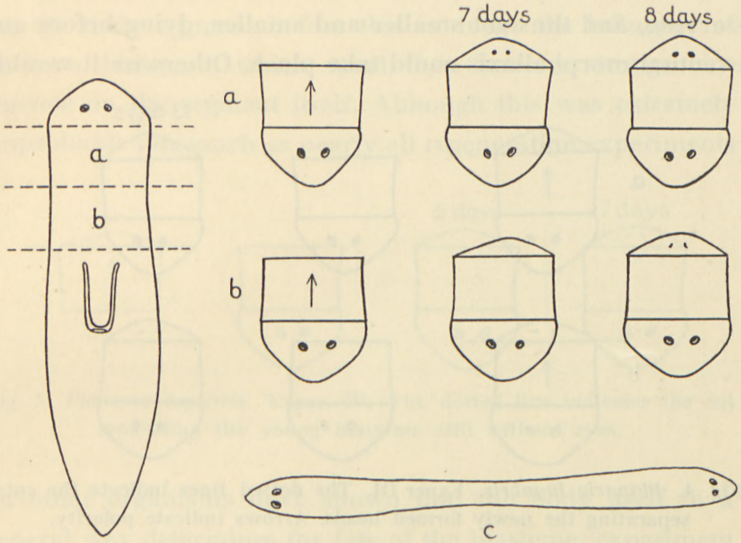


Fig. 3. *Planaria lugubris.* Exper. II. a and b as in fig. 2. c, chimaera formed by the old head transplanted to an a-piece, which has formed its own head according to its own polarity. The two animals are dragging one another out into a long shape by their efforts to go forward.

Experiment II. Essentially the same experiment as I: series a and b (fig. 3). After 7 and 8 days all body segments had developed heads in their blastemes. Of the 15+15 transplantations 10+8 chimaeras were now living.

Experiments I and II prove that the fully formed head is not able to prevent the formation of a head in the blasteme of the body segment into which it is grafted in the opposite direction of the antero-posterior polarity. Neither is it able to revert the neuro-muscular movements of the body-segment. Chimaeras are formed, animals with a head at

each end; and these two heads strive to get away from one another, thereby dragging the whole thing out into a rather long thin thread (fig. 3 c). As the animals had no pharynges, they were not able to take food, therefore they were starving, and thus got smaller and smaller, dying before an eventual morphallaxis could take place. Otherwise it would

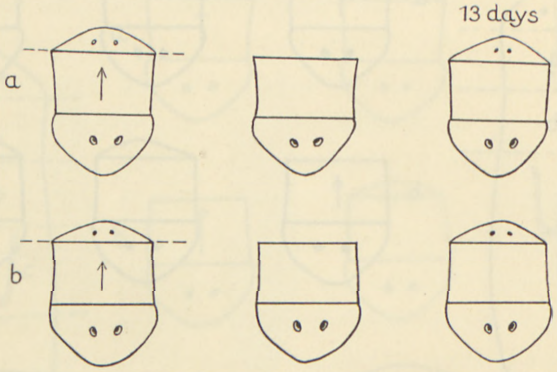


Fig. 4. *Planaria lugubris*. Exper III. The dotted lines indicate the cuts separating the newly formed heads. Arrows indicate polarity.

of course have been of considerable interest to see if the morphallaxis would proceed in the same order in the animal with the adult head and that with the head being built in the blasteme. In the future I hope I shall be able to look into this theoretically important question, using larger animals.

The likewise interesting question as to the histological connection between the two animals is also postponed.

Experiment III. As we have seen, new heads are formed in the blasteme of the body-segments. After 15 days these heads are not yet fully differentiated and pigmented. Now it might be argued that, if this new head were cut away, the grafted head might perhaps, so to speak, have a chance. This experiment (fig. 4) was made with three chimaeras of series a and b of experiment II. After

13 days all six animals had built heads in the blasteme formed on the newly cut front-surfaces of the body-segments.

Experiment IV. But perhaps an organising agent from the grafted head is present but has not had time to work on the body segment before this is under the influence of its own blasteme, which must then have a stronger influence on the segment itself. Although this was extremely improbable inasmuch as nearly all regeneration experiments

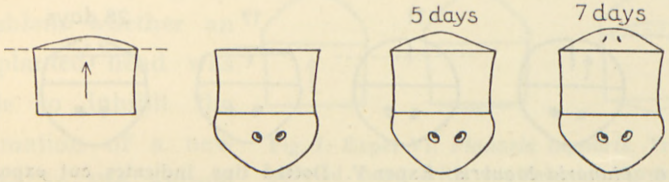


Fig. 5. *Planaria lugubris*. Exper. IV. The dotted line indicates the cut separating the young blasteme still without eyes.

on other organisms have shown that the body itself in a general way determines the fate of the blasteme, experiment IV was carried out.

To investigate this question the blasteme on 4 pieces with blastemes in which no traces of eyes were as yet to be discerned, were cut away. Then a head was grafted. But after 5—7 days new blastemes were formed with eyes in them (fig. 5).

Experiment V. Finally it may be argued that even the very first beginning of the new blasteme is able to resist an influence from the grafted head. In order to test this I proceeded as follows.

Not rarely it happens that a cut surface contracts from side to side so that the wound is closed in a median suture. If this happens, we get after the transplantation a curious sort of chimaera, consisting of a head and a piece of a body which do not build any blasteme and therefore

do not develop any further. Analogous facts are known from other experiments and are of considerable interest. But no further stress will be laid on this here.

Here we have body-segments which cannot have been influenced by any blasteme. Here, then, is a possibility for the head to exert its influence on the body-segment. And in order that this possible agency might have sufficient time to work, I waited 11 days before cutting away the closed

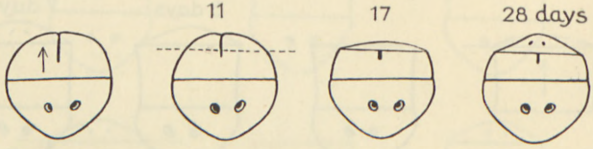


Fig. 6. *Planaria lugubris*. Exper V. Dotted line indicates cut exposing a free surface. Arrows indicate polarity. The dark line medially in the upper segment indicates suture of wound closure.

wound, so that a fresh cut surface was exposed. Blastemes were formed and they built heads. 4 samples were treated in this way, all showed the same result (fig. 6).

Experiment VI. The five foregoing experiments were conducted in such a way that a possible power of the head to reverse the antero-posterior axis was tested. After the negative results it did not seem likely that the head would be able to reverse the dorso-ventral axis. The experiment, however, was made. Three series were done, corresponding to the series in experiment I. 5 transplantations were made in each series. Only three transplantations from the a-series were successful. After 10 days the result was that on the only two now living chimaeras small blastemes had grown out from the body-segments forming heads with the dorsal side in accordance with the body-segment, and not with the grafted head (fig. 7).

Discussion.

Now two questions will at once arise. 1. What importance can be attached to these results? 2. How can the disagreement with the findings especially of SANTOS (1929) be explained?

In order to discuss these questions it is necessary to review the literature which touches upon these problems.

RAND and BROWN (1926) had 8 successful transplantations on testing the problem whether an implanted head was able to inhibit the formation of a new head in the animal into which the head was grafted. The head of the animal itself was removed after the grafting. In the two experiments new heads were built from the animal despite the implanted head, although the blastemes were removed several times. In two other experiments the blastemes were removed so close to the implanted head that after a time this became the head of the animal. In the other experiments the head of the animal was severed close to that of the graft, and in those cases the graft became the head of the animal.

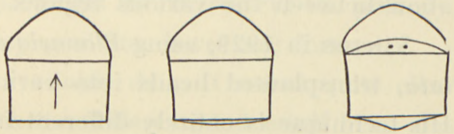


Fig. 7. Exper VI. *Planaria lugubris*. Transplanted head with ventral side upwards, no eyes are therefore seen. Arrow indicates polarity.

It is quite clear that we have here to deal with another problem than that with which we are here concerned. RAND and BROWN's experiments do not involve the problem of reversing the polarity, though there is some similarity in so far as RAND and BROWN deal with the question whether a new, implanted head may be able to replace the original head of a body-segment. And this has been proved to be

the case if only the implanted head comes to lie in the axis of the body into which it is transplanted. This is apparently the case in the experiments of Rand and Brown. So far there can be no question of an organising effect of the head as such. It is to be expected that a sort of reorganisation of the whole chimaera will thereafter take place. But we do not know from what part this reorganisation is started; most probably it takes place by a collaboration between the various regions of the body.

Santos in 1929, using *Planaria dorotocephala* and *maculata*, transplanted heads into various levels of the body. His technique is entirely different from that here recorded. Santos cuts out the part of the head containing the head-ganglion. This piece is inserted into the body in which a corresponding hole, triangular or round, has been made to receive the graft. Santos found that such a graft is capable of reversing the polarity if transplanted post-pharyngeally.

On comparing the findings of Santos with those recorded in this paper it must be emphasised that the techniques employed are entirely different. And this may be the cause of our different results. It is not clearly to be seen, but it seems most probable, that a certain amount of regeneration-blasteme is formed in the place where the head has been grafted. If this is the case, a new set of gradients are undoubtedly set up under the influence of this blasteme. A further complication as to the interpretation of the results of Santos is that *Planaria dorotocephala* at any rate is not the appropriate material to test these questions, because this species forms new heads by spontaneous fission post-pharyngeally.

Steinmann (1933) on *Planaria gonocephala* seems to

have used a similar technique and obtained similar results. But here also the formation of blastemes is likely to have taken place.

Okada and Sugino (1934) do not find any disturbance of the orientation of the host after the implantation of heads. Unfortunately it is not stated how the experiments were carried out.

Spirito (1935) using *Planaria torva* finds that foreparts implanted into the genital tracts do not reserve the polarity, but supplementary pharyngeal structures are formed.

Considering all the available data, it seems quite clear to me that in planarians the differentiated adult head has not the power to organise a grown-up body segment, at least not into the reversal of its polarity. To do this a blasteme has first to be formed. That this is the case has been proved various times, most extensively by Kahl (1935).

It would seem, therefore, that there exists a fundamental difference between the fully differentiated head and the head under differentiation in respect of organising powers.

If this is so, it would be a fact of considerable importance. It would show that only if planarian tissue has the opportunity of forming blastemes, that is, undifferentiated tissue, is it able to organise.

The blastemes would then resemble embryonic tissue in two respects: 1. it differentiates and 2. it influences by a kind of organisation-proces other tissues, and remoulds them.

So far I have not been able to transplant pure blastemes to try their effect in this respect, but all regeneration-experiments on planarians seem to show that this is the case.

Summary.

A new transplantation-technique on planarians is described.

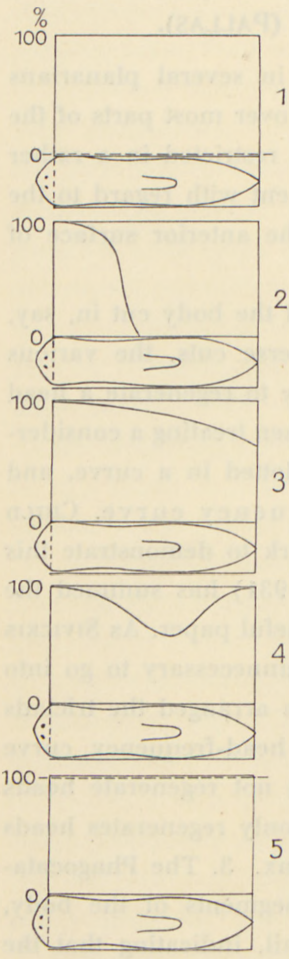
With this technique it is shown that the fully differentiated grown-up planarian head does not act as an organiser when transplanted to other parts of the planarian body with reversed polarity.

II. The Head-Frequency of the Fresh-Water Planarian *Bdellocephala* punctata (PALLAS).

Whereas the regenerative power in several planarians seems to be fairly evenly distributed over most parts of the body, this power is in some species restricted in a rather peculiar way. This is especially evident with regard to the ability to regenerate a head from the anterior surface of transversely cut pieces.

If a planarian be decapitated and the body cut in, say, 6 pieces of equal length by transverse cuts, the various segments will show a different ability to regenerate a head at its anterior surface. The results, when treating a considerable number of animals, may be plotted in a curve, and this curve is termed the headfrequency curve. CHILD and his school have done much work to demonstrate this curve in several species. SIVICKIS (1931) has summed the results up in a very suggestive and useful paper. As SIVICKIS gives a complete bibliography it is unnecessary to go into details. Suffice it to say that SIVICKIS arranged the triclads into 5 groups with regard to the head-frequency curve (fig. 8). 1. The Belloura-group does not regenerate heads at all. 2. The Dendrocoelum-group only regenerates heads from segments anterior to the pharynx. 3. The Phagocata-group regenerates heads from all segments of the body, but the curve slopes towards the tail, indicating that the

ability to regenerate heads from posterior segments is not always present. 4. In the Planaria dorotocephala-group, in which there is a fall in the curve a little beyond the middle of the body, the hind part regenerates heads quite as well as the forepart. It must be remembered that Planaria dorotocephala is able to develop heads spontaneously in the hind part of the body, in this way multiplying by natural fission. 5. In the Planaria velata-group every part of the body is able to produce heads with equal facility. The head-frequency curve is here constant.



The difference in the ability to regenerate heads in planarian species which are of about the same organisation grade is a warning against the too rigid generalisation of the current hypothesis that regenerative power is correlated with and proportionate to the degree of organisation.

It is obvious that the feature is

Fig. 8. Diagram of head-frequency curves of planarians after the grouping of Siwickis (1931). Dotted lines across the heads indicate the cuts separating the heads from the body. The curves indicate the ability of a cut surface, corresponding to each point of the curve, to regenerate a head. 1, the Belloura-group. 2, the Dendrocoelum-group. 3, The Phagocata-group. 4, The Planaria dorotocephala-group. 5, the Planaria velata-group. Dotted lines in 1 and 5 indicate absent (1) and absolute (5) power of regenerating heads.

of fundamental significance for our understanding of regeneration in general. The question is so important because it is likely to throw light upon the problem why some animals regenerate easely, others with difficulty or not at all. But this will not be discussed in this first paper in a series of studies on the phenomenon.

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With regard to other problems of significance for the understanding of regeneration on which I am engaged it was necessary to find a planarian species, the head-frequency of which was of about the same nature as that of the Dendrocoelum-group.

The problem to be solved had to be investigated by means of the transplantation method described before. Dendrocoelum proved unsuited for the purpose, because this animal is very active and had to be narcotised so heavily in order to be kept immovable sufficiently long that too many of the animals died after the operation.

One of the largest species of the European freshwater planarians is *Bdellocephala punctata* (PALLAS). It often attains a length of 30 mm. and a breadth of 5—7 mm. On account of its relatively slow movements, its size and its tolerance of operations this species proved very useful for a larger series of planned experiments. Since the head-frequency curve enters as an important element into the questions investigated in some of the experiments, it was necessary to find out this curve for the species.

The animals were taken in the freshwater-lake, Furesøen, near Copenhagen. Beneath stones by the shore Bdellocephala can be obtained in sufficient numbers throughout the summer and autumn, and if the winter is

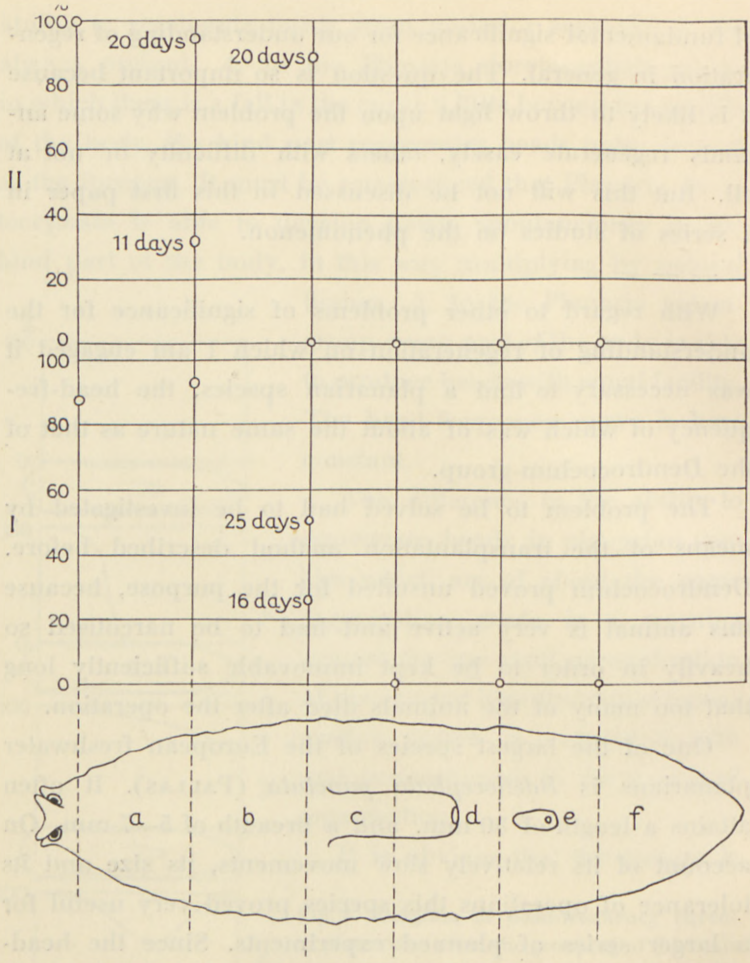


Fig. 9. *Bdellocephala punctata.* Dotted lines indicate cuts made transversely to test the power of various levels of the body to regenerate heads. In I (exper. 258) 86 % of the *a*-segments had regenerated heads after 16 days, the *b*-segments 92 %, the *c*-segments 26 % after 16 days, 52 % after 25 days. The d—f segments did not regenerate heads at all. In II (exper. 259 b) all *a*-segments had regenerated heads after 11 days, the *b*-segments 32 % after 11 days, 94 % after 20 days. The *c*-segments 0 % after 11 days, 89 % after 20 days. d—f segments did not regenerate heads.

mild enough big samples may be collected in March, most of them in process of laying cocoons.

Freshly collected animals were decapitated, the body cut in 6 segments by transverse cuts as indicated in fig. 9 (dotted lines). The result was the same with 20 animals which had been kept starving in the laboratory for a fortnight, and likewise with animals starving for a month or more.

All experiments showed that the ability to regenerate a head was lost in the body at a level just before the pharynx. Fig. 9 gives the result of two experiments, 258 and 259b from the protocol (20 animals in each experiment).

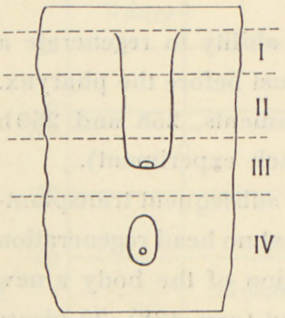
As it was of vital importance for subsequent transplantation experiments to be quite sure that no head regeneration occurred behind the pharyngeal region of the body a new series of experiments were carried out (exp. 428). 20 pieces of Bdellocephala comprising the body just before the beginning of the pharynx to the region behind the genital pore were divided by transverse cuts as indicated in fig. 10, that is: group I had its anterior surface just before the beginning of the pharynx, group II a little behind the beginning of the pharynx, group III just before the mouth, group IV between the mouth and the genital pore. No one of the four groups showed any sign of a regeneration of heads after 20 days, nor later. After the lapse of 20 days all pieces in the fore-region of the body had regenerated heads.

In order to be quite certain that the tail region around the genital pore is also unable to develop heads, a series of experiments were made as indicated in fig. 11 (protocol exp. 430): The cuts were laid just anterior to, II just posterior to the genital pore. No head formation took place.

This makes it certain that the head-frequency curve in Bdellocephala is of the same type as in Dendrocoelum, that

is Sivickis group II. The curve may be drawn as in fig. 12 (full line).

Before closing this brief report I wish to emphasise that the inability to regenerate heads in the hind part of the



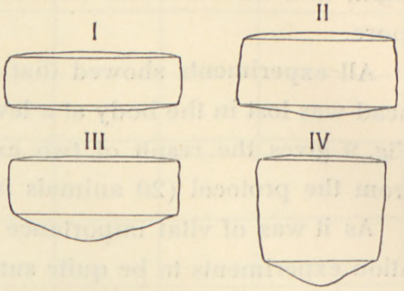
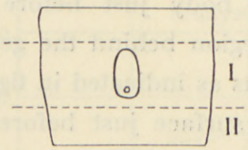


Fig. 10.



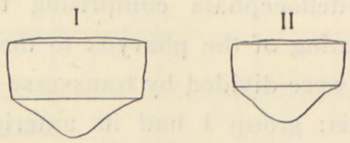


Fig. 11.

Fig. 10. *Bdellocephala.* Dotted lines indicate cuts separating the middle body in segments I—IV. After 18 days no trace of head-formation. Note greater tail-regeneration in IV. — Fig. 11. *Bdellocephala.* The tract around the genital pore. Dotted lines indicate cuts separating segments I and II. No head-formation. Note well regenerated tails.

body is by no means equivalent to loss of regenerative power. On the contrary, the ability to regenerate tails on the hind surface of a segment of the body is undoubtedly greater in segments from the posterior parts of the body (fig. 10, 11).

Whereas it is easy to ascertain the regeneration of heads because of the occurrence of pigmented eyespots, it is much more difficult to tell when a tail is regenerated. This is so

because an undifferentiated regeneration blasteme may easily be confounded with the earlier stages of a tail in regeneration. A sure sign of the regeneration of a tail is that branches of the intestine grow into the blasteme. Using this criterion I have been able with some certainty to ascertain

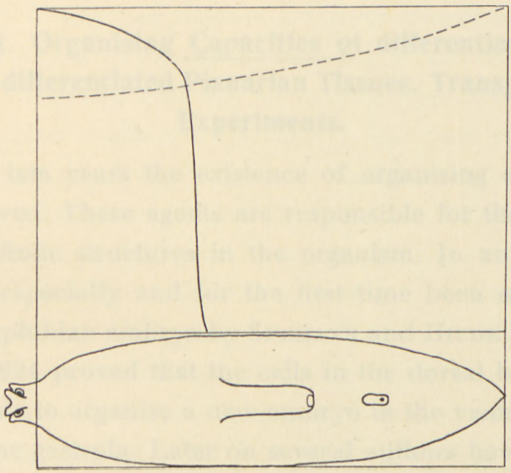


Fig. 12. *Bdellocephala.* Curve in full line: head-frequency curve. Dotted line: approximate tail-frequency curve.

that the ability to regenerate a tail is more pronounced in the posterior segments of the body than in the anterior segments. With some certainty the tail-frequency curve of Bdellocephala may therefore be depicted as in fig. 12 (dotted line).

It may be added that this observation in some measure sustains the hypothesis that in the planarian body there exists two gradients, one for the formation of the head and foreparts, and one for the formation of the tail and hindparts, the first generally sloping from head to tail, the other from tail to head. It is impossible to resists the suggestion

that the results reported here present a striking analogy to the gradient in eggs, e. g. the animal and vegetative gradient in the see-urchin egg as suggested by Runnström (1928) and borne out by him and his school (see e. g. Hörstadius 1935 and Lindahl 1936).

III. Organising Capacities of differentiated and non-differentiated Planarian Tissues. Transplantation Experiments.

1. Of late years the existence of organising agents has been proved. These agents are responsible for the building up of definite structures in the organism. In animals this fact has especially and for the first time been established in the amphibian embryo by SPEMANN and HILDE MANGOLD, who in 1924 proved that the cells in the dorsal blastopore-lip are able to organise a new embryo in the ventral epithelium of the gastrula. Later on several authors have contributed studies on the question (For literature concerning the amphibian embryo see BRØNDSTED 1936. For literature on the whole question of organisation, especially from the biochemical point of view, see J. NEEDHAM 1938).

The question of the nature of the organising agents has for the present come to a standstill. This is because it has been made clear that in all true organising agents we have to reckon not only with trigger-substances from the "organiser", but with agents generated in the reacting cells themselves. And this makes the problem so complicated and intricate that we have to wait for a closer examination of the finest metabolic processes in the cells under organisation before we can advance any further with regard to the chemical nature of the organising substances. In the mean

time it will be well to collect more facts concerning organisation processes on the biological level of research.

2. The processes in tissues under regeneration which have to do with the moulding of the tissues into the shape of the lost parts are directly comparable with processes in embryonic development.

Much evidence has been brought forward in support of the idea that the regeneration blasteme in later stages gives off substances which organise not only the blasteme's own tissues but may also reorganise the whole body. Thus a new head regenerating from a piece of a planarian is able to remould this piece to a complete worm: the well known phenomenon, called by Morgan morphallaxis. Here the head in regeneration is a true organiser. And it is quite conceivable that this influence of the head in the state of regeneration on the body is exercised by means of organising substances.

3. Polarity exists in almost every organism. The polarity of a body displays itself as a dynamic force when regeneration takes place, in so far as the polarity, and therefore the basic shape, of the regenerated bud is determined by the body. We do not know in what way this determination takes place. It may be by diffusing substances or by cells predetermined for the special task. The question therefore arises if this displaying of polarity when a blasteme is stamped is comparable with, say, the displaying of the forces in the blastopore lip. If so, it would be probable that dynamic polarity were due to organising substances.

4. In order to penetrate a little into these obscure problems the following question was raised: If a segment from the fore-part of a planarian which is able to regenerate a head from cuts in the fore-part is transplanted with reversed

polarity to a segment of the hind-part which is not able to regenerate a head, will the transplanted piece in some way induce the hind-part to regenerate a head, when the

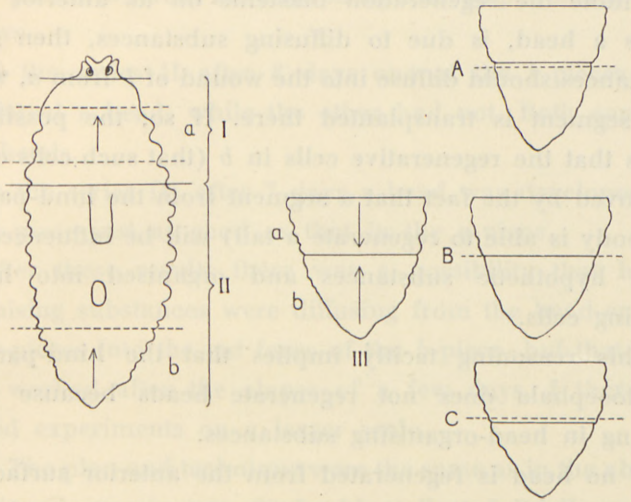


Fig. 13. *Bdellocephala punctata.* I, the part of the body which is able to regenerate heads. II, the part not able to do so. Dotted lines indicate cuts separating the segments *a* and *b*. Arrows indicate polarity. III, indicates the principle of the experiments: segment a is transplanted to the anterior surface of *b*, but with opposite polarity. A, B, C, the three ways of separating *a* from *b*, when these have been held together for some days. Dotted lines indicate cuts.

two parts have been in connection for some time and are then separated?

5. In Bdellocephala punctata I have found a planarian which possesses a head-frequency curve stopping a little before the pharynx. That is to say, only the fore-part of the body is able to regenerate a head. Moreover, this species proving very resistent to operations, my transplantation method could easily be employed on it.

6. The principle of the experiment is indicated in fig. 13. A piece *a* is cut out, transplanted autoplastically to the

piece *b*, but with reversed polarity (fig. 1, III). After a certain time it is again separated from *b*.

7. If the ability of the fore-part (when left to itself) to determine the regeneration blasteme on its anterior face to be a head, is due to diffusing substances, then such substances should diffuse into the wound of *b* from *a*, when this segment is transplanted there. If so, the possibility exists that the regenerative cells in *b* (that such cells exists is proved by the fact that a segment from the hind-part of the body is able to regenerate a tail) will be influenced by these hypothetic substances and organised into head-forming cells.

This reasoning tacitly implies that the hind-part of Bdellocephala does not regenerate heads because it is lacking in head-organising substances.

If no head is regenerated from the anterior surface of piece *b* after removal of the *a*-piece, this may be explained in different ways which will be discussed later.

8. A preliminary experiment was carried out as follows. 20 Bdellocephala which had been kept in the laboratory for about one month without food were handled as indicated above. Only 8 transplantations succeeded, and after 24 hours only 5 were left, the rest having cytolised. Two days after the transplantation the *a*-pieces were cut away as indicated in fig. 13c. I separated the pieces in such a way that the cut was laid in the *a*-piece but so as to leave only a brim of about $^1/_2$ mm. of the *a*-piece on the *b*-piece. By this procedure it is possible to prevent the cut face of the *b*-piece from contracting and closing up. If this takes place, no regeneration whatever occurs.

The separation was undertaken after the pieces had been held together for two days: 2 samples (series I); three days:

2 samples (series II); 4 days: 1 sample (series III). The cut-away *a*-pieces acted as controls.

In the series I after 9 days no head had appeared in the *b*-pieces, whereas the controls had developed heads after 6 days.

In the series II after 8 days one of the *b*-pieces had developed a head, while the other had not. Both controls had heads.

In the series III after 7 days a head was developed by the *b*-piece just as good as that in the *a*-piece.

After these results there was a possibility that head-organising substances were diffusing from the head-end of the *a*-pieces into the cut faces of the *b*-piece, but that they only worked after the elapse of a few days, I therefore started experiments on a larger scale.

9. The plan and technique were the same as in the above-mentioned experiments. 20 freshly collected Bdellocephala were operated on $^{21}/_{3}$-38. Ten of the grafts were successful. $^{22}/_{3}$, 20 Bdellocephala from the same stock were operated, 16 grafts were successful. These 26 grafts were allowed to adhere for 4 days. Then the *a*- and *b*-pieces were separated by cuts made in three fashions, grouped under A, B, C as seen in fig. 13.

In group A the cut was made in the *b*-piece, but as near as possible to the line of coalescense. In group B the cut was made in this line, and in group C it was made in the *a*-piece, but as near as possible to the said line.

The results that emerged were rather interesting.

In group A 8 experiments were made. 15 days after the separation of the *a*- and *b*-pieces all surviving specimens were fixed (Zenker with formol). At that time the control (pieces of other Bdellocephala from the fore-parts of the

body) had regenerated heads; this takes place after 6—8 days. None of the 8 *b*-pieces had regenerated traces of heads, several had a rather fine blasteme, but without any special stamp. Of the *a*-pieces 5 had died at this time. Of the three remaining specimens one had regenerated a fine head on its anterior surface, one had a blasteme, but no head, and

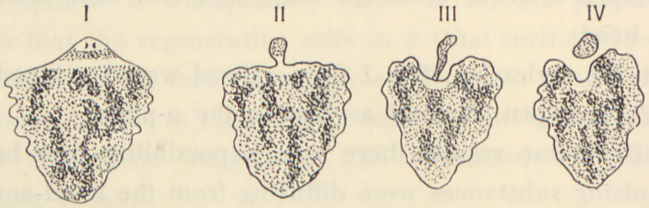


Fig. 14. *Bdellocephala.* 4 *b*-segments cut after modus fig. 13 C. I, a head is formed by the adhering *a*-brim. II—IV, various stages of a-brims being cast off from *b*.

one had no blasteme, but was rather cytolising at its anterior end.

In group B 11 experiments were made. The samples were likewise fixed after 15 days. Of the 11 *b*-pieces, none had heads, though several had beautiful blastemes. Only 5 *a*-pieces were alive. Of these 4 had made heads with eyes on their anterior surface.

Group C, 7 samples. After 15 days 6 *b*-pieces were alive. One had a beautiful head on its anterior surface (fig. 14 I), one had traces of eyes, 4 had no traces of head-building. 3 *a*-pieces were alive. One had a fine head on its anterior surface, one blasteme but without eyes, one cytolised on its anterior surface.

Only one or two *b*-pieces, in group C, had built a head. But on close examination it was quite clear that the head was made of and in the remaining tissues of the *a*-piece. This was also ascertained to be the case with the tail-pieces,

which had formed heads in the preliminary experiments. It will be remembered that in all these experiments the cut was made in the *a*-piece. That this is so can be clearly seen in fig. 14 I, where the light space between the *b*-piece and the head indicates the unpigmented tissue between host and graft.

After this it may be said with fair certainty that no agents from the *a*-pieces penetrate into the *b*-pieces, forcing these

Fig 15. *Bdellocephala*. *a*-segments. I, piece of *b*-brim in course of constriction. III, piece of *b*-brim only loosely adhering to *a*-segment, where *a* blasteme is formed. II, a new head is formed in the *a*-segment in accordance with the polarity, a piece of *b*-brim is pushed aside.

to organise heads. It is therefore in the highest degree improbable that dynamic polarity displays itself in the same manner as, say, the organising blastopore-lip, i. e. with organising substances.

10. Most of the pieces, after having been separated, had some tissue of the other piece adhering to them. This is of course the case in group A, where the *a*-pieces had some of the *b*-tissues fastened to them, because the cut was made in the *b*-piece. And so also—vice versa—in group C. In the B-group the line of coalescense was in most instances somewhat irregular so that the cut was not able to separate the two pieces cleanly.

The adherent tissue showed two distinct modes of behaviour. In some cases the strip of *a*-tissue covered the anterior surface of the *b*-pieces, and in one or two cases when *b*-tissue covered the surface of the *a*-wound, the

foreign tissue adhered so tightly and covered the wound so completely that no regeneration-blasteme was formed from the bigger piece.

It is very interesting to note that a very small brim of tissue from another part of the body is able to block the regenerating-powers of the piece on which it is grafted. This is shown in fig. 16, where a brim of tail tissue prevents the *a*-segment from forming head on its anterior surface.

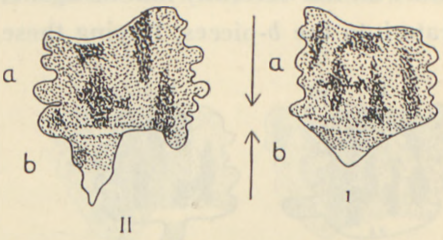


Fig. 16. *Bdellocephala*. I, an *a*-segment with *b*-brim, which covers the whole wound-surface. II, same, but *b*-brim only covering half of the wound-surface of *a*. Both *b*-brims are developing blastemes on their free (hind) surface, tending to become tail. The light spaces between *a* and *b* indicate unpigmented tissue on the border-line of the two segments.

But whereas the *b*-brim on an *a*-piece inhibit all regeneration from *a*, it is itself able, if big enough, to regenerate tail-shaped buildings (fig. 16 I, II). And an *a*-brim on a *b*-piece is itself able to build up a head, as we have seen. It should, however, be noted that this brim forms its head opposite to its own inherent polarity. That this phenomenon is certainly not due to directing forces from the *b*-piece is evident from the fact that very short pieces of planarians by Sivickis (1930) were shown to be able to develop heads on both anterior and posterior surfaces. It has by Morgan likewise long ago been demonstrated that very short head-pieces and tail-pieces sometimes regenerate their own doubles, "Janus-heads".

In other cases, the most numerous, the adherent strips gradually drew themselves together into rounded appendices

on the surface of the pieces to which they were grafted, eventually pushed aside by the growing blasteme (fig. 15 II). The connection between these lobes and the main-piece is gradually loosened, and in some of the samples the bases of the lobes were quite constricted and the lobes in a few cases had fallen off when the fixation took place. Several instances are shown in figs. 14 II—IV and 15 I—III.

This makes it clear that the remaining brim of tissue from the grafted but again separated piece is treated as foreign by the other piece, though it comes from the same animal, thus giving evidence of a chemical difference in the different parts of the body. But the experiments supply further evidence.

11. Why is the brim not always cast away? It is found that the brim-tissue is rolled off only when a blasteme is formed by the main piece. Now, is the blasteme formed because space is made for it by the active rolling off of the foreign tissue, or is the brim forced away by the blasteme? It has never been observed that the brim is fastened onto the blasteme. It is of course hard to say which is the effect and which is the cause. Perhaps the histological investigations will furnish the answer. But at the outset I should think that the formation of a blasteme is the primary cause of the pushing aside of the foreign tissue, for a clear line of demarcation is always seen between the blasteme and the lobes. And why should the brim not actively roll itself off when it covers the wound completely?

12. To serve as a control to these experiments the following were made. 10 Bdellocephala were decapitated on a level with the anterior surface of the *a*-pieces. Small blocks of sterilised 3 % agar (fig. 17 I) were laid firmly to the cut surface on Scotté-tables, the whole thing then

put into the cool room. The same day 5 Bdellocephala were transversally cut on a level with the anterior surface of the *b*-pieces (fig. 5 II). Agar-blocks were fixed to the wounds in just the same fashion as in the first experiment. This was to serve as a control for the following experiment.

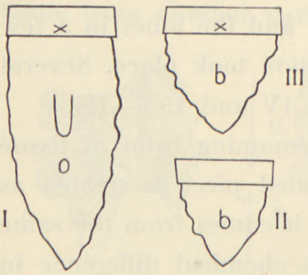


Fig. 17. *Bdellocephala*, I, agar-block firmly attached to wound made in the anterior part of the body. II, agar-block attached to a *b*-segment (tail), serving as a control to III: agar-block from I attached to wound of fresh *b*-segment to test whether hypothetical head-forming substances possibly taken up by the agar from I, are again given off in III to *b*-segments.

5 agar-blocks which had for 24 hours adhered to the cuts representing anterior surfaces of *a*-pieces, were now pressed against the wounds of 5 new *b*-pieces (fig. 17 III). If the agar-blocks had been imbued with head-forming substances, there would be a possibility that such substances would diffuse into the *b*-pieces and there act as organisers.

The agar-blocks were fixed to the *b*-pieces for two days and then removed. After 17 days the *b*-pieces were fixed. Neither these nor the 5 controls had formed heads in the blastemes.

Discussion.

The fact that the fore-part of the *b*-pieces does not react with head-formation in the blasteme which is built on the cut surface after the removal of the *a*-pieces may of course be explained in two essentially different ways.

1. The *b*-piece is unable either to suck up headforming

substances from *a*, or unable to respond to the summons of such substances after these have been sucked up.

2. The *b*-pieces do not react owing to the simple fact that no headforming substances are given off from the *a*-pieces.

We have to discern between these two possibilities.

My experiments on the possible organising capacities of the adult planarian head have shown that adult tissue does not react by reorganisation to possible influences from this head.

Only if a blasteme is formed is this in some way determined in accordance with the polarity or rather the possibilities (e. g. in the very short segments, where a head may be formed opposite to the natural polarity of the segment) of the adult pieces from which it is regenerated. Then the blasteme in its turn gives off forces which reorganise the adult tissues themselves.

Now, when piece *a* with reversed polarity is grafted into piece *b*, no formation of a blasteme takes place. Therefore no available tissue is able to respond to possible influences from either *a* or *b*. And the experiments show that the adult tissue does not react to eventually present summons from other adult tissue.

It therefore seems most probable that we have to look upon regeneration in planarians in different way from that usually adopted. It is commonly assumed that the various parts of the adult planarian body are able to organise, in fact it has been stressed (e. g. HUXLEY and DE BEER 1934) that the head of the planarian is equivalent to, say, the organiser of the amphibian embryo.

After the results of the transplantation experiments here presented, I think that the following working hypothesis may be formed.

Organising capacities in the adult planarian body are present only in a masked or latent manner. They can unfold themselves only when a blasteme is formed, and only in this blasteme, not in fully differentiated tissues. The blasteme is of course made of cells from the grown-up body itself, but only when the cells in the blasteme have been together for some unknown time are they made sensible to influences from the adult body, and then according to these influences determined in accordance with the polarity and capacities of said body. But then the blasteme takes the lead, and besides differentiating itself, gives off organising influences to the body, so strongly marked that they are able to remould the adult tissues (morphallaxis).

Therefore, only in the blasteme must we look for competent (Waddington) tissue, and for tissue containing organising forces comparable with the organisers in embryonic development. Adult tissue on the contrary is only competent when under the influence of forces generated in the blasteme.

So far, it has been impossible, with the new method, to transplant pure blastemes. I hope in the future to be able to develop a more refined technique, which should be able definitely to settle the question.

Further, and very strong, evidence that the formation of a blasteme is necessary for the rebuilding of the whole organism is afforded by the fact that when a wound is closed up, so that no blasteme is formed, no morphallaxis takes place. The forces necessary for morphallaxis therefore must be generated in the blasteme.

All this suggests that in the fully differentiated adult organism we have to deal with inhibiting factors controlling

the interaction of the various organs and cells. In the regenerating organism "organising" forces control the building up of the animal until a new level of interaction between the organs has been established.

If this idea be accepted, it is easily understood that the differentiated adult tissues do not exercise any organising influence on other adult tissue, as has now been shown for the head of *Planaria lugubris* and body-segments of *Bdellocephala punctata*.

Finally I should like to say a few words concerning the curious phenomenon of the rolling off of the tissue-brim grafted from another part of the body. When a blasteme is formed at the place of junction between the two grafted pieces, then the foreign tissues are pushed aside. Here again it seems that the blasteme has some qualities which are not present in the adult tissue.

Summary.

1. The head-frequency curve of Bdellocephala punctata is made out. Only the part of the body before the pharynx is able to regenerate a head.

2. The hind-part of the body is able to regenerate a tail with more energy than the fore-part; thus there also exists a tail-frequency curve.

3. Segments from the fore-part of the body of the planarian Bdellocephala punctata, which are able to regenerate heads on their anterior cut surface, are transplanted with the anterior surface to the anterior surface of tail-segments, which are not able to regenerate heads. This was done to test the question, whether headforming powers penetrate into the tail-pieces, forcing these to regenerate heads. The

segments were cut from the tails after they had been attached to them for a few days. The tails never regenerated heads.

4. If strips of the segments adhere to the tail after the removal, the strips may, if big enough, regenerate heads with a polarity in a reversed direction to the polarity of the segment itself.

5. Small strips either of segment or tail, or strips of tail-tissue on segments may inhibit further regeneration, if they cover the whole cut surface.

6. If the wounds on the anterior surface of either tail or segment are not closely covered by the strips of the other piece, a blasteme is formed, and then the strips are pushed aside and lastly cast off, being then treated as foreign tissue.

7. These results are discussed and their value for a working hypothesis concerning regeneration pointed out.

(Experimental-zoologiska avdelningen, Stockholms Högskola and Carlsbergfondets biologiske Institut).

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ON THE ORIGIN OF SOME ANGIOSPERMS THROUGH THE *GNETALES* AND THE *CONIFERAE*

IV. THE GYNAECIUM OF *PERSONATAE*

BY

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I. Introduction.

In my earlier phylogenetic works rather primitive phanerogams only were examined. In the present investigation, however, which is a direct continuation of the preceding ones, an attempt will be made to demonstrate that the results previously gained may be generalised so as to apply also to such a highly developed group of phanerogams as the *Personatae*. In this demonstration teratological features will be given special weight, which, again, is due to my having received from Dr. A. LEVAN a very valuable material of *Petunia*, for which I here beg Dr. LEVAN to accept my cordial thanks.

Owing to the primitive technique of earlier times the great morphologists of the last century, EICHLER, PAYER, VAN TIEGHEM, ČELAKOVSKÝ, PEYRITSCH, and many others, did not succeed in elucidating the morphology of the gynaecium. And the animated discussions then carried on gradually ebbed out for want of proofs.

However, with the technical progress of recent years the discussion has been resumed, very different views being set forth by numerous investigators, as for instance MCLEAN THOMPSON, GRÉGOIRE, TROLL, KOZO-POLJANSKI, JUHNKE and WINKLER, PARKIN, EAMES, SAUNDERS, HENNING, HUNT, STEFANOFF, RISCHKOV, THOMAS, HAGERUP, and several others. A good summary of the results of this debate will

be found for instance in GRÉGOIRE's comprehensive treatment of the literature, to which work it will suffice here to refer the reader.

It should merely be noted that there are, in particular, two different opinions sharply opposed to each other, whose main points may be briefly characterised as follows:

1) The gynaecium consists almost exclusively of leaves (carpels). This "classical theory", which originates from the last century (GOETHE), especially has its adherents among German scientists.

2) The gynaecium further includes larger or smaller parts of the floral axis.

For the purpose of forming an opinion on the greatly diverging views two methods especially will be adopted in the present work, viz. the histological and the teratological method.

It was during my study of the gynaecium of *Salix* that I realised the value of the histological examination of the various developmental stages of the gynaecium. In the present work this method has therefore been employed on a number of selected objects within the *Personatae*, which I have collected partly on various journeys in the Indies and Africa, partly under natural conditions in Denmark and in the Botanical Garden of the University of Copenhagen. The young flowers were cut into series of thin sections.

The *Personatae* are valuable as objects of investigation, because the gynaecia of the different families are very differently built. Thus in most of them the placenta is central, while in *Gesneriaceae* and *Orobanchaceae* the placenta is parietal. In most families the ovary contains septa; however, as is well known, a unilocular gynaecium is found in the *Lentibulariaceae* and the *Gesneriaceae*. Some selected

types of gynaecia will be examined below, special attention being given to the placenta and ovules, as I consider the "classical" interpretation of these organs incorrect. On the other hand, there is hardly any reason to doubt that an essential part of the carpels has been formed of leaves; this appears distinctly from their organogeny, as is especially shown in TROLL's most recent works (1939).

II. The Organogeny of the Gynaecium.

Among the various forms of gynaecia found within the *Personatae*, it is easiest to examine and understand such as have a central placenta; and among these, again, we first select a unilocular gynaecium, and in the first example will confine ourselves—for the sake of clarity—to outline drawings of a series of developmental stages (Figs. 1—6).

a. *Utricularia minor* L.

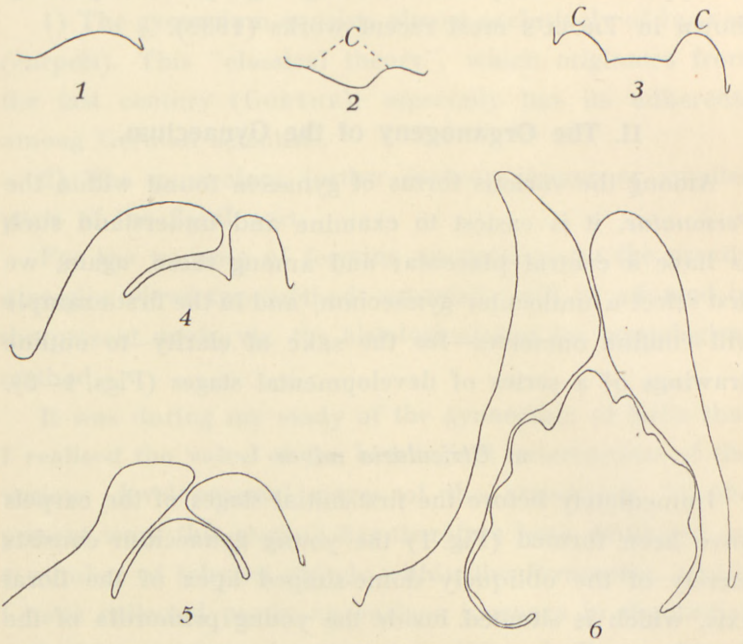
Immediately before the first initial stages of the carpels have been formed (Fig. 1) the young gynaecium consists merely of the obliquely dome-shaped apex of the floral axis, which is situated inside the young primordia of the stamens. Figs. 2 and 3 show very young initial stages of the carpels (C); they do not cover the whole apex of the stem, of which a distinct remnant is left; this remnant will continue to grow, increasing both in length and breadth (Fig. 4—5), to develop finally (Fig. 6) into the placenta. Accordingly the young placenta is the oldest part of the gynaecium, and the carpels begin as lateral leaves on the placenta.

In the following will be given, as a supplement, a histological analysis of the organogeny of some other gynaecia

which behave in the main like *Utricularia* and are provided with a central placenta.

b. *Plantago major L.*

Fig. 7 represents a median longitudinal section through the growing point of a quite young flower stalk; nearly

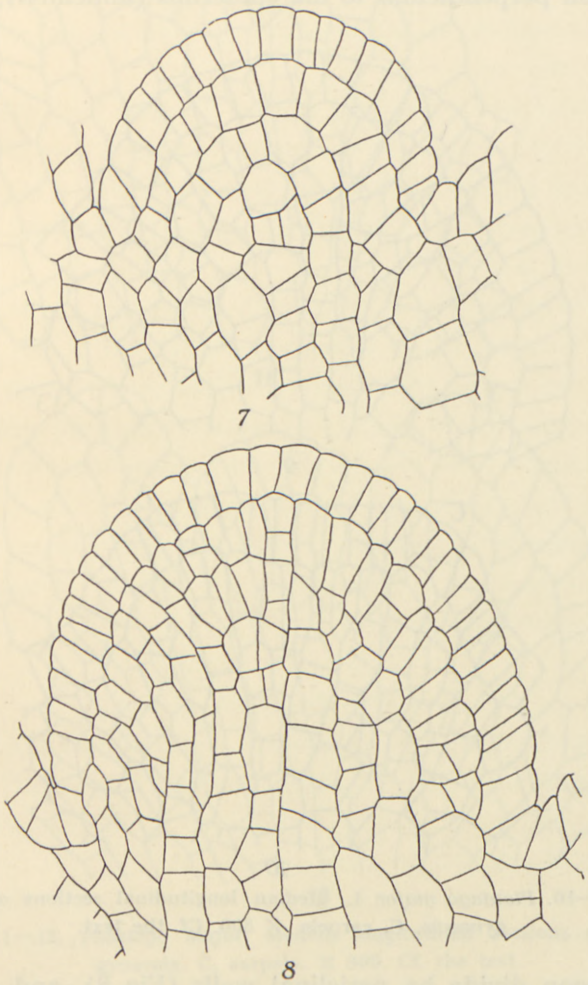


Figs. 1—6. *Utricularia minor* L. The organogeny of the gynaecium in median longitudinal sections. × 200. C, carpels. Cf. further the text.

three mantle-shaped cell layers are seen, one outside the other and parallel to the epidermis. The innermost central cell-rows do not reach the epidermis; and on the whole this growing point is built in a similar way to other stem apices in phanerogams.

However, during the further development of the young flower something happens which morphologically is very curious: the typical apical growth shown in Fig. 7, as it

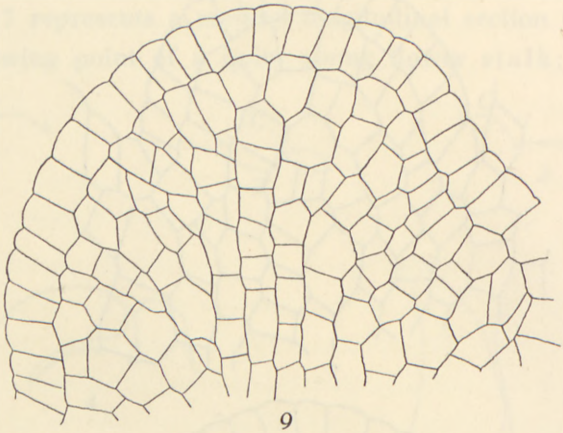
were ceases, the newly formed cells being situated in a way different from the usual one. This feature is shown



Figs. 7—8. *Plantago major* L. Median longitudinal sections of very young flowers. × 800. Cf. the text.

in Fig. 8: in the innermost and lowermost parts of the section figured the primary arrangement of the cells as

shown in Fig. 7 is still plainly distinguished. However, the cells of the subepidermal layer (Fig. 7) have elongated in a direction perpendicular to the epidermis (anticlinal); these



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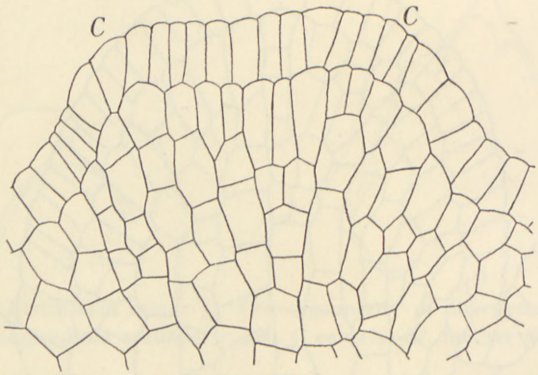

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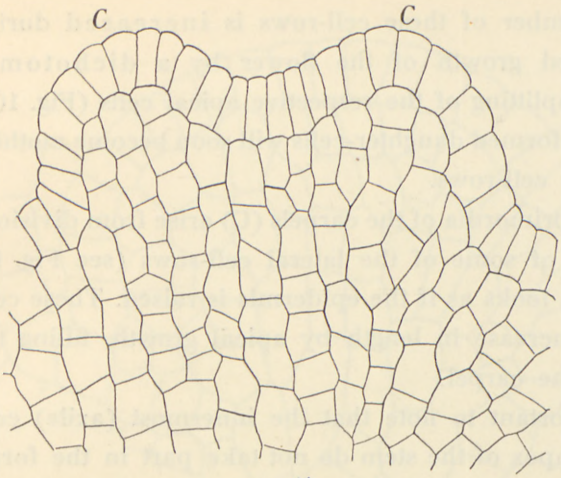
Figs. 9—10. *Plantago major* L. Median longitudinal sections of young gynaecia. C, carpels. × 800. Cf. the text.

cells soon divide by periclinal walls (Fig. 8), and during the continued growth (Figs. 9—12) the subepidermal cells (Fig. 7) will become mother cells of veritable cell-rows, which expand sideways as in a fan and are perpendicular to the epidermis.



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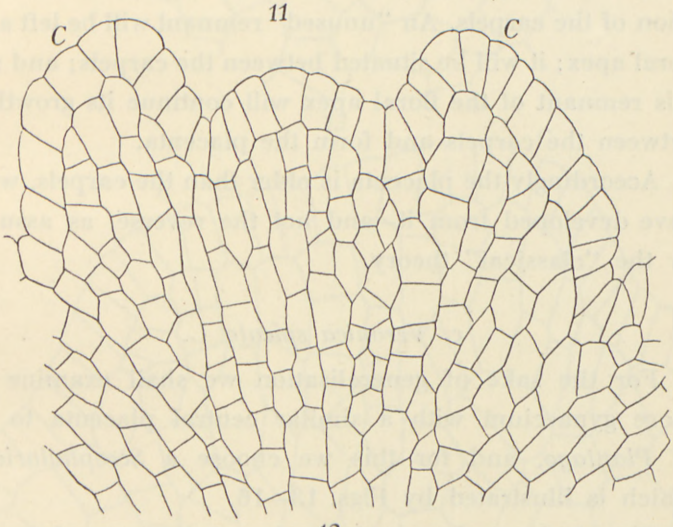

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Figs. 11—12. *Plantago major*. Median longitudinal sections of young gynaecia. C, carpels. × 800. Cf. the text.

In the succeeding developmental stages (Fig. 9—12) no cell layer parallel to the epidermis is found, all cells being arranged in rows perpendicular to the epidermis, as in the growing points of certain algae (e. g. *Furcellaria*).

And the number of these cell-rows is increased during the continued growth of the flower by a dichotomal (anticlinal) splitting of the respective apical cells (Fig. 10); and the new-formed daughter-cells will soon become mother-cells of new cell-rows.

The first primordia of the carpels (C) arise from divisions at the apex of some of the lateral cell-rows (see Fig. 10, right), and it looks as if the epidermis is raised. These cell-rows soon increase in length by apical growth, filling the interior of the carpel.

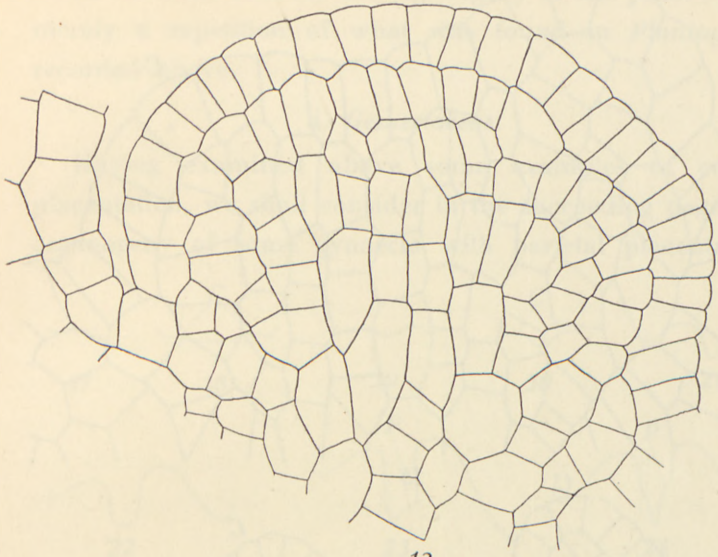
It is important to note that the innermost (axile) cell-rows of the apex of the stem do not take part in the formation of the carpels. An "unused" remnant will be left at the floral apex; it will be situated between the carpels; and soon this remnant of the floral apex will continue its growth up between the carpels and form the placenta.

Accordingly the placenta is older than the carpels, which have developed from it—and not the reverse, as assumed by the "classical" theory.

c. *Veronica spicata* L.

For the sake of generalisation we shall examine one more gynaecium with a similar central placenta to that of *Plantago*, and for this we choose a *Scrophulariacea*, which is illustrated by Figs. 13—16.

Fig. 13 shows the growing point of the apex of the axis of a flower so young that the primordia of the carpels have not yet been formed. It will be seen that here, too, the stem apex is built up of dichotomally branched cell-rows spreading sideways like fans. The carpels arise (Fig. 14) from the apex of some of the lateral cell-rows growing obliquely upwards, raising the epidermis from its original position.



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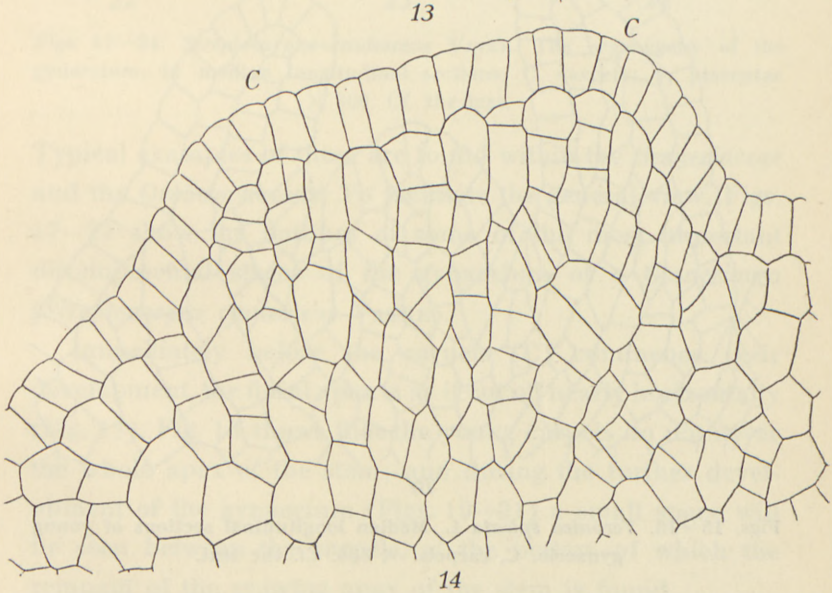

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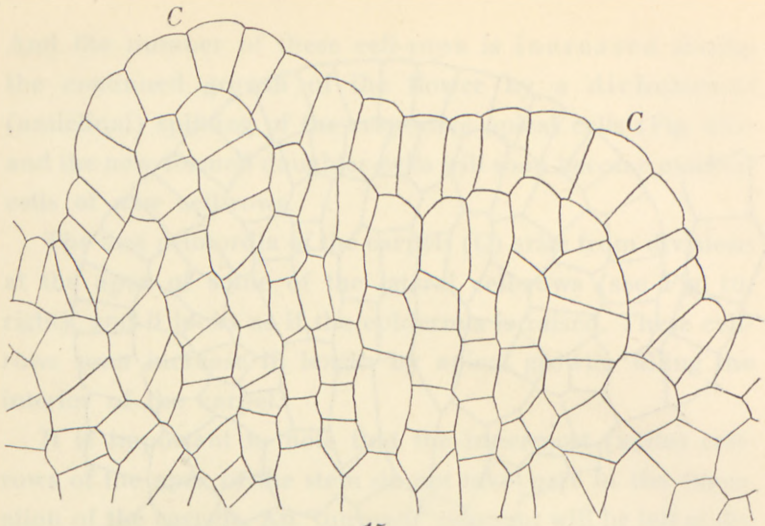
Figs. 13—14. *Veronica spicata* L. Median longitudinal sections of very young gynaecia. C, carpels. × 800. Cf. the text.



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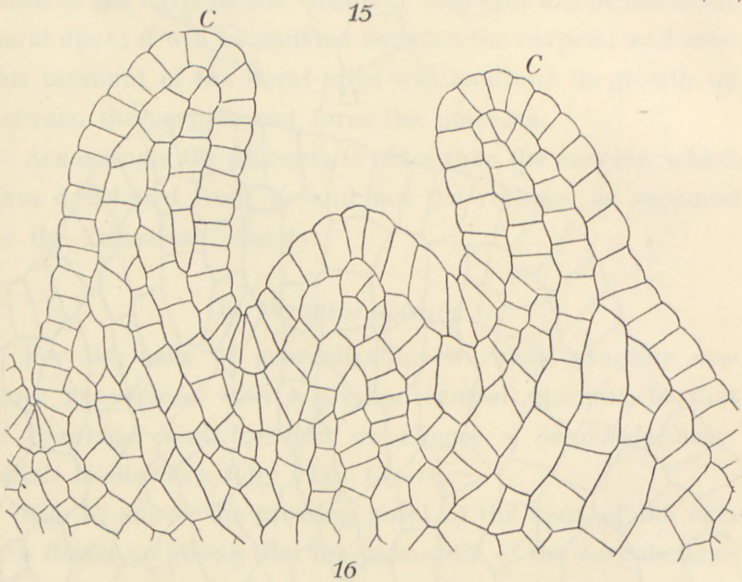

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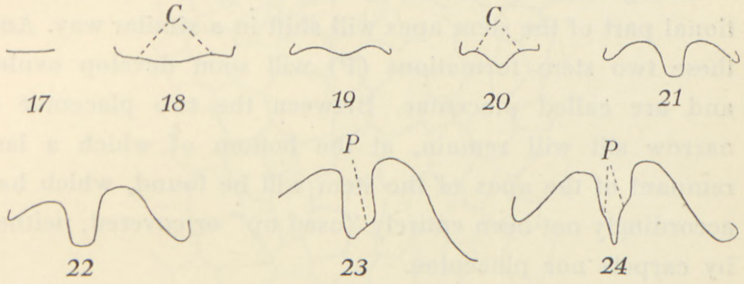
Figs. 15—16. *Veronica spicata* L. Median longitudinal sections of young gynaecia. C, carpels. × 800. Cf. the text.

Soon that part of the stem apex which is situated between the carpels will grow up between the latter, forming the placenta (Figs. 15—16).

In the main features the organogeny of this plant is thus merely a repetition of what was found in *Plantago* as recorded above.

d. *Gesneriaceae.*

Having examined above some examples of central placentation, we shall consider in the succeeding pages the organogeny of some gynaecia with parietal placentation.



Figs. 17—24. *Streptocarpus caulescens* VATKE. The organogeny of the gynaecium, in median longitudinal sections. C, carpels; P, placentae × 200. Cf. the text.

Typical examples of these are found within the *Gesneriaceae* and the *Orobanchaceae*. To facilitate the general view, Figs. 17—24 show the outlines of some of the most important developmental stages of the gynaecium of a *Gesneriacea* (*Streptocarpus caulescens* VATKE).

Immediately before the carpels (C) commence their development, the floral apex is as if cut off nearly horizontally (Fig. 17). Fig. 18 shows that the young carpels do not cover the whole apex of the stem; and during the further development of the gynaecium (Figs. 19—21) a small space will be seen between the carpels, at the bottom of which the remnant of the growing apex of the stem is found.

If we compare Figs. 22 and 23, it will be seen that the apex of the stem enclosed between the carpels has become

oblique because it has grown a little upwards on its right side. This peculiar growth will constantly continue, and since at the same time the carpels grow by an intercalary zone across their base, the result will be that the aforementioned oblique part of the apex of the stem will be shifted upwards along the inner side of the carpel, so that the characteristic parietal position will result.

Along the inner side of the other carpel, also, an additional part of the stem apex will shift in a similar way. And these two stem formations (P) will soon develop ovules and are called placentae. Between the two placentae a narrow slit will remain, at the bottom of which a last remnant of the apex of the stem will be found, which has accordingly not been entirely "used up" or covered, neither by carpels nor placentae.

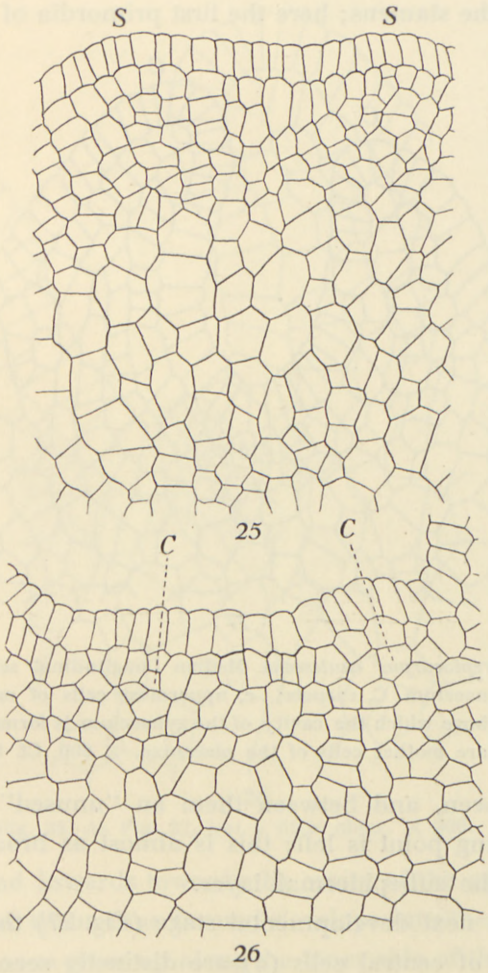
However, after these introductory remarks we shall give below a more detailed histological analysis of the peculiar organogenetic phenomena, and for this purpose we select the same species.

Fig. 25 represents a median longitudinal section through a flower, so young that only the first tiny primordia of the stamens (S) are distinguishable. From the portion of the stem apex situated between them the gynaecium will later develop.

In the middle lower part of Fig. 25 some vertical cell-rows are seen which show part of the original arrangement of the tissue of the young flower stalk. However, it appears from the uppermost left part of the phase figured here that below the epidermis cell-rows in fan-shaped arrangement are present, similar to those characteristic also of the young gynaecia with central placentae described above.

Now the apex of the floral axis increases in thickness

(Fig. 26), which causes fresh alterations in the arrangement of the tissue; i. a. there gradually develop some more or



Figs. 25—26. *Streptocarpus caulescens.* Median longitudinal sections through the apex of young flowers. S, stamens; C, carpels. × 600. Cf. the text.

less distinct cell-rows which are parallel to the epidermis; this applies especially to the subepidermal layer, in which

the other organs of the gynaecium are initiated, as will be seen in the following figures.

Fig. 26 represents the part of the stem apex that is found between the stamens; here the first primordia of the carpels

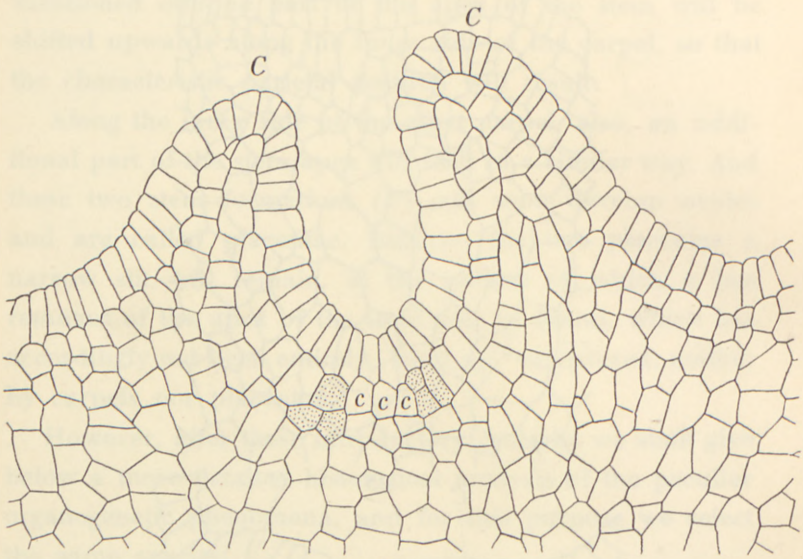


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Fig. 27. *Streptocarpus caulescens.* Median Longitudinal section through a young gynaecium. C, carpels; c, uppermost cells of median vertical cell-rows, above which the cavity of the gynaecium is formed. The dotted cells are mother cells of the placentae. × 600. Cf. the text.

(C) are seen, and between them an "unused" portion of the growing point is left; this is almost as broad as seven cells of the subepidermal layer.

In the next developmental stage (Fig. 27) three vertical cell-rows of central cells (c) are distinctly seen, extending upwards to the epidermis and continuing downwards into the axile rows of medullary cells of the flower stalk. The cells of the subepidermal layer situated nearest to the said axile cell-rows are dotted in the next figures, and it soon

becomes evident (Figs. 27—29) that these (dotted) cells divide rapidly by periclinal walls so as to become the mother-cells of cell-rows which are directed obliquely

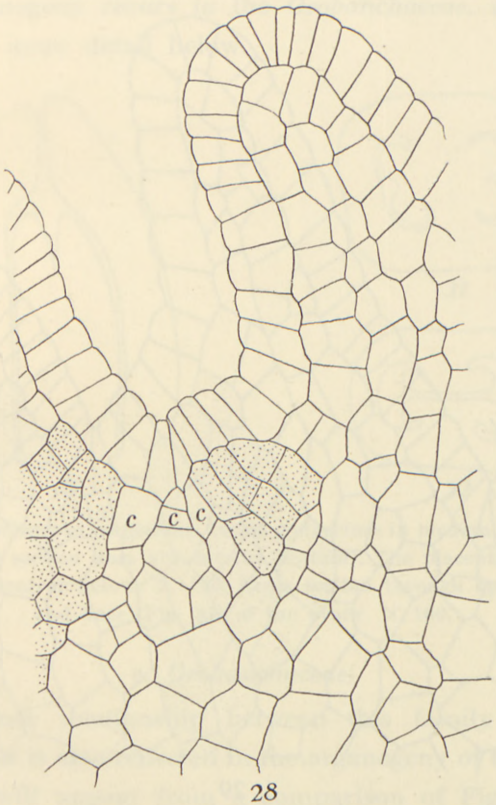

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Fig. 28. As Fig. 27, but a little older. × 800.

upwards and inwards towards the cavity of the gynaecium. The mass of tissue thus formed develops into the placenta, which will soon grow up along the inner side of the carpels. The divisions in the placentae continue, and new cell-rows are initiated by anticlinal divisions in the subepidermal layer.

The axile cell-rows also (c) continue to divide for some time yet, though at a slower rate than in the surrounding

tissue (dotted in the figures). As a consequence of this peculiar unequal growth the apex of the stem will be cleaved, as shown in Fig. 30. This, again, is distinctly seen in cross

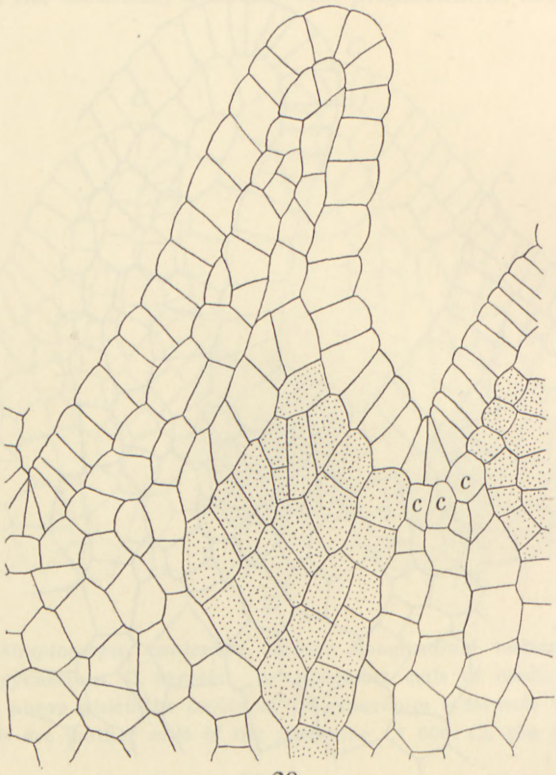

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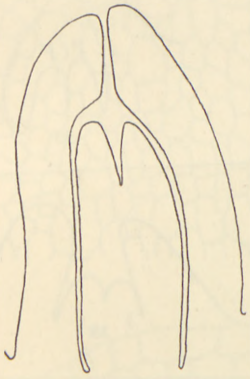
Fig. 29. As Fig. 27, but somewhat older. × 800.

sections, which show that in the upper part of the ovary the placentation is parietal (Fig. 32); but in the lower part there is a central placenta as in most other *Personatae* (Fig. 31).

For the sake of generalisation we show in Figs. 33—35 and Figs. 36—38 some of the most important developmental stages of the gynaecium of two other *Gesneriaceae*.

And it will be seen that the carpels (C) and the placenta (P) are developed in a way which corresponds in the main features to that found above in *Streptocarpus* (Figs. 17—24). This organogeny recurs in the *Orobanchaceae*, as will be shown in more detail below.



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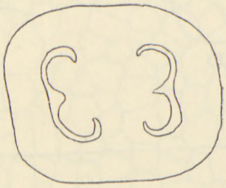

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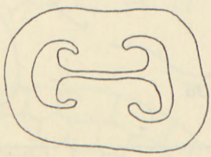

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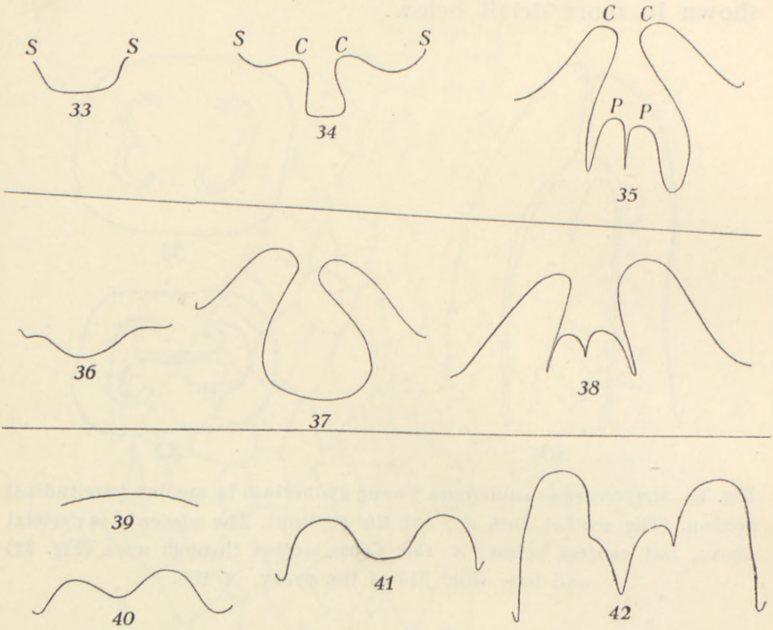
Fig. 30. *Streptocarpus caulescens*. Young gynaecium in median longitudinal section. (The section does not cut the septum). The placenta is parietal above, but central below. × 150. Cross section through apex (Fig. 32) and base (Fig. 31) of the ovary. × 100.

e. *Orobanchaceae.*

The close relationship between this family and the *Gesneriaceae* is also reflected in the organogeny of the gynaecium, as will appear from a comparison of Figs. 33—42. Of these, Figs. 39—42 have been drawn from sections through young gynaecia of *Orobanche lucorum*.

Immediately before the carpels are initiated (Fig. 39), the growing point of the flower is only slightly convex. This stem apex is not entirely covered by the young carpels (Fig. 41), but a portion of the central part of the growing apex will remain at the bottom of the cavity of the gynaecium. This portion of the stem will increase somewhat in

thickness, and soon it will also grow in length and "move upwards" along the inner side of the carpels. This young placenta will be deeply cleaved because some few of the axile cell-rows of the floral axis cease to grow (cf. Fig. 46).

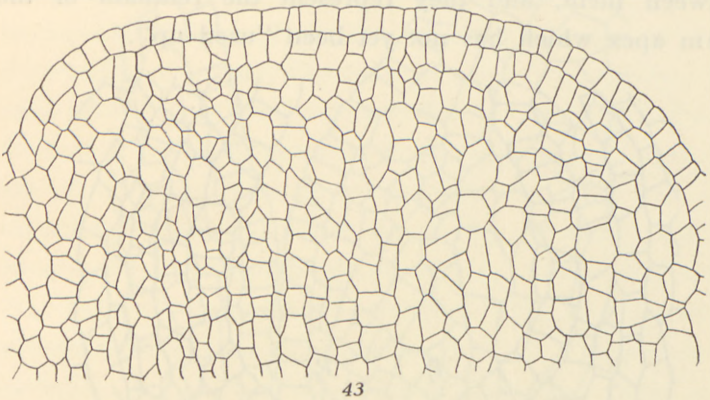


Figs. 33—35. The organogeny of the gynaecium in *Achimenes candida* Lindl. in median longitudinal sections. S, stamens; C, carpels; P, placentae. × 160. — Figs. 36—38. *Smithiantha zebrina* (Paxt.). × 160. — Figs. 39—42. *Orobanche lucorum* A. Br. × 80.

Figs. 43—47 give histological details of some of the most important developmental stages of another *Orobanchacea* (*Aeginetia indica*) which I brought home from the Indies (Sumatra).

It will be seen that immediately before the corolla is initiated (Fig. 43) the cells of the growing point are not arranged in layers parallel to the epidermis, as in the growing points of ordinary vegetative stems. In the lower

middle part of the figure, however, some short vertical cell-rows are seen which were formed at the time the



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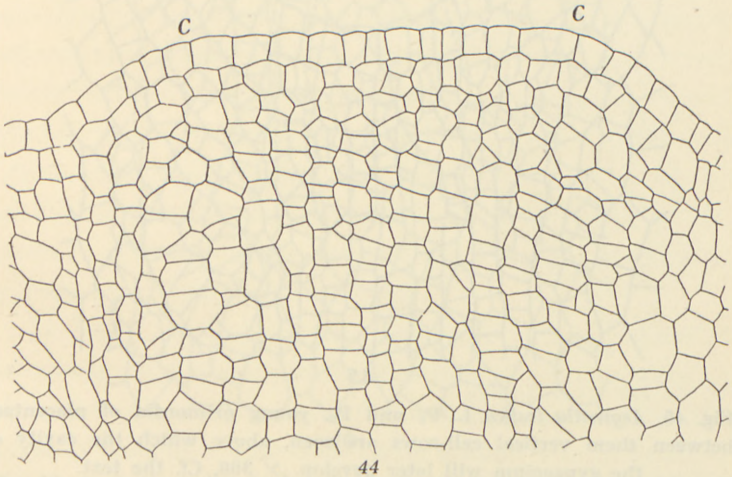

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Figs. 43—44. *Aeginetia indica* L. Median longitudinal sections through young flowers, Fig. 43 immediately before the corolla is initiated. C, carpels. × 240. Cf. the text.

flower stalk was growing by a typical vegetative growing point. The cells subsequently formed (Fig. 43, top) are arranged in irregular fan-shaped cell-rows almost perpendicular to the epidermis.

The carpels (C in Fig. 44) arise in a distinctly lateral position; many (about 6—8) vertical cell rows are situated between them, and they represent the remnant of the stem apex which has not yet been "used up".

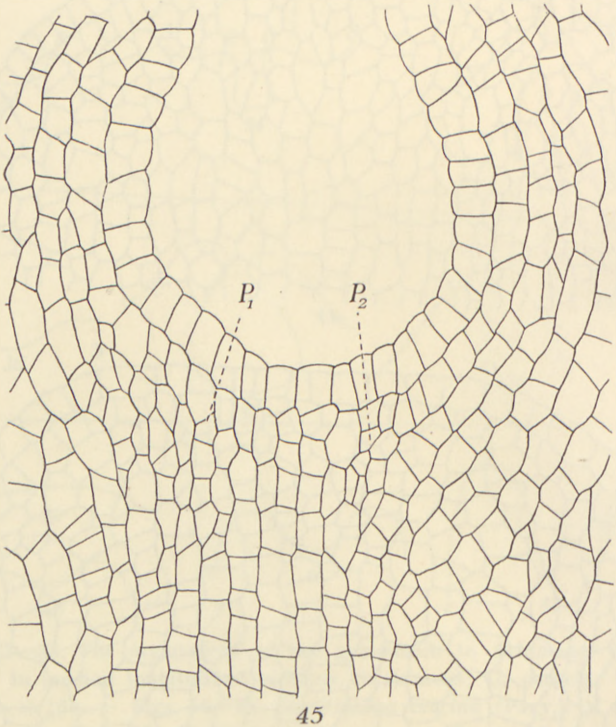

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Fig. 45. *Aeginetia indica* L. P_1 and P_2, young primordia of placentae; between them vertical cell-rows are seen, above which the cavity of the gynaecium will later develop. × 300. Cf. the text.

Fig. 45 shows a still older stage, in which the axile vertical cell-rows are distinctly seen; above they reach right up to the epidermis at the bottom of the cavity of the gynaecium. And a microscopical examination of the whole section (not drawn in Fig. 45) will show that these cell-rows continue downwards into the medulla of the flower stalk.

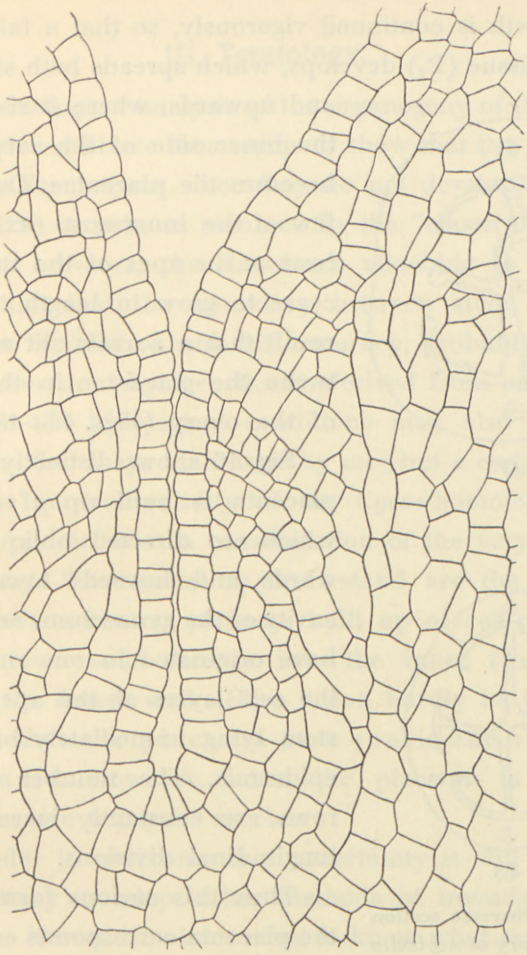

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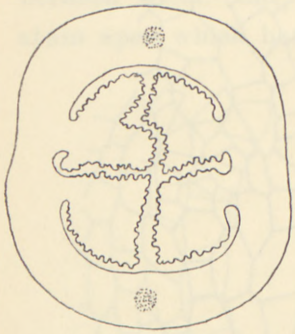
Fig. 46. *Aeginetia indica* L. Longitudinal section through young placentae formed of cell-rows which are directed obliquely upwards and inwards. × 300.

It is of special interest to note the place where the placenta is originally initiated. It will be seen (at P_2 in Fig. 45) that several cell divisions have taken place in the outermost part of the remaining portion of the stem apex.

This growth is continued vigorously, so that a fairly large mass of tissue (P_2) develops, which spreads both sidewards and upwards, where it rises along the inner side of the carpels and becomes the placentae. Only some few of the innermost (axile) cell-rows at the apex of the stem soon cease to grow in length, with the result that a narrow slit arises between the placentae in the centre of the ovary (Figs. 46—48).



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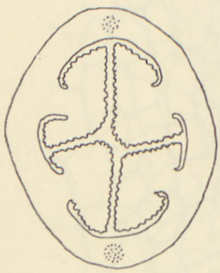

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Fig. 47. Transverse section of young ovary of *Aeginetia indica*. × 20. — Fig. 48. *Cistanche lutea* Lk. et Hoffm. Transverse section of young ovary, showing four parietal placentae on the lamina of the two carpels. × 20.

Fig. 46 shows distinctly that the placenta is built up of cell-rows which are directed obliquely upwards and inwards towards the cavity of the gynaecium, and which have originated in one or two of the cell layers at the apex of the stem lying immediately below the epidermis. The number of these rows are constantly increased by longitudinal divisions.

Thus this curious formation of the placenta corresponds entirely to that found above in the *Gesneriaceae*, except that in *Aeginetia* (Fig. 47) there are four placentae, and they are not situated on the place where the edges of the carpels are joined together, but some distance out on the lamina. This is even more distinct in *Cistanche* (Fig. 48)—which shows that the placentae are not formed of the margins of the carpels.

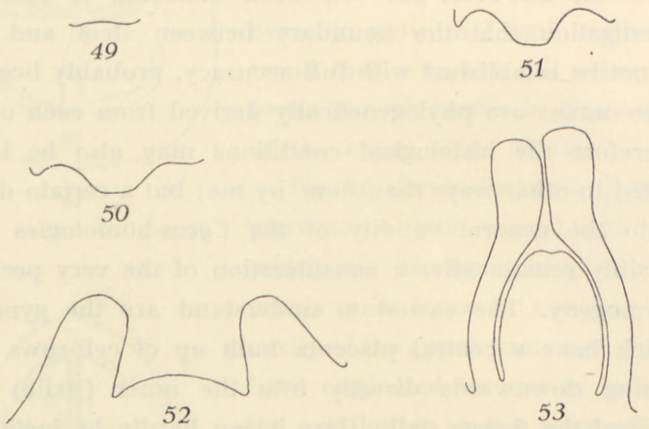
III. Teratology.

The histological analysis of the organogeny of the gynaecium recorded above thus seems to show that the placentae are stem formations, since they do not develop from the edges of the carpels, as assumed by the "classical" theory. There is, however, the drawback attaching to such an investigation that the boundary between stem and leaf cannot be established with full accuracy, probably because these organs are phylogenetically derived from each other. Therefore the histological conditions may also be interpreted in other ways than done by me; but a certain doubt as to the general validity of the *Cycas*-homologies may possibly remain after a consideration of the very peculiar organogeny. The easiest to understand are the gynaecia which have a central placenta built up of cell-rows continuing downwards directly into the inner (axile) cell-rows of the flower stalk. Here it can hardly be justifiable to doubt that the placenta is a continuation of the floral axis. However, when the placenta is parietal, conditions are not quite so clear.

In order to overcome this uncertainty it will therefore be necessary to adopt other methods of investigation. In particular it would be interesting to know what organs may develop from the placenta. Unfortunately the morphological value of the ovules has not been definitely recognised. But could they be found transformed into other organs of known morphological value, we should obtain a valuable body of evidence. That such a possibility is within the bounds of probability cannot reasonably be doubted if for instance we utilise PENZIG's ample information about "oolysis". Personally I have previously (1938) described

numerous examples showing that ovules (in *Salix*) may be transformed into stamens; and similar observations have been made within the *Personatae* (Rischkow), for instance in *Petunia*.

That the carpels are homologous with leaves, has been doubted by Grégoire; but that this doubt is unfounded



Figs. 49—53. The organogeny of the gynaecium in a normal *Petunia nyctaginiflora* Juss. Figs. 49—52, × 200. Fig. 53, × 60. The placenta is slightly parietal at the apex, otherwise central.

has recently been shown by Troll and his school, who by a histological analysis of the various developmental stages of the carpels found good agreement with the general organogeny of the leaves. We will therefore in the following pages attempt to throw light on the morphological nature of the carpels and ovules. And for this purpose we shall employ Levan's valuable teratological material of *Petunia* and his work on it, to which the following considerations are merely to be regarded as a supplement.

However, we shall begin the teratological examination of *Petunia* by tracing the organogeny of a normal gynaecium

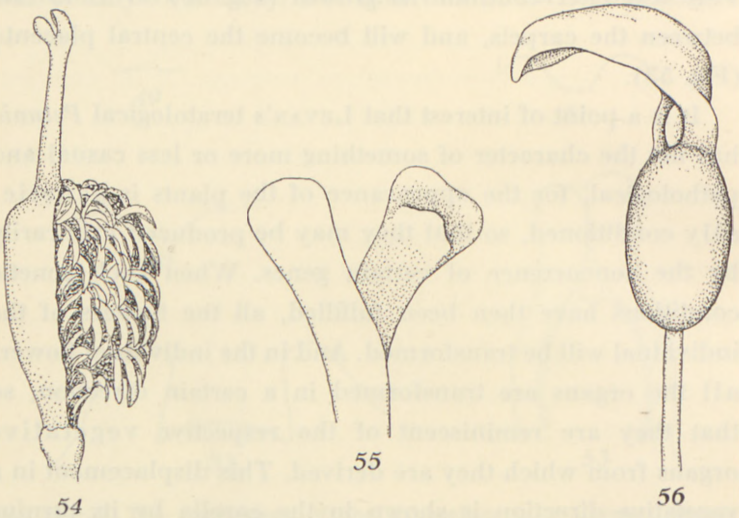
(Figs. 49—53) in order to show that in the main features the development is the same as was found above in *Personatae* that have a central placenta: At the apex of the floral axis (Fig. 49) the carpels arise laterally (Figs. 50—51) so that an unused remnant of the apex of the stem will be left. This will later continue its growth (Fig. 52) so as to rise between the carpels, and will become the central placenta (Fig. 53).

It is a point of interest that LEVAN's teratological *Petunia* has not the character of something more or less casual and pathological, for the appearance of the plants is genetically conditioned, so that they may be produced arbitrarily by the concurrence of certain genes. When such genetic conditions have then been fulfilled, all the flowers of the individual will be transformed. And in the individual flowers all the organs are transformed in a certain direction, so that they are reminiscent of the respective vegetative organs from which they are derived. This displacement in a vegetative direction is shown in the corolla by its turning greenish or whitish. Stamens (Fig. 56) and stigma (Fig. 55) also show their leaf-nature.

However, special interest attaches to the transformation of the gynaecium, which is very conspicuous in the later developmental stages of the flower, as will appear from LEVAN's photographs.

After flowering all the ovaries swell considerably although none of the ovules have been fertilised. The ovary will soon burst lengthwise, and numerous ovules will project (Fig. 54); they have a very peculiar appearance, as shown in Figs. 54 and 57—66. As each of the many ovaries of the plant contains several hundred seeds, it will be understood that an exceedingly rich teratological material may easily

be procured. This abundance is further augmented in that the individual ovules of an ovary are transformed in very different manners, so that all conceivable stages of transition may easily be found between nearly normal ovules (Fig. 57) and green leaves even (Fig. 66) with nerves and glandular



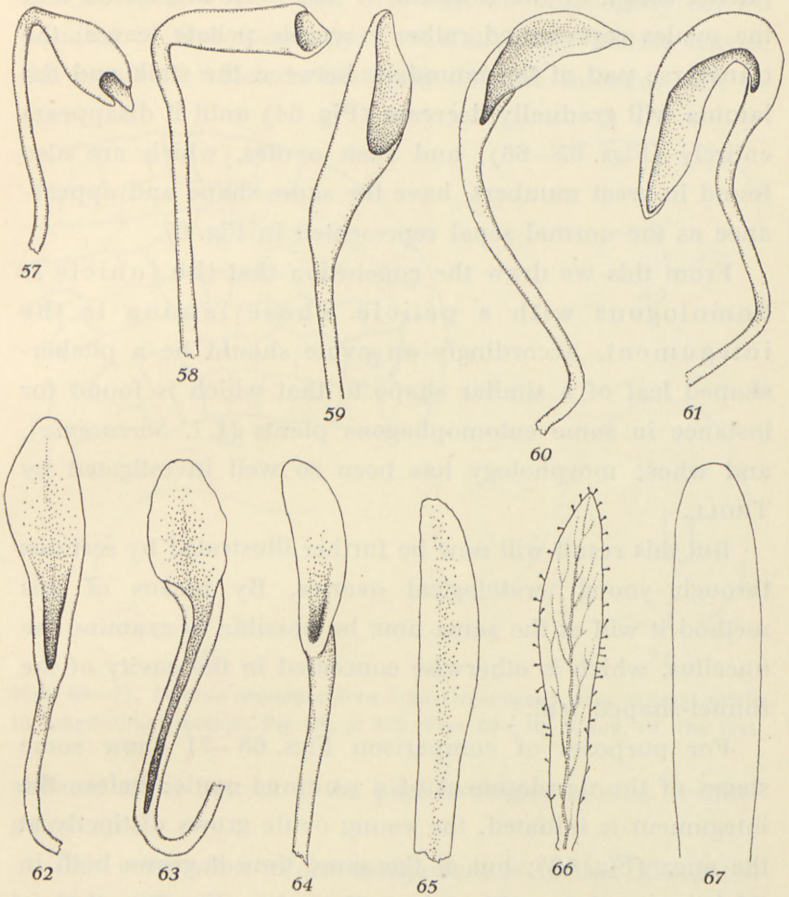
Figs. 54—56. Teratological *Petunia nyctaginiflora* Juss. — Fig. 54. The transformed ovules have caused the ovary to burst. × 2. — Fig. 55. Transformed stigma. × 5. — Fig. 56. Teratological anther with a large connective. × 5.

hairs and of an entirely similar appearance for instance to that of normal sepals (Fig. 67), as to whose leaf-nature there is hardly any reason to doubt.

All the ovules represented in Figs. 57—66 have been dissected out from the same ovary and have been drawn by means of a drawing apparatus at slight magnification. Special attention should be given to the size of the micropyle, which is indicated in the figures by heavy dotting.

Fig. 57 shows an almost normal ovule, whose micropyle is relatively small and but slightly oblique. The following

figures show an increase of the size and obliquity of the micropyle; and such ovules are present in the ovaries in



Figs. 57—66. *Petunia nyctaginiflora* Juss. Older teratological ovules showing transitions from nearly normal ovules (Fig. 57) to leaf-like "ovules" (Figs. 65—66). — Fig. 67, leaf of calyx. — Micropyle heavily dotted. × 8. Cf. the text.

particularly large numbers. At the same time the funicle becomes short, broad, and flat (Figs. 65—66), assuming the character of a leaf stalk, while the micropyle attains such

a large size that the integument will assume the shape of a normal lamina. Figs. 62—64 will show that the original pitcher-shape of the integument has been altered so that the ovules represented rather resemble peltate leaves; the transverse pad at the boundary between the stalk and the lamina will gradually decrease (Fig. 64) until it disappears entirely (Figs. 65—66); and such ovules, which are also found in great numbers, have the same shape and appearance as the normal sepal represented in Fig. 67.

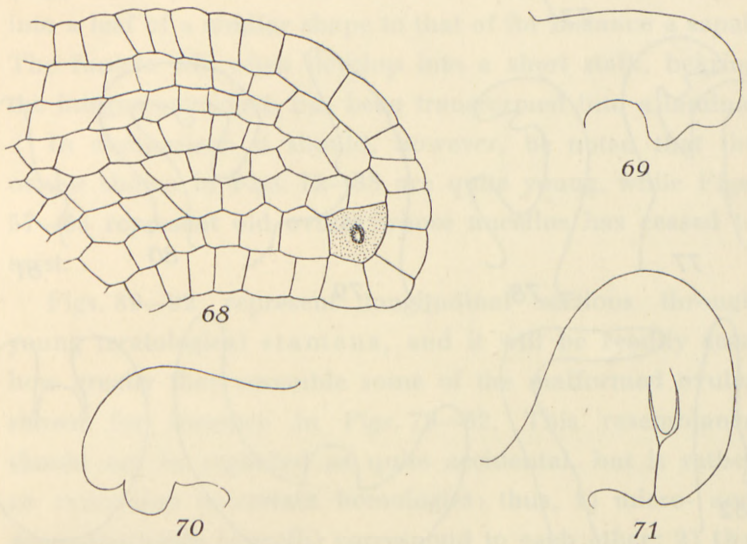
From this we draw the conclusion that the funicle is homologous with a petiole whose lamina is the integument. Accordingly an ovule should be a pitcher-shaped leaf of a similar shape to that which is found for instance in some entomophagous plants (f. i. *Sarracenia*), and whose morphology has been so well investigated by Troll.

But this result will now be further illustrated by sections through young teratological ovaries. By means of this method it will at the same time be possible to examine the nucellus, which is otherwise concealed in the cavity of the funnel-shaped leaf.

For purposes of comparison Figs. 68—71 show some stages of the development of a normal ovule: before the integument is initiated, the young ovule grows distinctly at the apex (Fig. 68); but at the same time it grows both in thickness and breadth (along the edge, Fig. 88)—that is to say, in the same way as many vegetative leaves grow (cf. Troll, 1939).

The cell which is dotted in Fig. 68 is the mother cell of the nucellus; but the young ovule constantly continues its apical growth (Fig. 69) beyond the nucellus, and the youngest apex of the lamina will soon bend up around it.

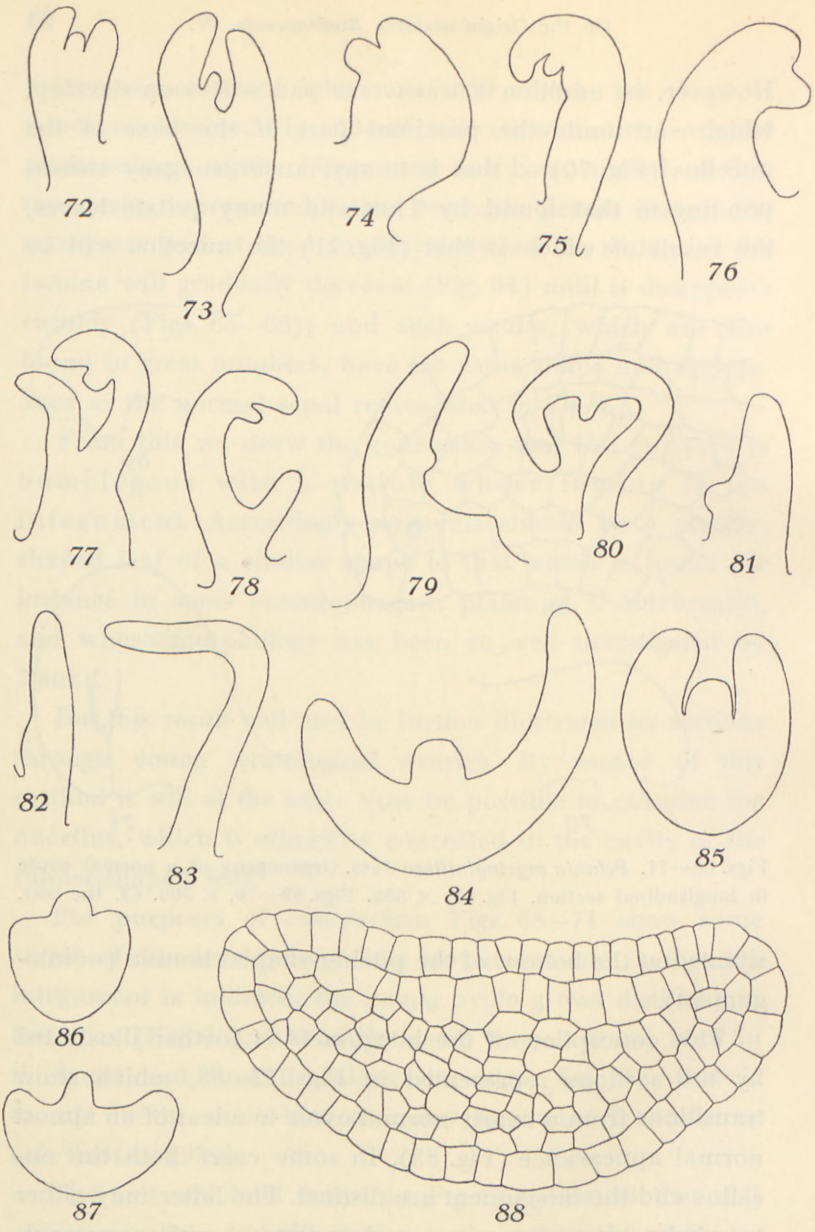
However, in addition a transverse pad will soon develop, which surrounds the proximal part of the base of the nucellus (Fig. 70) — that is to say, an organogeny corresponding to that found by TROLL in many peltate leaves, the result of which is that (Fig. 71) the nucellus will be



Figs. 68—71. *Petunia myctaginiflora* JUSS. Organogeny of a normal ovule in longitudinal section. Fig. 68, × 800. Figs. 69—70, × 300. Cf. the text.

situated at the bottom of the pitcher-shaped lamina (= integument).

This conception of the integument is further illustrated by the sections represented in Figs. 72—88, which show transitions from a nearly normal ovule to a leaf of an almost normal appearance (Fig. 83). In some cases both the nucellus and the integument are distinct. The latter may either be pitcher-shaped or more or less flat, or with a crescent-shaped transverse section; either the lamina is almost straight (Figs. 81—82), or it may (most frequently) be



Figs. 72—88. *Petunia nyctaginiflora* Juss. Section of young teratological ovules. Figs. 72, 73, 75, 77, 78, 80, 82, × 100. Figs. 74, 76, 79, 84, 85, 86, 87, × 200. Fig. 88, × 600. Figs. 81 and 83, × 75. Above, slightly transformed ovules with small micropyles; below, ovules with nearly normal leaf-like shape. Cf. further Figs. 57—66 and the text.

incurved at the apex in a similar way to that of a young fern-leaf.

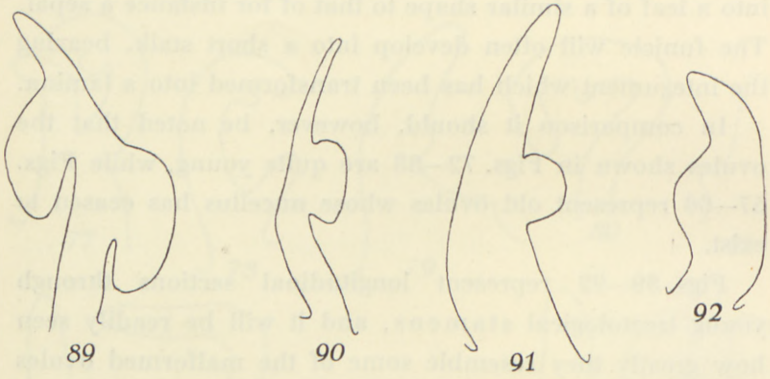
The transverse pad which conditions the peltate shape of the young integument is in some cases large and distinct (Figs. 77—78), but often it is partially or entirely absent (Figs. 79—81), and if so, the ovule has been transformed into a leaf of a similar shape to that of for instance a sepal. The funicle will often develop into a short stalk, bearing the integument which has been transformed into a lamina.

In comparison it should, however, be noted that the ovules shown in Figs. 72—88 are quite young, while Figs. 57—66 represent old ovules whose nucellus has ceased to exist.

Figs. 89—92 represent longitudinal sections through young teratological stamens, and it will be readily seen how greatly they resemble some of the malformed ovules shown for instance in Figs. 79—82. This resemblance should not be regarded as quite accidental, but is rather an expression of certain homologies: thus, 1) micro- and macrosporangia (nucelli) correspond to each other; 2) the connective is homologous with an integument; and 3) the filament is homologous with the funicle. And as a whole an ovule is homologous with a microsporophyll and is itself a monosporangiate macrosporophyll.

Penzig and Rischkow record many other malformations which create a presumption that the malformations described above do not illustrate conditions special to *Petunia*, but express morphological rules of general validity. Thus in *Datura stramonium* the ovules may be transformed into complete carpels, and in *Scrophularia vernalis* Penzig has found handsome oolyses of a similar kind to those in *Petunia* described above. It is of special interest that in

Petunia the ovules may also be transformed into stamens, whose leaf-like nature there is hardly any reason to doubt. Similar transformations have previously been described by me in a plant which is placed in the system very far from *Petunia*, viz. *Salix*. Penzig further mentions similar oolyses in numerous other plants belonging to quite different groups,

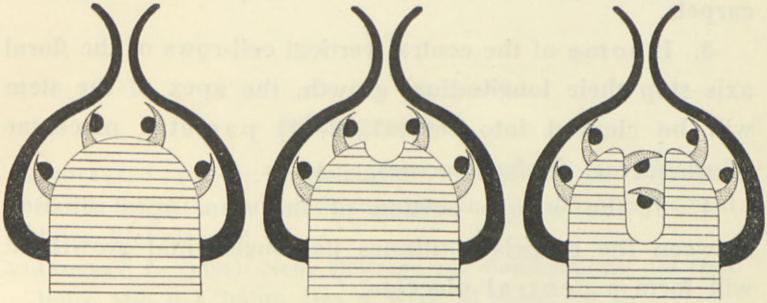


Figs. 89—92. *Petunia nyctaginiflora* Juss. Longitudinal section through teratological stamens, showing similarity to malformed ovules (Figs. 81—82). Slightly enlarged.

and showing that the ovules may often be transformed so as to resemble microsporophylls, calyx, corolla, or carpels.

The placenta also may in several plants be transformed into a shorter or longer axis whose leaves are more or less distinct ovules. Such malformations have been figured for instance by Peyritsch (1878) and especially distinctly by Rischkow, who has also examined i. a. many genera of the *Solanaceae*, in which he has produced experimentally very convincing malformations of ovaries: "In antholytic tomato-flowers the placenta acquires the character of a sprout, in the lower part of which there are leaflets, next come stamina, while above ovules are still present." (Rischkow, 1937, p. 131). Similar transformations were

found for instance in *Solanum, Datura, Atropa, Capsicum, Nicotiana, Physalis, Convolvulus, Allium, Brassica,* etc., which warrants the assumption that the phenomena described above in *Petunia* have a more universal validity.



93 94 95

Figs. 93—95. Diagrams showing the morphology of the gynaecium. The axial parts are horizontally ruled; the carpels are black; integument and funicles dotted; nucelli black. Fig. 93, Central placenta (e. g. in *Solanaceae*). Fig. 94. Placenta parietal above, central below (e. g. in some *Gesneriaceae*). Fig. 95. Placenta entirely parietal (*Orobanchaceae*). The floral axis elongated and forming the placentae; it bears the following leaves: 1) barren carpels, and 2) ovules which are monosporangiate macrosporophylls.

Thus both the organogeny of the gynaecium and the teratological conditions seem to point in the same direction, namely towards the view of the gynaecium which is expressed in the accompanying diagrammatic figures (93—95) and in the subjoined summary.

IV. Summary.

The present work is an inquiry into the morphology of the gynaecium in some selected types within the *Personatae*. They were examined partly organogenetically, partly (*Petunia*) teratologically with the following results:

1. The carpels are barren leaves, which do not bear ovules at their edges (as assumed by the "classical theory").

2. The placentae are direct continuations of the floral axis, whose cell-rows grow up between the carpels.

3. If some of the central vertical cell-rows of the floral axis stop their longitudinal growth, the apex of the stem will be cleaved into several (2—4) parietal placentae (*Gesneriaceae, Orobanchaceae*).

4. If the whole portion of the stem apex situated between the carpels continues its longitudinal growth, it will form a central placenta.

5. As lateral organs on the floral axis the ovules are homologous with leaves; they are monosporangiate macrosporophylls of a similar nature to those occurring in *Lycopodiales*.

6. The malformations show that the funicle is homologous with a leaf-stalk whose lamina is the pitcher-shaped integument.

7. In many *Solanaceae* Rischkow found the ovules to be transformed into: flat leaves, carpels, or stamina (i. e. organs homologous with leaves); and the placenta is often transformed into a sprout, which forms a direct continuation of the floral axis.

8. The view maintained in the present paper as to the morphology of the gynaecium is illustrated in Figs. 93—95, and in addition it is described at greater length in my previous works and in those of J. M. Thompson.

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STUDIES ON THE PLANT-GEOGRAPHY OF THE NORTH-ATLANTIC HEATH-FORMATION

I. THE HEATHS OF THE FAROES

BY

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1. Introduction.

A journey to the Faroes in 1935 gave me an opportunity to study the heath vegetation[1] more closely, and later (1938) I saw allied heaths in Norway. The material collected forms the basis of a number of comparisons of heath vegetation at the northern Atlantic, followed by an attempt to compare heath vegetation in various localities along the whole of the Atlantic Coast of Europe, especially within the area of distribution of *Erica cinerea*. Finally, the ecology and plant geography of some heath plants from the Faroes and western Norway will be subjected to a more detailed investigation.

2. Analyses of Heaths "heathermoors" in the Faroes.

Ostenfeld (1907) was the first to deal with the heath of the Faroes. He pointed out some of its essential features: "The appearance and occurrence of the heath absolutely

[1] Tansley (1939) makes a distinction between heath and heather-moor. The latter is distinguished by a "marked preponderance of species of non-vascular plants which depends closely on damp air"; it is most frequent at higher altitudes on deep acid peat, whereas the heath is developed on sandy or gravelly soil with a minimum of peaty humus. However, it seems difficult to maintain a definite distinction between heath and heather-moor, as according to Tansley there is a very "substantial community" between them. The Faroes heath is undoubtedly rather of the character of heather-moor, but nevertheless we will in the following only use the one designation, *viz.* heath, which thus also will be made to include heather-moor.

makes the impression on the spectator that it is a formation which does not thrive particularly well in the Faroes, and in consequence it must be considered exacting and particular. It requires a fairly dry soil and sun, both of which are rare commodities." These requirements can be satisfied on south-facing slopes in the lowlands. At heights of less than 300 m above the sea the vegetation is locally characterized by the heath or by related grass-communities (*Nardeta, Agrostideta*) full of oceanic species (cf. Böcher 1937b).

In the Faroes it is in particular possible to distinguish between two kinds of heath: a type rich in *Empetrum* or *Vaccinium* with relatively few oceanic species (see later) and a type with many of these species.

A. The Heath rich in *Empetrum-Vaccinium*.

This type was examined in an area west of Ejde on Østerø, where there is a rather level undulating stretch surrounding a large lake. The greater part of this area was covered by grass-vegetation, most frequently dominated by *Nardus* (cf. Ostenfeld's "Hedekær" (acidic grassland)). On small slopes there were heath-sociations rich in mosses, especially *Calluna-* or *Empetrum* heath; more rarely the *Vaccinium* species were met with in larger quantities. The whole of the area was very moist, and with the exception of the drier heath-covered knolls it all presented the appearance of a large, greatly variegated heath bog. The variation was due to the inhomogeneity of the substratum. *Eriphorum polystachyum* and *Sphagnaceae* occurred in the most moist localities (e.g. turf pits); however, *Eriophorum* could also grow in the mosses on tops of low, flat hills. It is peculiar that *Eriphorum vaginatum* does not play any great part in such vegetation, seeing that this species

dominates the vegetation in large parts, for instance of the British Isles ("Cotton-grass Moor", see Moss 1913).

Time did not permit to make a thorough investigation of the distribution of all sociations. The exposition seemed to exercise a rather considerable influence. OSTENFELD (l. c. p. 77) mentions how the various heath shrubs can be closely associated with southern slopes. In table 1 there is an example of the difference which can exist between a heath-covered north and south side of a low wall in that locality. The bottom layer of the heath mainly consisted of thick moss; *Cladonia*-species only occurred as extremely scattered individuals. The moss chiefly consisted of *Hylocomium* (*Rhytidiadelphus*) species, locally also with *Rhacomitrium hypnoides* as a dominant. In some cases the tops of the flat rocky knolls were covered by a cap consisting of *Empetrum-Rhacomitrium hypnoides*-soc., whereas the sides where the peat and the soil were much thicker, were covered by *Calluna-Hylocomium*-soc.

The north-facing wall (No. 3, Table 1) was particularly rich in bilberries and marsh wortleberries, the latter and *Cornus suecica* were almost only to be found here; bilberries were extremely dense (100.100.4)[1] whereas on the southern slope (No. 4, Table 1) they were scattered (90.60.0)[1] in the heather. A peculiar feature of the south-facing slope examined was the great density of *Juncus squarrosus*; I have very rarely seen this species so dense. The cause of this density is probably due to the exposition combined with favorable edaphic conditions (a thick raw humus layer and a higher degree of humidity in the soil) than in the steeper south-facing slopes, where the other type of heath occurs (cf. Table 2). The Faroes are situated at

[1] See explanation p. 38, footnote.

the northern limit of the species (fig. 132 in Böcher 1938); it hardly ever enters into the alpine vegetation, but has a pronouncedly vertical limit of frequency in the islands, at the same time as the heath decreases in frequency. The examined vegetation lay at about 200 m. above the level of the sea, in the very part where the heath disappeared as a dominant formation.

From the list of species and the spectrum (pp. 40—41) it appears that the vegetation is rather rich in boreal species. This Faroes heath type agrees very well with the English and Scotch "heathermore" rich in *Vaccinium*, as well as with the humid heath types in western Norway (see Nordhagen 1917, Goksøyr 1938), but also farther north and south there are allied types of vegetation. A subalpine *Empetrum-Vaccinium* heath, rich in mosses with *Dryas* and many temperate and arctic species (Kodlur at Ejde), forms a transition to the *Empetrum-Vaccinium* heath of south-eastern Greenland (Böcher 1937b, p. 190). There is also some resemblance with Norwegian subalpine-oceanic *Empetrum-Vaccinium* heaths; an analysis of a heath of this kind is to be found in Table 1, No. 5. It is distinguished from the Faroes-heath by being richer in lichens and boreal-arctic species. There is a rather considerable similarity with many mossy heaths in more temperate regions e. g. Denmark and western Sweden. Here such heaths, however, are closely connected with northern slopes. We find them on the heather-covered hills in central and eastern Jutland, where *Hylocomium* species (however, with the exception of *H. loreum*) and in certain regions *Cornus suecica* dominates *Calluna*-heaths rich in *Vaccinium*. The vegetation which in Danish latitudes approaches most nearly to the one mentioned above from the Faroes, is the

heath on the north-facing rocky slopes of the Halland Ridge towards the Kattegat (Hovs Hallar). Here also *Hylocomium loreum* enters as a dominant in the heath. These Danish and Swedish heaths will be mentioned in a later treatise.

B. The heath rich in *Erica cinerea*.

This type of heath was especially examined on the south-western slopes at Bordö Vig, south of Klaksvig (Plate 1) and also in the area behind Thorshavn. It is most typically developed (with *Erica cinerea* dominating) in the driest and warmest localities; with increasing humidity the vegetation merges into a *Calluna*-heath without *Erica cinerea*, and this vegetation again approaches the type rich in *Empetrum-Vaccinium* (No. 4, Table 1). The six analyses from the Faroes (Table 2) are arranged in such a manner that partly at Thorshavn (1—3), partly at Klaksvig (4—6) they form the transition from *Calluna* to *Calluna-Erica cinerea* heath; Nos. 1 and 4 are *Calluna* heaths, Nos. 3 and 6 *Calluna-Erica cinerea* heaths and Nos. 2 and 5 transition heaths.

The driest and warmest places in the Faroes are the lower parts of the south-facing slopes, where inclination and exposition cause better drainage (and so less humidity and greater warmth in the soil) and a stronger influence of the rays of the sun. In the Faroes literature I have only found one indication of *Erica cinerea* outside the southern lowland heath and this (Ostenfeld 1908, p. 955) in faintly sloping acidic grassland, where it was of rare occurrence, probably in some slightly drier spot.

The *Calluna-Erica cinerea* heath is nowhere on the Faroes so luxuriously developed as e. g. at Kvala in western Norway (p. 20); this is presumably due both to climatic

Table 1. The heath rich in *Empetrum-Vaccinium.*

	The Faroes				Norway
Analysis No.	1	2	3	4	5
Method (see p. 38)	D.	—	S.	S.	S.
Height above the sea	220	230	200	200	500
Exposition	N.	SW.	N.	S.	NW.
Inclination	15—20°	rather strong	30°	10—15°	20°
Northern species:					
Empetrum nigrum (+ hermaphroditum)	5	!	100.90.3	90.30.0	100.100.4
Vaccinium myrtillus	1	!	100.100.4	90.60.0	40.10.0
— uliginosum	1	!	90.20.0	—	100.60.2
— vitis idaea	—	—	—	—	50.20.0
Arctostaphylos alpina	—	—	—	—	100.60.2
Cornus suecica (O.)	—	!	80.50.0	—	100.40.1
Carex rigida	—	—	—	—	70.20.0
Andromeda polifolia	—	—	—	—	50.30.0
Alchemilla alpina (O.)	1	—	—	—	—
Trientalis europaea	—	—	—	—	10.0.0
Temperate oceanic species:					
Calluna vulgaris	—	!	30.10.0	100.90.3	20.10.0
Juncus squarrosus	—	!	+	100.70.3	—
Narthecium ossifragum	—	!	+	20.0.0	—
Galium saxatile	1	+	—	—	—
Polygala serpyllacea	—	+	—	—	—
Blechnum spicant	+	+	—	—	—
Carex binervis	—	—	—	+	—
Temperate species:					
Potentilla erecta (O.?)	1	!	100.30.1	100.30.1	—
Nardus stricta	—	!	90.40.0	70.20.0	—
Deschampsia flexuosa	1	+	—	20.10.0	80.30.0
Molinia coerulea var. (O.?)	1	—	—	70.20.0	—
Carex stellulata	—	—	60.20.0	—	—
— panicea	—	+	—	—	—
— Goodenoughii	—	+	—	—	—
Luzula multiflora	1	+	20.0.0	10.0.0	—

Table 1 (continued).

	The Faroes				Norway
Analysis No.	1	2	3	4	5
Method (see p. 38)	D.	—	S.	S.	S.
Height above the sea	220	230	200	200	500
Exposition	N.	SW.	N.	S.	NW.
Inclination	15—20°	rather strong	30°	10—15°	20°
Festuca ovina vivipara (O.?)	—	—	40.20.0	+	—
Anthoxantum odoratum (O.?)	—	!	10.0.0	—	—
Agrostis canina var. mutica	—	—	20.0.0	—	—
Scirpus caespitosus (O.?)	—	!	—	30.10.0	+
Eriophorum polystachyum	—	!	30.0.0	10.0.0	—
Orchis maculatus	1	—	10.0.0	60.0.0	—
Rumex acetosa	—	!	+	—	—
Thymus serpyllum var. (O.?)	1	—	—	—	—
Plantago maritima (O.?)	1	—	—	—	—
Lycopodium clavatum	—	—	—	—	20.10.0
Solidago virgaurea	—	—	—	—	+
Mosses and lichens:					
Hylocomium loreum (O.)	**4**	!	**100.100.5**	**100.70.1**	10.0.0
— parietinum	1	!	50.10.0	**80.50.1**	**90.50.0**
— proliferum	**3**	!	**80.50.0**	50.20.0	—
Plagiothecium undulatum (O.)	—	!	60.20.0	**70.50.0**	—
Rhacomitrium hypnoides (O.?)	+	!	—	—	**100.80.2**
Polytrichum commune	—	!	**100.50.1**	—	60.20.0
— alpinum	—	!	—	—	—
Dicranum scoparium	1	!	30.10.0	—	40.10.0
Stereodon cupressiformis	1	—	—	30.0.0	—
Andraea petrophila	—	—	—	—	40.10.0
Frullania tamarisci (O.)	1	!	—	—	—
Blepharozia ciliaris	—	—	—	—	**80.50.0**

Table 1 (continued).

	The Faroes				Norway
Analysis No.	1	2	3	4	5
Method (see p. 38)	D.	—	S.	S.	S.
Height above the sea	220	230	200	200	500
Exposition	N.	SW.	N.	S.	NW.
Inclination	15—20°	rather strong	30°	10-15°	20°
Cephalozia divaricata	—	!	—	—	—
Diplophyllum albicans (O.)	—	!	—	—	—
Jungermannia quinquedentata	—	!	60.30.0	—	—
Jungermannia Floerkei	—	—	—	40.10.0	50.10.0
— ventricosa	—	—	50.20.0	—	—
Martinellia gracilis (O.)	—	!	—	—	—
Mylia Taylori (O.)	—	—	—	—	40.10.0
Kantia trichomanis	—	—	—	40.20.0	—
Cetraria islandica	—	—	—	—	100.80.2
Cladonia silvatica	1	—	—	—	90.30.0
— uncialis	—	—	—	—	30.20.0
— cf. chlorophaea	—	—	—	—	10.0.0
— rangiferina	—	—	—	—	+

No. 1: Heath at Ejde (at the lake) on Østerø. No. 2: Western side of Skaalefjordsdalen, Østerø (Jensen 1897, page 208). Nos. 3—4: Ejde (at the lake). No. 5: Blåmannen at Bergen; vegetation in a strongly exposed place with rocky ground below a thin layer of soil. In more sheltered places *Calluna* heath was found here with very nearly the same species, in more humid soil on the transition between meadow and heath: *Erica tetralix*, *Molinia*, *Nardus* and *Scirpus caespitosus*.

(O.) = Oceanic species.

(O.?) i. e. possibly represented by a special oceanic race.

and biotic conditions. In the summer the climate is cooler in the Faroes as compared with that of western Norway; add to this, that the heaths e. g. at Kvala are hardly so

Table 2. The heath rich in *Erica cinerea*.

	The Faroes						Norway	
Analysis No.	1	2	3	4	5	6	7	8
Method (see pag. 38)	D.	D.	D.	S.	S.	S.	S.	S.
Height above the sea	200	50	50	150	200	150	ca. 50	ca. 50
Exposition	SE.	S.	S.	SW.	WSW.	S.	SW.	S.
Inclination	30°	20°	20°	30°	40°	35—40°	10—15°	30—40°
Northern species:								
Alchemilla alpina (O.)	—	—	—	30.10.0	+	—	—	—
Thalictrum alpinum	—	—	—	30.10.0	—	10.0.0	—	—
Selaginella selaginoides	+	—	—	10.0.0	—	—	—	—
Empetrum nigrum	1	1	1	+	—	—	—	—
Arctostaphylos uva ursi	—	—	—	—	—	—	—	30.10.0
Vaccinium myrtillus	—	—	—	—	—	—	10.10.0	—
Silene acaulis	—	—	—	+	—	—	—	—
Temperate oceanic species:								
Erica cinerea	—	**4**	**5**	—	20.20.0	**100.100.4**	**90.50.0**	**100.100.4**
Calluna vulgaris	**4**	3	1	**100.100.4**	**100.100.4**	**100.80.3**	**100.100.4**	**70.30.0**
Polygala serpyllacea	1	1	+	30.10.0	30.10.0	40.20.0	30.0.0	+
Hypericum pulchrum *)	1	1	1	50.0.0	30.0.0	40.10.0	40.10.0	50.20.0
Carex binervis	—	—	—	—	10.0.0	20.0.0	+	—
Narthecium ossifragum	1	—	1	+	—	10.10.0	—	—
Juncus squarrosus	1	1	—	—	30.10.0	—	—	—
Galium saxatile	1	1	1	—	—	—	30.0.0	+
Blechnum spicant	1	—	—	—	10.0.0	—	—	10.0.0
Erica tetralix	—	—	—	—	—	—	—	40.0.0
Temperate species:								
Potentilla erecta (O.?)	**3**	1	1	**100.50.2**	**80.20.0**	**90.20.0**	**100.70.2**	**100.30.1**
Agrostis canina (incl. var. mutica)	2	1	1	**80.30.0**	**90.50.0**	**90.60.0**	—	—
Deschampsia flexuosa	—	—	—	—	10.0.0	—	**90.40.0**	—
Scirpus caespitosus (O.?)	—	—	—	40.20.0	50.10.0	**70.20.0**	—	+
Carex pulicaris (O.*)	—	—	—	40.10.0	—	30.10.0	—	**70.10.0**
Viola Riviniana (O.?)	1	1	—	**80.10.0**	—	30.0.0	—	40.10.0
Thymus serpyllum (O.?)	1	2	1	60.0.0	—	50.10.0	—	—
Nardus stricta	1	1	1+	20.10.0	10.10.0	40.0.0	10.0.0	—
Carex pilulifera (O.*)	1	1	1	20.0.0	20.0.0	30.10.0	10.0.0	20.0.0
Carex panicea	1+	—	—	20.0.0	—	20.10.0	—	10.0.0

Table 2 (continued).

	The Faroes						Norway	
Analysis No.	1	2	3	4	5	6	7	8
Method (see pag. 38)	D.	D.	D.	S.	S.	S.	S.	S.
Height above the sea	200	50	50	150	200	150	ca. 50	ca. 50
Exposition	SE.	S.	S.	SW.	WSW.	S.	SW.	S.
Inclination	30°	20°	20°	30°	40°	35—40°	10—15°	30—40°
Festuca ovina vivipara (O.?)	1+	1	1	50.20.0	40.10.0	40.20.0	10.0.0	+
Anthoxanthum odoratum (O.?)	1+	1	1	20.10.0	30.10.0	40.20.0	+	—
Orchis maculatus	—	—	—	—	20.0.0	10.0.0	30.0.0	+
Pinguicula vulgaris	—	—	1	20.0.0	10.0.0	+	—	—
Plantago lanceolata (O.?)	+	—	—	—	—	10.0.0	—	—
Euphrasia cfr. scotica	1	1	1	20.0.0	+	—	—	—
Luzula multiflora	—	—	—	+	+	—	—	—
Agrostis tenuis	2	1	1	—	—	—	20.0.0	—
Juniperus communis	—	—	—	—	—	—	10.0.0	50.10.0
Agrostis alba	—	—	—	—	—	—	50.0.0	—
Campanula rotundifolia	—	—	—	—	—	—	40.0.0	20.0.0
Sieglingia procumbens (O.*)	—	—	—	—	—	—	50.0.0	20.10.0
Antennaria dioeca	—	—	—	—	—	—	30.0.0	20.0.0
Molinia coerulea (O.?)	—	—	—	—	—	—	—	40.10.0
Sanguisorba officinalis	—	—	—	—	—	—	20.0.0	—
Succisa pratensis	—	—	—	—	—	—	50.10.0	—
Solidago virgaurea	—	—	—	—	—	—	30.0.0	—
Plantago maritima (O.?)	—	—	—	—	—	—	50.0.0	+
Brunella vulgaris (O.?)	+	—	—	—	—	—	—	+
Lotus corniculatus	—	—	—	—	—	—	10.0.0	—
Mosses and lichens:								
Hylocomium loreum (O.)	4	2	1	**100**.50.1	60.60.0	60.40.0	10.0.0	—
— parietinum	—	1	1	40.20.0	20.0.0	**70**.30.0	**100**.50.2	—
— proliferum	2	1	1	60.50.0	30.10.0	—	40.10.0	—
— triquetrum	—	—	—	10.0.0	—	—	—	—
— squarrosum	—	+	1	—	—	—	40.20.0	—
Stereodon cupressiformis	—	1	1	40.30.0	**80**.60.0	60.20.0	**100**.80.3	**80**.50.0
Thuidium tamariscifol. (O.)	—	—	—	**70**.60.0	—	20.10.0	**90**.20.0	20.0.0

Table 2 (continued).

	The Faroes						Norway	
Analysis No.	1	2	3	4	5	6	7	8
Method (see pag. 38)	D.	D.	D.	S.	S.	S.	S.	S.
Height above the sea	200	50	50	150	200	150	ca. 50	ca. 50
Exposition	SE.	S.	S.	SW.	WSW.	S.	SW.	S.
Inclination	30°	20°	20°	30°	40°	35—40°	10—15°	30—40°
Dicranum scoparium	—	—	—	20.20.0	—	40.0.0	50.0.0	—
Rhacomitrium hypnoid.	—	—	—	20.10.0	20.10.0	10.10.0	—	10.0.0
Leucobryum glaucum (O.)	—	—	—	—	—	—	20.0.0	—
Polytrichum juniperinum	—	—	—	—	—	—	10.0.0	—
Blepharozia ciliaris	+	1	—	60.10.0	30.10.0	50.20.0	—	—
Frullania tamarisci (O.)	+	—	+	70.30.0	40.20.0	20.0.0	80.20.0	30.10.0
Diplophyllum albicans (O.)	+	1	+	50.20.0	40.40.0	20.0.0	—	—
Nardia scalaris (O.)	—	1	—	—	—	—	—	—
Mylia Taylori (O.)	—	—	—	—	40.10.0	—	—	—
Martinellia gracilis (O.)	—	—	—	—		—	—	—
Scleropod. purum (O.)	—	—	—	10.10.0	—	—	10.0.0	—
Cladonia silvatica	—	—	—	—	—	—	+	+
— uncialis	—	—	—	—	10.0.0	—	—	—
— fimbriata	—	—	—	—	—	—	—	10.0.0
Bæomyces roseus	—	—	—	—	—	—	—	+

No. 1: At Thorshavn very heavily grazed. Nos. 2—3: At Thorshavn (Husaraun). Nos. 4 and 6: Bordø Vig, Torvadalsá. No. 5: Heath at Anir, Bordø. No. 7: Heath at Skudenæshavn in Karmø. The place lay near a vertical rocky wall and had, as its downward limit, a heath with *Narthecium, Carex pulicaris, stellulata, binervis, Erica tetralix*. The upper part of the soil below the heath was very rich in humus, perhaps rather of the character of peat. No. 8: Kvala at Haugesund (Plate 2). At the foot of the scree *E. tetralix* and also *Carex viridula* and *Pinguicula* were very frequent. Below the heath there was only very little humus (12,6 %), the soil was brownish down to a depth of 25—30 cm. No distinct podsolation. P_H see page 27).

O.? = possibly represented by a specially oceanic race.

O.* = slightly oceanic (suboceanic) according to Hård av Segerstad (1935).

*) = var. *procumbens* in Nos. 1—6.

heavily grazed as those of the Faroes. The extensive *Nardeta* which form a kind of mosaic with the heath patches in the Faroes, dominates entirely when the grazing becomes particularly intensive (cf. Scarth (1911), Nordhagen (1921, pp. 61—62)). That there is no heath near the villages may be due to the human exploitation of the heather plants. Thus Hornemann (1821, p. 431) writes: "In the Faroes they are used for the smoking of the meat of sheep"; more particularly, however, the heath was used as winter fodder for sheep.

A characteristic feature of the transition from *Calluna* to *Calluna-Erica cinerea* heath is the great decrease of the mosses, which fact presumably has some bearing upon the increasing desiccation, and recurs in western Norway (Table 2, Nos. 7—8) and in alpine English heaths (see p. 21).

The abundance of species in the heaths of the Faroes is striking when comparing it with the greater number of those of western Jutland, both moraine sands and fluvioglacial plains. This undoubtedly first and foremost has some bearing upon edaphic conditions. Investigations of the hydrogenion concentration in the soil of Faroes heaths (Table 3) shows that both *Empetrum-Vaccinium* and *Calluna-Erica* heaths, have p_H values which compared with Danish heaths (Weis 1933, and my own unpublished analyses) is rather high. Whereas the raw humus layer in the heaths of Jutland on an average is situated at p_H 3.8—4.5, we find that the upper soil layers of the Faroes heath has p_H 4.6—5.3 (6.5). The cause of the high value must be looked for in the greater base-content of the Faroes soils. The basalt contains rather large quantities of calcium which in spite of the heavy precipitation are not totally

Table 3. Hydrogenion concentration and organic content in some Faroes heath-soils.

Sample No.		p_H	Humus (loss on ignition) percentage
1.	Empetrum-Vaccinium uliginosum-Soc. (subalpine with Dryas, Böcher 1937 b p. 191)	6.5	
2.	Empetrum-Vaccinum-Soc.(Table 1, No. 3)	4.6	high value
3.	Empetrum-Soc. (rich in mosses: Hylocom. loreum, Stereodon, Polytrichum alpinum) Thorshavn	4.9	51.4 (28.2[1])
4.	Vaccinium Myrtillus-Soc. (rich in mosses: Nardia scalaris, Diplophyllum albicans, Dicranella heteromalla, Pogonatum polytrichoides, Polytrichum alpinum) Thorshavn	4.6	71.7
5.	Calluna-Juncus squarrosus-Soc. (Table 1, No. 4)	4.7	high value
6.	Calluna-Soc. (with Nardus, Agrostis tenuis and mosses, particularly Dicranum). Thorshavn	5.3	67.5
7.	Calluna-Soc. (with Erica cinerea, Table 2, No. 5)	5.1	very high value
8.	Calluna-Erica cinerea-Soc. (Table 2, No. 6)	5.2	rather high value
9.	Erica cinerea-Calluna-Rhacomitrium hypnoides-Soc. (with Potentilla erecta, Nardus, Carex Oederi, Agrostis tenuis, Stereodon, Dicranum Starckei, Polytrichum alpinum, Diplophyllum albicans, Nardia scalaris) Thorshavn. Upper 2—5 cm of the soil	5.2	67.0
10.	In the same place, but 7—10 cm. further down in the soil	4.9	60.3
11.	Calluna-Erica cinerea-Soc. (rich in mosses) Thorshavn. Upper 2—5 cm of the soil	4.7	81.1
12.	In the same place, but 10—13 cm. further down in the soil	5.2	80.2

[1] 4 greater particles of gravel included.

washed away. Even though the organic content of the soil can rise to considerable values, the p_H figures are not so low as those quoted for Danish heaths. This is presumably partly due to the fact that the vegetation does not make quite such acid humus as the Danish one, partly to the presence of earth worms (observed in sample 9—12). Besides well-water in the soil may lead to an admixture of basic substances from the underground, and finally particles of gravel due to the weathering of the rocks (as observed in samples 3—4) can add mineral components of basic influence to the soil.

It is a well-known fact, however, to what an extent the volcanic rocks in Iceland form basic compounds during their weathering so that only extremely few soils here have a very acid reaction. Besides the investigations of Weis (1933) reference may here be made to Steenbjerg & Gunnarsson's exhaustive analyses (1938). The latter on p. 82 makes the following mention of the Icelandic heaths: "These heather vegetations are extremely mixed and do not bear the slightest resemblance to Scandinavian heaths with their often entirely unmixed heather vegetation; besides, it was not possible with the naked eye to prove any podsolation or destruction of the upper layer of earth which, when viewed in connection with the above-mentioned reaction of the soil, is probably due to the fact that the Icelandic soils, so rich in minerals, and more particularly calcium and magnium, counteracts the formation of humic acid sol which presupposes the comprehensive destruction of the upper layer, resulting in the formation of distinct podsol-profiles." After this are mentioned two measurings of p_H in Icelandic *Calluna* heaths, being situated in p_H 5.7 and 6.4. Also Weis states that podsolation is extremely

rare on Iceland. Nor have I on the Faroes, even under a thick layer of raw humus, seen anything, in the line of a bleached podsol layer.

Finally, it must be borne in mind that the great abundance of species is probably not merely due to the high p_H values and the resulting greater Ca-contents (cf. Tovborg Jensen 1936). There is every probability that a greater quantity of other nutritive substances will be present in the heaths of the Faroes than in those of Denmark (cf. the large kali-content of Icelandic uncultivated "mo" soils (Steenbjerg & Gunnarsson l. c.)).

As opposed to the greater part of Danish, South-Swedish and North-German heaths the Faroes heath (both type A and B) can in the main be regarded as a natural type of vegetation; perhaps it is even a climax vegetation in great parts of the lowland (particularly on south-facing slopes), but here it has to a very large extent been replaced by *Nardeta* (p. 14) and possibly further reduced by the humid climate of present days. In all probability the heath had a greater distribution in the Faroes during the warmer and drier subboreal period (Jessen & Rasmussen 1922, Jessen 1925).

3. Types of Vegetation rich in *Erica cinerea* outside the Faroes.

Before making a plant-geographical comparison of vegetations containing *Erica cinerea*, we will first of all have to orientate ourselves as regards the distribution of the species. The distribution in Europe appears from fig. 1. Outside the closed area it is said to exist in western Germany (further details see Grevellius & Kirchner 1923, p. 162), the western Alps and the Ligurian Appenines (Braun-

Blanquet 1923, p. 121). It is absent in low-lying regions in the Mediterranean area. Outside Europe it is according to Allorge (1927, p. 204) to be found in Algiers, and besides it is represented by a special subspecies on Madeira (*Erica maderensis* Bornm. = *E. cinerea* var. *maderensis* D.C.). As shown on fig. 1 the eastern limit of the species coincides with the January isotherm of 2° (cf. *Ilex* and other species mentioned in Holmboe's works). At the southern limit in Spain the winter temperature may also possibly exercise a certain influence, but here—as in many localities in the Mediterranean (and in Madeira), where the species is rare or entirely absent in the lowland—the summer heat, together with edaphic conditions, is the factor which more than any other helps to limit the area. We are surely justified in defining the species as temperate-thermic-oceanic (see further p. 23) as opposed to northern-oceanic (e. g. *Lobelia dortmanna*) and southern-oceanic species (*Rubia peregrina*) cf. Matthews (1937).

After this we will examine in detail the occurrence of *Erica cinerea* in the most important parts of its area:

Norway. The plant-geography and vegetation of the western-most part of Norway has in particular been examined by Nordhagen (1917, 1921), Holmboe (1926, 1927), Degelius (1935) and Goksøyr (1938).

The plant-geographic zonation on the western coast of Norway is exceptionally definite. During a few hours it is possible, when travelling in a motorbus from the interior to Haugesund out at the sea, to pass at any rate two important plant-geographical boundaries; there is an *Erica tetralix* zone and an *Erica cinerea* zone. Already at Kyrping (50 km. from the sea) one enters the coast land, where the lowland is characterized by foliferous trees (ash, elm) and where *Digitalis purpurea* grows as weeds along the road or is scattered in grass plots in open shrubs. Close west of Ølen (35 km. from the sea) the heaths begin to

appear; *Erica tetralix* and *Myrica* occur in great quantities in wet heath, rich in mosses and frequently tussocky. At Etne the country is still rich in foliferous trees (small woods with grass plots). These glades become greater and greater towards west and are gradually replaced by heaths. At Skjold (20 km. from

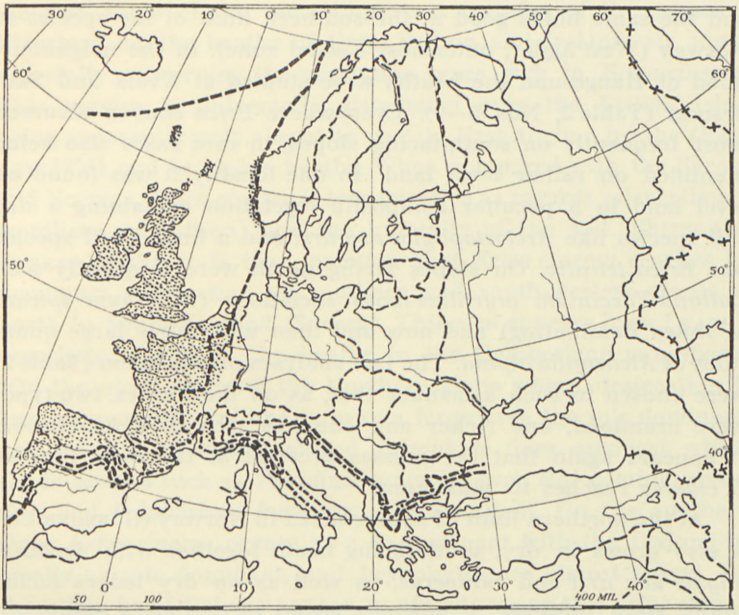


Fig. 1. The distribution in Europe of *Erica cinerea* L. (dotted area; the arrow north of Corsica indicates the localities in the Ligurian Appenines). The stippled line = January isotherm of 2° (Celsius). The line with dots and dashes interchanging = July isotherm of 22° (according to Alt (1932) and Birkeland and Føyn (1932)).

the sea) the heath begins to predominate, though frequently with a good deal of birches and junipers. At the most westerly branch of Skjold's Fjord (10 km. from the sea) *Erica cinerea* suddenly occurs together with *Calluna* in firm soil on the southwestern slopes, being raised above the moist heath with *Erica tetralix, Narthecium, Orchis maculatus* etc. At Haugesund the region is pronouncedly oceanic; the vegetation bears a considerable resemblance to that of the Faroes, though with a far more southerly character (*Vicia orobus, Sanguisorba).* In the gardens *Sarothamnus*

scoparius, Picea sitkensis and *Araucaria araucana* flourish. Immediately east of Haugesund the January isotherm of 2° is passed (Skudesnæs and Utsire have the January mean temperature of 2.2°) and at the northern limit of *Erica cinerea* in Norway (Rundøy) the January mean temperature is 3.1°; on the islands northeast of Rundøy the January mean temperature quickly approches 1°, and the same holds good at the southern limit of the species in Norway (West Agder, outermost coastal zone). In the neighbourhood of Haugesund the heaths were studied at Kvala and Skudesnæs (Table 2, Nos. 8—9). Everywhere *Erica cinerea* occurred most frequently on south-facing slopes, in rare cases also being identified on rather level land. In one locality it was found on level land in a peculiar composite vegetation containing a dry soil species like *Arctostaphylos uva ursi* and a humid-soil species like *Erica tetralix*. On slopes facing north were frequently seen *Calluna-Vaccinium myrtillus*-moss sociations (*Blechnum spicant* at times dominating) and now and then with fairly large quantities of *Alchemilla alpina*. The two analyses of vegetation (Table 2) were chosen in such a manner that, as on the Faroes, two types were examined, one richer and one less rich in *Erica cinerea*. It appears again that the mosses decrease at the same time as *E. cinerea* reaches its maximum.

At the northern limit of *Erica cinerea* in Norway (Goksøyr l. c.) it also grows in dry, south-facing heath together with *Arctostaphylos uva ursi* and *Juniperus*, as well as on dry ledges facing south. Here, however, it nowhere seems to become a dominant. The small patch of heath at Utsire, which the sheep have not been able to exterminate (Nordhagen 1921) is also very like the Faroes vegetation, and we see again that the mosses decrease considerably with the appearance of *Erica cinerea*.

Vierhapper (1927, p. 156) mentions from the island Tysnes south of Bergen an interesting "Laubmischwald", the character of which is Atlantic. In drier localities with *Pinus silvestris* and *Juniperus* he found *Erica cinerea, Hyperichum pulchrum* and *Galium saxatile*. There is much evidence of a number of heaths in western Norway (like those in Denmark) having arisen after destruction of such woodlands (sheep-grazing, the cutting down of trees); see Holmboe 1926, p. 63. On Karmø where there are many *Erica cinerea* heaths, it is still possible to find scattered oak trees and a few hazels; in the 16th century there were still

forests here (Norske Rigsregistranter II 565, see TROELS LUND: Dagligt Liv i Norden vol. I). FÆGRI (1935, page 32) proves that Jæren was partly covered with heath during the late glacial period, and it is not excluded that certain heaths in western Norway (the most exposed coast heaths) are natural.

The British Isles[1]. In this place it is only possible to mention some of the most important features of the extensive literature on the heaths of Great Britain and Ireland, *viz.* those especially concerning the vegetation types rich in *Erica cinerea*. This species is universally distributed over the British Isles, being apparently only absent in certain East-Anglian heaths (FARROW 1925) and in alpine heaths. When compared with the Faroes and Norway, *Erica cinerea* is less selective as regards microclimate (southern exposition) and the humidity of the soil. FRITSCH & PARKER (1913), it is true, mention that *Erica cinerea* reaches its maximum domination on southern and south-western slopes in heath in South-England (Surrey). This also appears from LEACH's description (1925) of the heaths in the Cumberland mountains: "On the upper parts of the Northern slope where desiccation is less pronounced, *Calluna* is over a large area the sole dominant, and there is a well-developed *Bryophyte* flora amongst which shade mosses such as *Thuidium tamariscinum*, *Hylocomium splendens* and *Polytrichum formosum* are abundant. On the southern slope *Erica cinerea* occurs as a co-dominant with the *Calluna* or locally as sole dominant, and bryophytes are almost absent. On the other hand lichens appear in abundance on this drier slope". It appears, however, from numerous descriptions of the heath in other regions that *Erica cinerea* does not require a southern exposition. Even though it is beyond a doubt most frequent in dry soil, it may occur scattered in moist heaths (WATSON 1931) or in *Molinia* or *Scipus cæspitosus*-sociations together with *E. tetralix* and *Drosera rotundifolia* (ARMSTRONG, INGOLD & VEAR). *Erica tetralix* may for that matter exist locally without being associated with humid soil (FRITSCH & PARKER l. c. p. 155).

The heaths rich in *Erica cinerea* sometimes floristically very much resemble those of the Faroes; most frequently, however, they contain a good deal of *Ulex* (*U. Gallii, minor, europæus*). In Ireland *Dabeocia polifolia* (see fig. 7) may furthermore enter into

[1] A summary of English heaths and heather moors is to be found in TANSLEY (1939).

the vegetation (Praeger 1934). A special heath type is to be found on sand dunes and raised beach (Moore 1931, Good 1935). In such localities *Erica cinerea* occurs as a pioneer of the heath during the succession from dune or shore field to dune-heath. The floristic composition of the dune heath corresponds very well with that of the Danish dune-heath; only, in the Danish the *Ulex*-species, and *Erica cinerea* and in the English *Empetrum* are lacking; on the other hand there is hardly any resemblance between these English dune-heaths and the Faroes heaths.

Many heaths have arisen by the cutting down or burning of trees (e. g. Scotch pine forests with an undergrowth of *Calluna* and scattered *E. cinerea*, see Wyllie Fenton 1935). Where owing to the structure of the ground grazing animals are kept away, there is in Ireland instead of heath a copse wood in which *Taxus* frequently dominates, and in which *Erica cinerea* is to be found among *Ilex, Hedera, Calluna, Juniperus, Arctostaphylos uva ursi, Hymenophyllum* etc. (Praeger l. c.).

France. *Erica cinerea* enters as a dominant in the heaths of northern France (*Calluneto-Ericetum cinereae*). These comparatively dry heaths (Lemée 1937, p. 162) largely resemble the heaths of the Faroes (*Polygala serpyllacea, Hypericum pulchrum, Sieglingia, Nardus, Potentilla erecta, Galium saxatile*) but like most of the English ones, they are distinguished from them by dominating *Ulex* (*europæus*). Tüxen & Diemont (1936 analysis 1—3, p. 175) calls this vegetation the "*Erica-cinera Ulex europæus*-Assoziation".

South of Gironde the character of the heath vegetation (according to Mentz (1911) and Allorge) changes; the heaths of les Landes contain many Mediterranean plants (*Erica scoparia, vagans, Helianthemum alyssoides* and *Quercus Tozza*) but lack e. g. *Galium saxatile* and *Genista pilosa*. (cf. Weevers' analyses (1938) and of older investigations see those of Mentz (1911)). Both the heaths of northern France and les Landes have arisen after the destruction of foliferous woods (*Quercion roboris sessiliflorœ*, see Tüxen & Diemont, Lemée and Weevers).

Near the eastern limit of *Erica cinerea* in the southernmost part of France the latter, according to Braun-Blanquet (1915), appears as a constant together with *Calluna* in the undergrowth of the *Quercetum Ilicis* which has been almost destroyed by the agency of man. In advanced stages of destruction a heath of the character of a macki arises, with *Erica arborea, cinerea, Calluna,*

Sarothamnus and *Cistus*-species, *Buxus Teucrium scorodonia, Helianthemum* etc. a vegetation which out of above fifty species has only four in common with that of the heaths of the Faroes.

Spain and Portugal. In the north-western corner of the Pyrenees *Erica cinerea* occurs in great quantities in mountain heaths; together with *E. vagans* and *scoparia* it dominates the mountain heaths of the Cantabrian-Asturian mountains; ALLORGE (1927) has made an analysis from Lugo in north-western Spain, and here it i. a. occurs together with *E. umbellata* as also in the mountains of northern Portugal (WILLKOMM). Somewhat south of the northern range it appears in the upper part of the *Quercus Tozza* Wood on Sierra de Moncayo (WILLKOMM) and towards east RIKLI (1907) mentions it from a macki at a height of 900—1200 m. at Barcelona. (Here *Quercus ilex, Buxus* and *Viburnum tinus* were dominants.) According to WILLKOMM it dominates in mountain heaths in Portugal between a height of 750 and 1450 metres; it may, however, also occur in the lowland near the ocean, *viz.* in the undergrowth of the great pine forest Pinhal del Meiria, where it is i. a. found together with the Azorian *Myrica Faya.* It most frequently seems to be absent in the heaths of the eastern Pyrenees (the French part); here it may, however, be found in an open beech wood (GAUSSEN 1926, p. 357).

Madeira. VAHL (1904) mentions *Erica cinerea maderensis* from the upper shrub region (12—1800 m.) among a sparse rock-vegetation (cf. LOWE's (1868) statement: "The craggy summits of the highest central peaks"). The vegetation is here Mediterranean in character, and the Climate, from a thermic point of view, reminds of that of south-western Europe.

4. Contributions to the Aut-ecology of *Erica cinerea.*

A number of important features regarding the aut-ecology of *Erica cinerea* can be extracted from the preceding summary. The distribution of the species was designated as temperate-thermic-oceanic. As to this a further explanation is required. When going over measurings from climatologic stations within the area of the species and along its limits it appears that the yearly precipitation may vary con-

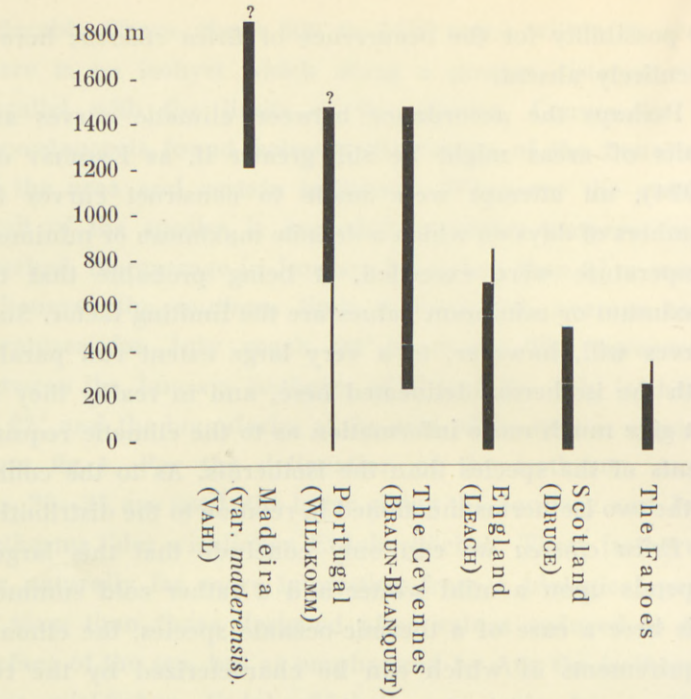
siderably (from about 600 to 1450 mm.) within its area; there is no isohyet which along a greater distance runs parallel with the limits of the species. Conversely, an accordance is found between the course of the boundary of the area and certain isotherms. Whenever the eastern limit of the species is exceeded, a winter temperature is reached, the average in January being less than 2° or more. Whenever the southern limit is exceeded, average temperatures for July reach 22° or more. The agreement between the January isotherm of 2° and the July isotherm of 22° and the boundaries of the area of the species appears from fig. 1. For the delineation of the isotherms Alt's figs. 30—31 are used, the latter giving the January and July isotherms "der wirklichen Erdoberfläche". These isotherms are naturally far more interesting from a biological point of view than those designed after values reduced to the surface of the sea, but as emphasized by Alt the isotherms "der wirklichen Erdoberfläche" can only be roughly drawn, seeing that they are to a very large extent dependent upon the height curves of the land; both local and certain regional details can often not be rendered in the small scale. The agreement between isotherms and boundaries of area is rather remarkable, when taking into account how difficult it is to make the small scale comprise details. In certain localities in the Ligurian coastal mountains the species has been regarded as a kind of Atlantic relic (Braun-Blanquet 1923, p. 133); it appears, however, that this area is cut off from the occurrences in Dauphine by an inflection in the January isotherm of 2°. Where the two said isotherms deflect from each other, the species begins to be common; where the two isotherms in Italy and on the Balkans, as it were, lie on top of each other, there is

no possibility for the occurrence of *Erica cinerea*; here it is entirely absent.

Perhaps the accordance between climatic curves and limits of areas might be still greater if, as ENQUIST did (1924), an attempt were made to construct curves for numbers of days on which a definite maximum or minimum temperature were exceeded, it being probable that the maximum or minimum values are the limiting factor. Such curves will, however, to a very large extent run parallel with the isotherms delineated here, and in reality they do not give much more information as to the climatic requirements of the species than the isotherms. As to the course of the two isotherms mentioned in relation to the distribution of *Erica cinerea* we can only conclude that this largely depends upon a mild winter and a rather cold summer; it is here a case of a thermic-oceanic species, the climatic requirements of which can be characterized by the two above-mentioned isotherms, though in such a manner that the limiting effect is not thought to be excercised by the mean temperatures themselves but by certain climatic conditions, or edaphic conditions dependent upon the climate (see later), which run parallel with the isotherms (cf. FÆGRI 1937).

As the northern limit of the species seems to be controlled by the winter temperature, it is probable that this also applies to its vertical limits. The graphic production given p. 26 shows the vertical distribution of the species from south to north within its area; a thin line indicates a rare occurrence. Both in Madeira and in Portugal the plant reaches the mountain tops for which reason the upward limit of growth is here uncertain.

Apart from the climate, *Erica cinerea* makes certain



edaphic requirements. In the first place it seems to be associated with relatively dry localities, as e. g. in the Faroes the south-western slopes. It is here worth mentioning that another thermic-oceanic species, *Carex binervis*, in the Faroes is not particularly associated with the southern slopes; this plant, however, can stand a much greater humidity of the soil. It is to be found in the heaths (see the tables) and furthermore in acidic grass land (Ostenfeld's *Carex binervis—Luzula silvatica*-Soc. Nordhagen's *Macroluzuletum*) and in *Nardeta* and bogs. In France it is a character plant of the moist heath series (Allorge, Lemée). It must, however, be borne in mind that the localities where *Erica cinerea* grows are frequently rather moist; in the thick peat which is a frequent habitat for it on the Faroes the

water capacity is very high. However, the soil under *E. cinerea* is only temporarily quite soaped, and the water does not stagnate in its habitats; this agrees with the fact that e. g. in England it thrives on the tops of tussocks in mosses and on brinks along watercourses running through the bogs where there is also a good drainage.

In this context it should also be borne in mind that *Erica cinerea* may occur in particularly dry dune-vegetation and e. g. in France in *Corynephoreta* (LEMÉE p. 38).

All authors are agreed to classify the species as a plant belonging to acid soil. We have the following measurings of the hydrogenion concentration in its habitats:

	p_H	
The Faroes	4.7—5.2 (Table 3)	(T. B.)
Western Norway	4.5—5.1 (Table 2, Nos. 7—8)	(T. B.)
Isle of Man (dune-heaths)	5.8—6.6	(MOORE 1939 p. 126)
England (Herefordshire)	4.8—6.2	(De SILVA 1934 p. 539)
Northern France (Perche)	4.4—5.0	(LEMÉE 1937 p. 191)
Southern France (Heath plantation)	4.4—5.8	(WEEVERS 1938)

All in all, it seems that the species is able to stand p_H values from 4.4 to 6.6; in proportion to *E. tetralix* the values are high; *E. tetralix* is most frequently found in soil with p_H round 4.0. *E. cinerea* both thrives in podsolated soil (France according to TÜXEN & DIETMONT (pp. 176—179) and LEMÉE (p. 183)) and in non-podsolated soil (the Faroes). In southern regions it looks as if the species is associated with montane soils and avoids the Mediterranean

neutral-basic ones[1]. A glance at the maps figs. 115a and b in Braun-Blanquet (1928) gives one the impression that the southern limit of the species is very largely conditioned by climatic-edaphic factors. The boundary of the Mediterranean soils and the course of the lines with the same N—S quotient[2] in southern France practically coincides with the July isotherm of 22°. It also appears that the absence of the species in the upper Rhône Valley practically falls within an area of "brown earth" on the transition to Mediterranean soil, whereas to the west in the Cevennes and to the east in the westernmost Alps it occurs in montane soils.

5. Some studies of the other species of the *Calluna-Erica cinerea*-heath.

After this summary of the ecology of *Erica cinerea* we will, in greater detail, go over the list of plants in Table 2 and try to give an account of the biological distributional type to which the various species belong. We may divide the species into three groups: the northern, the temperate and the temperate-oceanic; among the northern there are a few which may be called northern-oceanic (see the table). The justification for these terms will appear from the literature regarding the distribution of the species. The groups might naturally be further subdivided: among the temperate-oceanic ones it was possible to separate a northern sub-group (*Calluna, Erica tetralix, Hypericum pulchrum,* var. *procumbens, Narthecium*) and a southern one (*E. cinerea, Carex binervis*). One of the principal difficulties in the

[1] p_H 6.8—7.4 (Braun-Blanquet 1928, p. 149). As to p_H measurings in similar (North-African) soils see Lüdi (1939).

[2] Niederschlag: Sättigungsdefizit.

way of a proper classification of species in a system of distributional types is the variation of the species, *viz.* their contents of races with a different ecology (see BÖCHER 1938). It is, however, a very interesting task to try to conquer this difficulty. Beyond a doubt, many species occur in the Faroes in races which in various ways are adapted to an oceanic climate. Such species should then on the Faroes also be termed oceanic, seeing that biologically they belong to the species which may upon the whole be termed oceanic (*Erica cinerea*). In the table I have for the time being elected to mark with an O? the species which I think occur in oceanic races, while O is used in the sense of oceanic.

Below a list will be given of some of the species which in various ways have caused difficulties with regard to classification of the biological distributional types to which they belonged. Mention is partly made of studies of their variation, partly of their distribution.

1. *Anthoxantum odoratum* L. Eurasiatic species which towards west reaches Greenland, the Azores and Madeira. Material from two localities in the Faroes was transplanted to Denmark. As compared with Danish and Norwegian races the Faroes plants flower 2—3 weeks later and has fewer panicles.

2. *Molinia coerulea* (L.) MOENCH. occurs on the Faroes in small individuals. TURESSON (1930, p. 124) has shown that the progeny of Faroes plants in culture attain an average height of 61 cm., whereas plants from Scotland on an average reached 96 cm. Also *Succisa pratensis* occurs on the Faroes in a genetypically low growing race (TURESSON l. c.).

3. *Carex pilulifera* L. The Faroes plant deviate from the normal one by not being cæspitose; it has up to 10 cm. long rhizomelike stems, hidden in the peat (fig. 12 A in BÖCHER 1937 b). Besides the individuals collected by myself on Klakken similar individuals were identified by OSTENFELD (Trangisvaag) and C. JENSEN (Strømø). No really cæspitose individuals exist. It is

evidently the same plant which Beeby (1887) found on the Shetlands. Beeby's collection (No. 757 west slopes of Saxa North Hill Unst) is in full accordance with the material from the Faroes; in his paper it is mentioned as "a form with slightly creeping and rooting stems, not at all cæspitose". After having seen Beeby's material I dare not deny that we are dealing with a special race, not a modification. Cultivation experiments are here greatly needed. The type mentioned above from the Faroes and the Shetlands bear a considerable resemblance to *Carex azorica* Gay (Gay Annales des Scienc. Nat. II Series, Vol. 11 1839 p. 185, and illustration in Trelease 1897, plate 48). This species also sometimes has a rhizome-like subterranean stem, but is clearly distinguished from the rest by having more closely joined spikes, two styles and in consequence flatter fruits.

4. *Potentilla erecta* (L.) Hampe. This species is particularly polymorphous, special studies on the polymorphy of Danish individuals being found in H. E. Petersen (1926). It is an Eurasiatic, widely distributed plant, which towards west reaches the Faroes, the Azores and Madeira. In the Faroes it is of particularly common occurrence. The Faroes plants resemble Danish types, but are on an average smaller (modifications?). Plants from western Norway, Lizard Point, the Hebrides and the Shetlands entirely resemble the Faroes individuals. Large, robust, and large types with narrow leaves I have seen from the region of Oslo, from Finland, Russia and France. Cultivation experiments with different types have been commenced.

5. *Hypericum pulchrum* L. As to the distribution see in particular Braun-Blanquet (1923) and Køie (1939). A map of the most northerly localities along the coast of Norway is found in Nordhagen (1917, fig. 12).

The typical plant particularly belongs to the oak region of western Europe; it grows in oak forests, oak shrubs and in hilly, mossed heaths in Jutland. In the Faroes it is rather rare, being f. inst. found in the lowland at Thorshavn. It may be defined as a temperate-suboceanic species.

As opposed to the principal type, *H. pulchrum* var. *procumbens* Rostr. is of common occurrence in the Faroes, particularly in the lowland in heaths (Table 2), in *Nardus* and *Anthoxanthum* sociations (Jensen 1897, p. 183, Ostenfeld 1907, p. 79) and more rarely in the mountains; here it may be met with in south-facing

herb-fields rich in species, at a height of 500 m. above the sea (Böcher 1937 b, p. 178). The distribution of this variety is rather peculiar; besides in the Faroes it is to be found in the Shetlands, where Beeby (1887) mentions it as frequently occurring on "serpentine hills about Baltasound"; later (1888) he also mentions a find of the upright main type from "a deep and sheltered ravine". In the Orkneys it is known from three localities (Bennett 1915, Johnston 1921). In Scotland var. *procumbens* according to Williams (1901—12, p. 558) occurs near Holburn Head, Caithness and, according to G. Cl. Druce (1929) on a mountain 743 m. high at West Ross immediately at the sea. Wilmott states that it occurs on the outer Hebrides (communication by letter from Dr. H. G. Pugsley). In Ireland it occurs, according to Praeger (1934), in two of the westernmost localities, on Clare Island (in the highest point together with several Alpine species) and on Achill Island (on the promontory Croaghan). The variety ought to be looked for in western Norway where it is not known (Dr. K. Fægri, communication by letter). It consequently looks, as if var. *procumbens* has an extremely narrow area of distribution, which is pronouncedly oceanic and relatively northern (fig. 7).

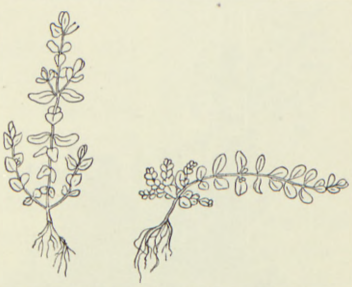


Fig. 2. 11 weeks-old individuals of *Hypericum pulchrum* var. *procumbens* Rostr. (to the right) and a typical *Hypericum pulchrum* (to the left). Both figures × 1/2.

Beeby (1887) writes: "The var. *procumbens* remains unaltered in cultivation; entirely maintaining its prostrate habit and three-flowered cyme." In spite of this observation Brown (1891) regards var. *procumbens* as a modification, but my investigations agree with those of Beeby. Plants which were transplanted from the Faroes in 1935 have still kept their characteristic appearance. Comparative investigations of progeny individuals of typical plants from Denmark (two localities) and from the Faroes (var. *procumbens* from two localities) yielded the same result; already quite small, three months-old individuals of the Faroes type prove to be prostrate, and as compared with the main type they have a far greater number of small basal shoots (figs. 2 and 3). The growth takes place somewhat more slowly in var. *procumbens* than in

the main type. In the autumn a characteristic difference appears, in that the Danish type at the beginning of September dies down to the perennial parts in the surface of the soil, whereas var. *procumbens* remains green until November and, in a green-house free from frost, for the greater part of the winter. Both types have the same chromosome number (2 n = 18, material from the Faroes, see fig. 4, and from Randbøl Heath in Jutland). The number

Fig. 3. Progeny individuals (cultivated under the same conditions and sowed at the same time) of *Hypericum pulchrum* var. *procumbens* Rostr. from Husaraun at Thorshavn (T. B. 110) and a typical *H. pulchrum* from Kaas in Jutland (T. B. 48). The plants are three years old, No. 48 is 10—15 cm. high.

corresponds with former countings of *H. pulchrum* (Chattaway 1926, in Brit. Jl. Exp. Biol. 3, p. 141).

6. *Arctostaphylos uva ursi* (L.) Spr. In the Finmark and several other localities this species has a continental character (see Böcher 1937 a); it is, however, frequently found in dry localities along the southern part of the west coast of Norway, frequently on southern slopes, i. e. in similar localities as *Erica cinerea*. I saw great quantities of this species at Haugesund and Mandal, and it also occurs in Scotland and Ireland and in several localities along the western coast of Europe. The fact that it avoids the coast of northern Norway may perhaps be due to its requiring a relatively high summer heat. The species may be regarded

as a boreal dry-soil plant, without any connection neither with oceanity nor with continentality.

7. *Calluna vulgaris* (L.) Salisb. The species, it is true, penetrates far towards east, but occurs most frequently in north-western Europe. Towards east it is particularly selective as regards the microclimate of the habitat. Its eastern boundary (MATTFELD 1929, BEIJERINCK 1936) runs parallel with the oceanic coast of Europe from northeast to southwest. The species is northerly-temperate and suboceanic.

8. *Brunella vulgaris* (L.). This species may be found scattered in the oceanic heaths of the Faroes and in western Norway, but is most frequent in different types of meadow. The Faroes plant is a genotypically low, early-flowering race (BÖCHER 1940), the closer relationship of which with other races is to be examined in detail, but which shows certain points of similarity with Mediterranean plants.

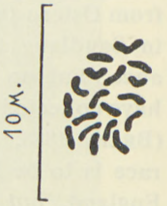


Fig. 4. Mitosis from the root-tips of *Hypericum pulchrum* var. *procumbens* Rostr. 2 n = 18.

9. *Plantago maritima* L. Very strongly polymorphous (Literature in BÖCHER 1938, p. 180). A number of transplantings of Faroes plants to Denmark shows thats the many Faroes types very largely maintain their characteristic appearance. In the Faroes the species enters into heaths, fell-fields and snow-patches; besides it is found on the beach and in meadows in the lowland. The investigation of the geographical and ecological conditions of the many Faroes types is still so defectively examined that I only dare to consider it probable that a number of the many races are oceanic and western-European.

10. *Plantago lanceolata* L. This species occurs in the Faroes in several varieties, some of which resemble Danish types. Particularly deviating from the Danish material is a nearly lanuginous type. Cultivation and transplant-experiments show that this race is particularly different both from Danish and Faroes races belonging to var. *dubia* (L.) Wahlenb. (cf. figs. 5—6). All the races examined have the same chromosome number (n = 6). When dealing with *Plantaginaceae* (in »das Pflanzenreich«) PILGER (1937) has subdivided *P. lanceolata* into a number of varieties; the hairy Faroes variety must be classed with var. *dubia* subvar. *eriophora* PILGER. Var. *dubia* is as a rule regarded as a southern group of races, and subvar. *eriophora* is said to belong in Middle France

and in the Mediterranean area. The most important difference between my *eriophora*-race from the Faroes and my cultures of var. *dubia* subvar. *eudubia* (= *eriophylla*) lies in the much greater hairiness and the thicker and stiffer leaves of the Faroes plant[1].

The herbarium of the Botanical Museum of Copenhagen contains plants of the above-mentioned hairy Faroes type from four localities (Strømø and Bordø), to which I may i. a. add a locality from Østerø (see below). Only one plant, collected by Steenstrup in Saudlaugsdalur on Iceland, seems to approach it in appearance; but on Vestmannaeyjar Helgi Jónsson and Ove Paulsen have collected entirely corresponding plants. Also in the Shetlands (Beeby 1886, Nos. 661—662) and in Scotland (Druce 1929) this race is to be found, and Dr. Pugsley suspects "that it grows in England, but has not been distinguished" (communication by letter). As to southwestern Europe I have seen it from Les Sables d'Olonne in France and from Coruña and Pontevedra in Galizia. In the Azores the subvar. *eriophora* is widely distributed; the material from there at one time led to the setting up of a new species, *Plantago azorica* Hochst, which entirely corresponds with the Faroes plant (see Plate VII, fig. 2 in Seubert (1844)); according to De Candolle (Prodromus Pars XIII, p. 715) types corresponding with *P. azorica* are to be found "in arenosis Europæ mediæ", in sand-dunes in western France, on the top of the 900 m. high mountain Foia in Algarve, Portugal, and in the Azores, Madeira and the Canaries. If we limit ourselves to the plants which distinctly resemble those of the Faroes[2], it looks as though we face a pronouncedly Atlantic distribution; when considering Pilger's distribution of subvar. *eriophora* (and De Candolle's central-European material, in all probability from France) the race rather becomes Mediterranean-Atlantic. The Danish material of var. *dubia* only slightly approaches the Faroes type from the point of view of hairiness and must thus be referred to "*eudubia*". No Norwegian individuals are so longhaired as subvar. *eriophora* (Fægri, communication by letter).

Ecologically there is some resemblance between *eudubia* and *eriophora*. In Denmark the former is often found in dry fields and on hills, whereas the large, smooth-leaved upright races belong near roads and in inhabited places. Smooth-leaved races are

[1] See further Pilgers Diagnoses (pp. 322—23) of subvar. *eudubia* and *eriophora*.

[2] Black dots without signs of interrogation on fig. 7.

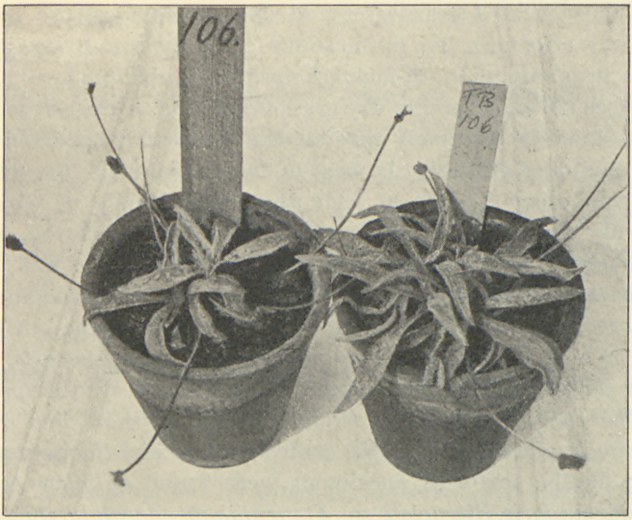


Fig. 5. *Plantago lanceolata* subvar. *eriophora* PILGER from the Faroes (Kodlur) transplanted to Copenhagen. The upper diameter of the flower-pots: 10 cm.

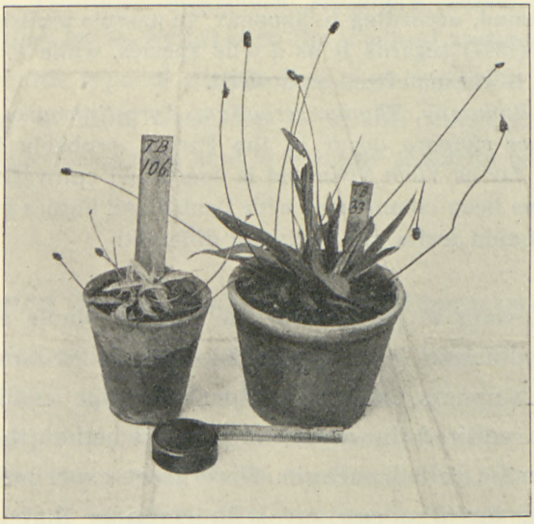


Fig. 6. *Plantago lanceolata* subvar. *eriophora* PILGER (to the left) and *Pl. lanceolata* subvar. *eudubia* PILGER (to the right); both from the Faroes and cultivated in Copenhagen. The tape measure stretched to about 10 cm.

found in cultivated grass-land and near villages on the Faroes, while var. *eriophora* live in the south-facing *Calluna-Erica cinerea*-heaths, in subalpine south-facing *Empetrum-Vaccinium* heaths, rich in species (*Dryas!*), and in dry grass-vegetation. A grass-vegetation of this kind is extremely rare in the Faroes; it occurred on a slope inclining 40° towards west in the direction of the sea, on the promontory Kodlur at Ejde the local climate of which is relatively dry. The vegetation was almost entirely closed. There were hardly any mosses, and the soil was here and there visible among the plants. Dominants were *Holcus lanatus* and *Vicia cracca* (in a peculiar race, which reminds of the one to be found in our sand-dunes). Common were: *Succisa pratensis, Polygonum viviparum, Thymus serpyllum, Ranunculus acer, Empetrum, Angelica silvestris, Armeria vulgaris, Festuca ovina* and *rubra, Plantago lanceolata* (*eriophora*) and *maritima* and *Botrychium lunaria*; finally, scattered specimens were found of *Silene acaulis, Brunella vulgaris, Hieracium* sp., *Athoxanthum odoratum, Habenaria viridis, Trifolium repens, Rumex acetosa, Euphrasia* sp. and *Equisetum arvense*. This vegetation was not very much influenced by the sheep, which in this place are kept off during the whole of the summer season. In the Azores *Plantago lanceolata* var. *eriophora* is found, according to Seubert "in pascuis pratisque siccis" Trelease (1897) regards it as a wild species, while *P. lanceolata* (*genuina*) is characterized as a weed.

Viola Riviniana, Thymus serpyllum, Agrostis tenuis and *canina* and *Festuca vivipara* occur in the Faroes, probably in special races; as far as *Viola Riviniana* is concerned cultivation experiments have been commenced with Danish and Faroes plants. The transplantation series are distinctly different.

If we want to prove that the Faroes plants are genotypically different from the Danish ones, is the work before us, as it appears, rather considerable; but a still greater work is required in order to prove whether the Faroes races can be called oceanic. Here great experiments must be made, for who can say with certainty that a race is oceanic, before he has cultivated it from numerous both oceanic and continental habitats, and perhaps besides has

made various physiological experiments with the races found?

When using the biological types of distribution in the following, we must consequently bear in mind that the percentage of temperate species is undoubtedly too high, while the percentage of the oceanic ones is too low. In particular the percentage of the oceanic ones will rise, if *Potentilla erecta*, which occurs with F$^0/_0$ 80—100, is included in the oceanic species.

Among the above-mentioned distributional types of the Faroes heath-plants there is one, the relatively southern temperate-oceanic type which demands particular attention. It is represented by quite few species, *viz. Erica cinerea*, which forms a transition between the oceanic and the Mediterranean-Atlantic species (*Ilex*); besides there is *Carex binervis* and, in so far as may be seen from the information at hand, *Plantago lanceolata eriophora*. Also *Anagallis tenella* which grows by lakes in the lowland and *Scilla verna* belong to this type of distribution, but *Anagallis tenella* is according to Lüdi (see fig. 7) almost entirely Mediterranean-Atlantic, seeing that it extends towards east as far as Crete.

6. Biological Distributional Types and Character-Species.

On a former occasion (1938, pp. 289—290) mention was made of the agreement which sometimes exists between the geographical distributional types and the character species (in the sense of Braun-Blanquet), particularly such as were based upon the climatic demands of the species. Outside the Scandinavian literature Lemée in his monograph on Perche also makes use of types of distribution.

As to his *Corynephoretum occidentale* he says e. g. that it contains "11 espèces atlantiques (dont 4 caractéristiques) soit 14 % de l'ensemble, et 6 sub-méditerranéennes (dont 5 caractéristiques) soit 6.6 %. C'est une association d'aire atlantique à affinités méridionales". As basis for the classification of the vegetation Lemée, however, as usual makes use of the character species.

Meusel quite lately (1939) has attempted to create a foundation for the investigation of vegetation, which largely builds on the total distribution of the species. In the case of each plant community its "pflanzengeographischer Charakter" is mentioned, and special weight is attached to an examination of the area of the "Leitarten" of the plant community (i. e. species which within his area of examination are more or less closely connected with a special community).

In the following an attempt will be made to divide the Faroes heaths on the foundation of the biological distributional types (which term may appropriately be abbreviated to B.D. types). In the same manner in which Raunkiær calculates his biological spectrum of life forms, it is possible to calculate the biological spectrum of distributional types. This material of figures is in the case of the vegetation analyses here published grouped in Table 4. The B.D. percentage is partly based on the list of species partly on the frequency percentage within $^1/_{10}$ m^2 large circular planes (cf. Raunkiær), partly within small (0.006 m^2), the method followed being the modified Raunkiær method (Böcher 1935)[1].

[1] This method can briefly be described as follows: The frequency of the species is examined, not only within a number (here only 10) of circular planes of $^1/_{10}$ m^2, but at the same time within circular planes

From the spectrum of biological distributional types the following facts appear:—

1) In the material from Table 1 a considerable oscillation from dominance of northern to dominance of oceanic species, at the transition from a northern to a southern slope.
2) In the material from Table 2 a rise in the oceanic phanerogams at the transition from the more humid, mossy *Calluna*-heaths, to the drier *Calluna-Erica cinerea* heaths, somewhat poorer in mosses. This fact is interesting, because it reflects the different ecology of the two groups, the phanerogams and the mosses. The former are highly thermic-oceanic, whereas the mosses are highly hygric-oceanic.
3) The characteristic differences between the vegetations examined appear most distinctly when using the figures

of 0.056, 0.025 and 0.006 m^2, the one overlapping the other; the four different sizes of circles are designated 1 (0.1 m^2), 2, 3 and 4 (0.006 m^2). Besides the frequency is examined within a plane of about the size of a halfpenny (the "centre" of the circles); this very small circle which almost corresponds with a point in the New Zealand point method is termed 5. The densest species naturally obtain the highest frequency percentage within the very small circles; the species which almost entirely cover the soil become dominants, even when the size of the circle is as small as a half-penny. For each of the species in the tables (1—2) are indicated: 1) the frequency percentage within circle 1 (0.1 m^2); 2) the frequency percentage within circle 4 (0.006 m^2), and 3) the minimum area (i. e. the one of the 5 sizes of circles which must be used, in order that the species may get the frequency percentage 100). The figures **100**.50.2 thus mean: The species is a dominant within circle 1, gets 50 % within circle 4 and will not be a 100-percent-dominant, if the size of the circle becomes less than that of circle 2 (0.056 m^2). The average density of its shoots is then about 20 cm.

Analyses made by means of this modified RAUNKIÆR method is in the tables marked *S*, while determinations of degree of covering (HULT-SERNANDER) are marked *D*.

Table 4. Spectrum of

Analysis		Northern species %	Oceanic species %	Temperate species %
Table 1, No. 1...	Number of species	30.8	23.1*	53.8
Table 1, No. 2...	Number of species	20.0	35.0*	50.0
Table 1, No. 3...	Number of species	23.5	23.5*	58.8
	Frequency: Circle 1	47.4	14.1*	48.7
	Frequency: Circle 4	**68.4**	15.8*	28.9
Table 1, No. 4...	Number of species	13.3	26.7	60.0
	Frequency: Circle 1	23.4	28.6	48.0
	Frequency: Circle 4	26.5	**47.0**	26.5
Table 1, No. 5...	Number of species	64.3	14.3*	28.6
	Frequency: Circle 1	83.7	16.2*	13.5
	Frequency: Circle 4	**87.2**	12.8*	10.3
Table 2, No. 1...	Number of species	9.5	33.3	57.2
Table 2, No. 2...	Number of species	5.9	35.3	58.8
Table 2, No. 3...	Number of species	5.9	35.3	58.8
Table 2, No. 4...	Number of species	21.7	21.7*	60.9
	Frequency: Circle 1	8.5	25.6*	69.1
	Frequency: Circle 4	6.9	**41.4***	**55.2**
Table 2, No. 5...	Number of species	5.0	40.0*	60.0
	Frequency: Circle 1	0.0	38.9	61.1
	Frequency: Circle 4	0.0	**56.0**	44.0
Table 2, No. 6...	Number of species	4.8	28.6	66.6
	Frequency: Circle 1	1.2	35.6	63.2
	Frequency: Circle 4	0.0	**55.0**	45.0
Table 2, No. 7...	Number of species	4.0	24.0	72.0
	Frequency: Circle 1	1.1	31.9	67.0
	Frequency: Circle 4	3.4	**55.2**	41.4
Table 2, No. 8...	Number of species	4.4	30.4	65.2
	Frequency: Circle 1	4.3	39.1	56.5
	Frequency: Circle 4	4.2	**62.5**	33.3

* In the calculation the northern-oceanic species are included.

The percentage of lichens and mosses is calculated on the base of the percentage is a great help towards the classification of heaths. In this case mosses are of far greater importance in the vegetation than the lichens. The continental heaths, where the lichen percentage increases. If with regard to the percentage for northern (*Jungerm. Floerkei, J. qvinqvedentata, Polytrichum* to be a difference in the occurrence of these two types within heaths rich the former contains more northern species than the latter and this in its mosses are marked with an O.

Mosses %	Lichens %	Number of species		Points	
		Phanero-games	Mosses + Lichens	Phanero-games	Mosses + Lichens
92.3	7.7	13	8	—	—
100.0	0.0	20	13	—	—
100.0	0.0	17	8	—	—
100.0	0.0	—	—	780	530
100.0	0.0	—	—	380	300
100.0	0.0	15	7	—	—
100.0	0.0	—	—	770	410
100.0	0.0	—	—	340	220
64.3	35.7	14	14	—	—
68.9	31.1	—	—	740	740
64.9	35.1	—	—	390	370
100.0	0.0	21	5	—	—
100.0	0.0	17	8	—	—
100.0	0.0	17	7	—	—
100.0	0.0	23	12	—	—
100.0	0.0	—	—	820	550
100.0	0.0	—	—	290	310
90.9	9.1	20	11	—	—
97.3	2.7	—	—	590	370
100.0	0.0	—	—	250	220
100.0	0.0	21	9	—	—
100.0	0.0	—	—	870	350
100.0	0.0	—	—	400	130
91.7	8.3	25	12	—	—
100.0	0.0	—	—	910	550
100.0	0.0	—	—	290	200
57.1	42.9	23	7	—	—
93.3	6.7	—	—	690	150
100.0	0.0	—	—	240	60

number of species and points for mosses and lichens. This lichens-mosses it had naturally not been necessary to calculate it, as it is evident that the calculation has been undertaken to provide values for comparison with more the mosses the percentage is calculated for oceanic (Western-European) and *alpinum, Andræa petrophila, Mylia* cf. C. JENSEN 1901) there will also prove in *Empetrum-Vaccinium* and *Calluna-Erica cinerea* respectively, seeing that turn more oceanic species than the former. The oceanic western-european

computed on the strength of the frequency within circle 4, and it must be remembered that only the very densest species can attain greater values, when this small circular plane is used. On the other hand, dominants and scattered species have the same numerical value in the computation of the figures, which are based upon the number of species. The value of Raunkiær's method, and more particularly its modified form, is clearly very great, and too little attention has been paid to it. The usual life-form percentages based on numbers of species, which are contained in numerous works[1] written by foreign scientists, ought to be supplied with life-form percentages based on frequency figures.

4) By means of the biological types of distribution it is possible to distinguish between 3 types for the here examined heaths: 1. The heaths rich in *Empetrum-Vaccinium* (Nos. 3 and 5, Table 1) characterized through a percentage for northern species of 68 and 87. 2. *Calluna*-heaths (No. 4, Tables 1—2) with 47 and 41 per cent. of oceanic species, and 3. *Calluna Erica cinerea*-heaths (Nos. 5—8, Table 2) with 56, 55, 55 and 62 per cent. of oceanic species. If the oceanic species were divided into relatively northern and relatively southern ones, a clearer distinction of type 3 in proportion to type 2 would be obtained, seeing that *Erica cinerea* would cause the southern species to obtain a relatively high value in type 3, as distinguished from type 2, where the southern species would hardly obtain any values worth mentioning.

[1] Cf. e. g. the critical remarks of Diemont (1938, p. 42) to the effect that the proportion of quantity of the species is not expressed in life-form spectra, as the latter are computed on the strength of the number of species. The original life-form spectra of Raunkiær at any rate do not suffer from this deficiency! (cf. Raunkiær 1934).

It should not always be a dominans of a given distributional type which is to make the foundation of the division; only a constant occurrence of a certain type of distribution in a series of analyses should be used to divide these into a special type of vegetation. It will, however, then be evident that there is no very great difference between the use of distributional types and the use of character species in the division. It therefore also appears that the character species, which are used for the division of heaths in different countries, very often belong to the same type of distribution (oceanic character species in western European heaths, continental in "Pontic" etc.).

However, in spite of the similarities, this is not merely an argument about words i. e. whether one wants to use the one or the other term in a division of vegetation. There is a great difference in the degree of objectivity obtained. Many character species are classified in the following manner: first the plant community is examined and the species found, which are supposed to be more or less closely connected with it; some species of the community are selected, which seem to have an ecologically narrow amplitude within the given area; finally, the community (association) is characterized by means of these species. Here it is not possible to a certain extent to avoid circular conclusions, and besides many character species can only be used within a limited area, "regional character species". Conclusively the choice of character species is naturally more or less subjective, being e. g. dependent upon the experience of the individual botanist. If on the other hand B. D. types, which are based on the total distribution of the species, are used in the division, this will be more objective, and at any rate one does not commit any offence to way

of circular conclusions. It is a different matter that the B.D. types are sometimes difficult to cope with, because the work required in the geographic-racebiological investigation of the species is so very great. However, I am of the opinion that all plant-geography and ecology in a greater or lesser degree suffers from the drawback that the unities used for the investigations (i. e. the species) are far too little known. It may thus be said with perfect justice that the road is long and arduous, but on the other hand I think that it is necessary to go on, in order to attain a greater exactitude and stringency of the problems bearing upon biologic plant-geography. For that matter, the character species used are surely only serviceable within a given area, because they here occur in comparatively few biotypes (races). The race-biological studies are likewise very valuable for the further substantiation of character species; often the best character species are found among the microspecies and races of the species (ecotypes).

The classification principles by means of B.D. types, which have been sketched above, apparently suffer from the drawback that the B.D. type of the species, in a way, is apt to be relative: a northern type in the flora of the Faroes will become southern in Greenland. Still, the oceanic and the continental requirements of the species will be determined once for all. If one only always knows the exact total distribution of the species (the variety), it is easy to say whether it is northern, southern etc.

7. The Faroes heath in relation to other heath-types.

It appears from the preceding studies that the Faroes heath types may recur in western Norway and in the British

Isles (particularly in the north or montane). How then does the heath-vegetation change when starting from the Faroes-West-Norwegian heaths, we pass to 1) more northerly, 2) more southerly, 3) more continental regions?

1—2). Along the Atlantic coast of Europe, a distinction may be made between at least 5 different heath types (not taking the moist heath series into account). Each of these consists of a number of sociations which we cannot here deal with in detail. The northernmost type is a *Rhacomitrium hypnoides* heath, in which dwarf shrubs frequently may be present in rather large quantities; the transition e. g. to *Empetrum Rhacomitrium* heath is quite gradual. Already at Lofoten, in sheltered places, *Empetrum*, *Calluna*- or *Vaccinium myrtillus-Hylocomium* sociations occur (Du Rietz 1925, pp. 47—50). Farther south (in Møre) there is a transition from heaths rich in *Rhacomitrium* to heaths rich in *Hylocomium*, and at the same time *Calluna* and *Erica cinerea* play the principal part in drier soil, while *Vaccinium myrtillus* rather keeps to moist northern slopes. The development of the heath farther south (British Isles, France, Spain, Portugal) has been mentioned earlier in this work. In England and northern France one passes from *Calluna-Erica cinerea* heath to *Calluna-Erica cinerea-Ulex* heath, which again in southern France are replaced by the heaths of Les Landes (e. g. *Erica scoparia-Molinia*-Soc.) which forms a transition type to Macki. South of France the heath becomes montane (the Pyrenees, northern Portugal, the Azores); on the Continent *Erica vagans, cinerea, scoparia* and *umbellata* are the dominants in heaths; in the Azores there is on the other hand a characteristic *Calluna-Thymus-Dabeocia* heath (Guppy 1917).

The vertical zonation corresponds very well with the

horizontal one. In his description of the mountains of Cumberland Leach (l. c. p. 89) says that the heath (*Calluna*, frequently with *Erica cinerea*) is the dominating type of vegetation between a height of 400 and 650 m. At 650 m. above the sea *Calluna* reaches a vertical frequency limit, seeing that it is only found in sheltered localities higher up in the mountains. The heath here consists of *Empetrum* and *Vaccinium myrtillus* and *vitis idœa*, but is otherwise restrained by Alpine grass-land (*Festuca vivipara-Alchemilla alpina*). On the tops fell-field and *Rhacomitrium hypnoides* heaths are found. In the mountains of Wales similar conditions prevail according to Price Evans (1932). *Calluna* here reaches its optimum between 335 and 488 m. above the sea. The *Ulex*-species are associated with the lowland. Above 488 m. *Empetrum*, *Vaccinium myrtillus* and *Festuca vivipara* become more frequent. Nearest the top (762—884 m.) there are lichen or *Rhacomitrium* heaths, frequently with *Empetrum* and *Vaccinium*. There is no doubt that the English *Calluna* zone corresponds with the Faroes Atlantic-cold-temperate zone (0—300 m. above the sea) which higher up is replaced by a transition zone, dominated by grass-vegetation (*Nardus*, *Festuca vivipara*), and then again at a height of 500 m. above the sea merges into the Alpine zone with fell-field, *Rhacomitrium* heaths and snow patches (Böcher 1937 b, p. 196).

3) The heath in the Faroes and W.-Norway (partly) is situated within the "maritime Kahlregion" (Du Rietz); in an easterly, more continental direction the heaths are frequently alpine or conditioned by culture, which makes comparisons difficult. If, however, we keep to the coast zone, allied heaths will be found in more continental regions in about the same latitude as the Faroes. The more

continental type corresponding with the *Calluna-Erica cinerea* heath with a southern exposition seems to be a *Calluna* or a *Calluna-Arctostaphylos uva ursi* heath. At Mandal and in several other places in southern Norway there were such dry heaths on southern slopes, while the northern slopes had a *Calluna* vegetation with much *Vaccinium myrtillus* (at Mandal f. inst. *Empetrum, Vaccinium vitis idœa, Cornus suecica, Arnica, Deschampsia flexuosa, Potentilla erecta* and *Hylocomium* species). Such heaths are, however, practically not to be distinguished from most of the Danish ones, and therefore it is surely justifiable to regard our *Calluna-Arctostaphylos* and perhaps also *Calluna-Empetrum* or the *Calluna-Empetrum-Vaccinium vitis idœa* heath with *Hylocomium* and *Cladina* (cf. Mølholm Hansen, 1932) as sub-oceanic types corresponding with the euoceanic heaths of the Faroes and western Norway.

Tüxen & Diemont (1936) describe the transition in the heath-vegetation from west to east in the lowland of north-western Europe. The western *Erica-cinerea-Ulex europœus*-Assoziation (with *Calluna*) is, in Germany, replaced by the sub-oceanic *Calluneto-Genistetum*, which in its turn further east gives way to a still more continental *Calluna-Antennaria*-Assoziation. In Jutland there is an interesting transition between the Scano-Danish heath (*Calluna, Empetrum, Vaccinium vitis idœa, Arctostaphylos uva ursi* and northerly exposed *Vaccinium myrtillus*, now and then *Cornus suecica*) and the more southerly *Calluna-Genista* heath.

It is evident that both in the direction east to west and north to south it will be possible to distinguish these heath types objectively by means of the computation of the B.D. percentages, and at the same time it will be possible in that way to obtain an ecological-geographical characterization

of the heaths in question. It is the idea to attempt something in this line in a future work on Scano-Danish heaths.

8. Problems regarding the History of the Heath Flora of the Faroes.

Ostenfeld (1901 b) and Warming (1903) were of opinion that the Faroes during the glacial epoch had been entirely devoid of plants and that the flora had immigrated postglacially by a landbridge (Ostenfeld), or across the sea (Warming) from the British Isles. The investigations of later years have to some extent changed the idea of conditions during the glacial age. A number of botanical and zoological arguments have been advanced in support of the idea that, during the last glacial epoch, a rather large fauna and flora must have been able to live in several localities of the North. As to the Faroes the zoological material (Wolff 1928, Spärck 1929, West 1929, Lindroth 1931) point very much in the direction that the Faroes cannot have been entirely glaciated during the last ice age; a number of animals must be supposed to have lived in the islands since the last interglacial epoch. In the freshwater fungus *Heteromeyenia ryderi* Spärck has further found an American element in the fauna of the Faroes[1]. This species is at present widely distributed in Atlantic America (from Nova Scotia to Florida), but in Europe it is only found in the Faroes, on the island Mull off western Scotland and in western Ireland. There is here a parallel of the American element in the flora of Ireland, and this element is regarded as a relic of the last interglacial period (Fernald, Nordhagen, Praeger), where there is supposed to

[1] In *Habenaria albida* var. *straminea* Morris we have an example of an American element in the flora of the Faroes (see Fernald 1929).

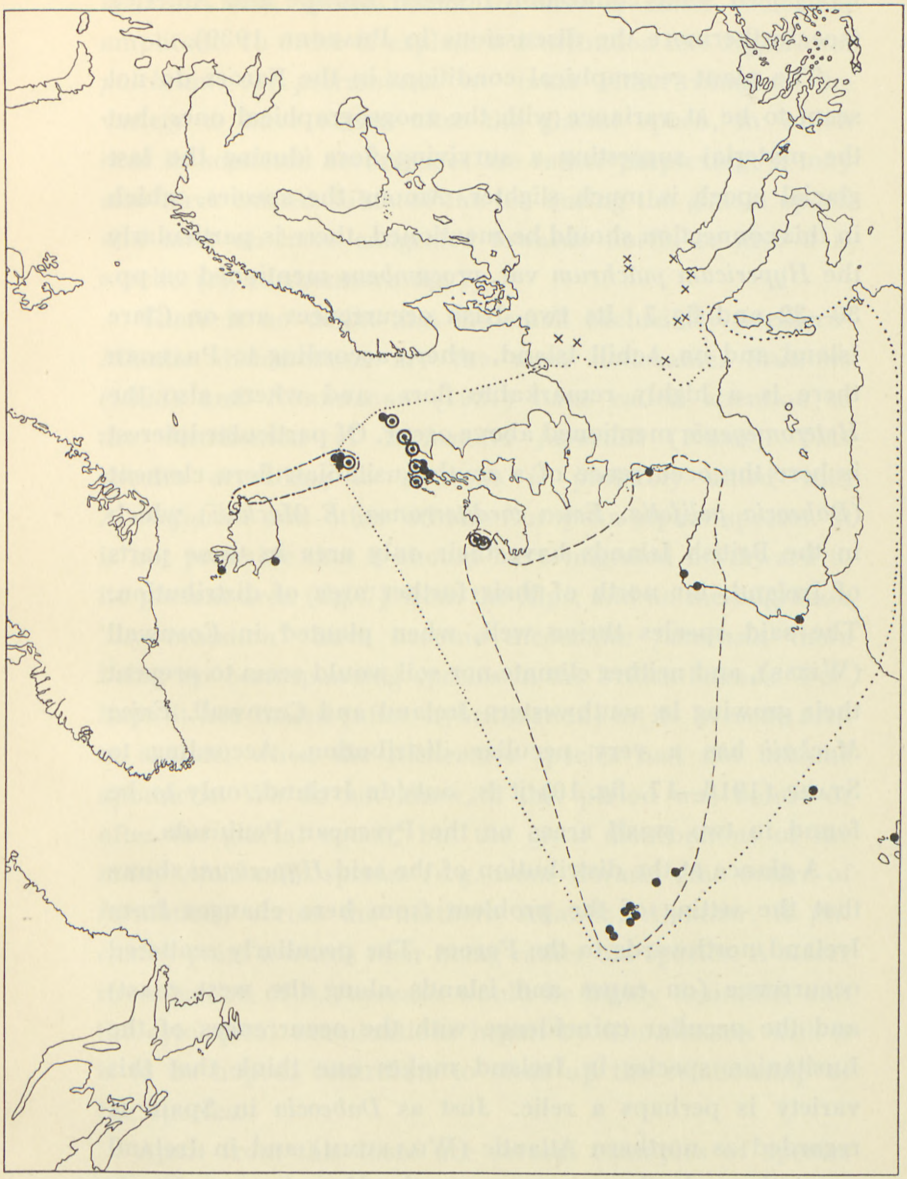


Fig. 7. Distribution of *Anagallis tenella* (·····; ×: scattered occurrences) according to Lüdi (in Hegi's Flora), *Dabeocia polifolia* (— — —) according to Stapf, *Alchemilla færöensis* (—·—·—), *Hypericum pulchrum* var. *procumbens* ⊙ and *Plantago lanceolata* var. *dubia* subvar. *eriophora*: ● (see for this type the text pp. 33—36).

have been some connection between Europe and America (see furthermore the discussions in Praeger 1939).

The plant-geographical conditions in the Faroes do not seem to be at variance with the zoogeographical ones, but the material suggesting a surviving flora during the last glacial epoch is much slighter. Among the species, which in this connection should be mentioned, there is particularly the *Hypericum pulchrum* var. *procumbens* mentioned on pp. 30—32 and fig. 7. Its two Irish occurrences are on Clare Island and on Achill Island, where according to Praeger there is a highly remarkable flora, and where also the *Heteromeyenia* mentioned above occur. Of particular interest is here the occurrence of a south-Lusitanian flora element (*Dabeocia polifolia, Erica mediterranea, E. Mackaii*) which in the British Islands have their only area in these parts of Ireland, far north of their further area of distribution. The said species thrive well, when planted in Cornwall (Weiss), and neither climate nor soil would seem to prevent their growing in southwestern Ireland and Cornwall. *Erica Mackaii* has a very peculiar distribution. According to Stapf (1916—17, fig. 10) it is, outside Ireland, only to be found in two small areas on the Pyrenean Peninsula.

A glance at the distribution of the said *Hypericum* shows that the setting of the problem from here changes from Ireland northwards to the Faroes. The peculiarly scattered occurrence (on capes and islands along the west coast) and the peculiar coincidence with the occurrences of the Lusitanian species in Ireland makes one think that this variety is perhaps a relic. Just as *Dabeocia* in Spain is regarded as northern Atlantic (Willkomm) and in Ireland as southern, in the same manner the *Hypericum* variety is regarded as southern on the Faroes, but as northern in

Ireland. Both species seem to have a small ecological amplitude. In order to explain a distribution like *Hypericum pulchrum* var. *procumbens* we must either imagine the variety to have arisen after the glacial epoch, in which case its scattered occurrences are rather perplexing; it may also have existed south of the ice during the glacial epoch and later on have emigrated towards north, or also it is a relic (cf. furthermore fig. 7).

There is no doubt that the small exclusively northern-Atlantic distributions are the most noticeable. SIMMONS (1898) and NORDHAGEN (1931) have called attention to the distribution of *Alchemilla fœrøensis* (Buser). This species according to SIMMONS is probably allied with *A. conjuncta* and other middle-European-alpine species. It is not probable that it should have migrated northward to its present area (fig. 7) from the Alps, and on the migration "by mutation" have become *Alchemilla fœrøensis*, there being no corresponding species in the British Islands. Perhaps it has arisen (after hybridization) in its present area at a time, when the *Alchemilla* species had not become apomictic. We do not know, if this period was before or after the glacial epoch, but the great distributions of the many *Alchemilla* species (e.g. from Eurasia (the centre of formation) across the northern Atlantic: *A. minor*, *A. filicaulis*) point towards their being rather old species. A closer investigation of *A. fœrøensis* would be highly desirable, and the cytological examinations begun by GUDJÓNSSON will, it is to be hoped, contribute to clear up the relationship of the species.

Apart from this there are several examples of species the distribution of which falls within the northern-Atlantic area. DEGELIUS (1935, p. 192) mentions a good deal of

liver mosses and some lichens, *Juncus Kochii* and *Hymenophyllum peltatum*. These species must have arisen after the glacial epoch, or else they must have made the journey out and back to the south during the glacial epoch, without leaving traces of this journey in the form of scattered finds towards south or occurrences in more southerly mountain regions, or also they are relics. Degelius rather leans to this opinion: "Mehrere Umstände sprechen dafür, dass das ozeanische Florenelement, das im grossen und ganzen als ein Reliktelement des Tertiärs in Europa aufzufassen sein dürfte, an eisfreien Küstenstrichen der vereisten Partien von Nordwesteuropa (wenigstens im Süden) weiterleben konnte."

Besides the "good" species, there are also some varieties which are associated with the northern-Atlantic area. In reality some of these varieties (races) are well-defined, and the transition from species to variety often depends upon an estimate, so that the use of them as plant-geographical arguments only meets one difficulty, *viz.* that our knowledge of them is frequently defective. Besides the above-mentioned *Hypericum* variety I will call attention to the following varieties:

Veronica officinalis var. *glabrata* Fristedt; "an excellent form" (clear variety) according to Rostrup 1870. Material from the Faroes (transplant series and progeny individuals) maintain their appearance in culture. Individuals from alpine localities are difficult to cultivate in Copenhagen. The variety is distributed over Scandinavia (Ångermanland (Fristedt), the Stockholm region, southern Norway near Oslo and several other localities, farther north in Ranen), but it is hardly alpine in any of these places; besides it is to be found in the Faroes and Iceland and probably also in Scotland ("a nearly glabrous form" on Glas Thulachan, Perthshire (Druce) according to Williams, Loch Maree side, West Ross accord-

ing to DRUCE 1929 "var. *glabrata* Bab"[1]). A closer examination should be made of the fact, whether the type which in HEGI's Flora is mentioned as "*f. glabrescens* Bolle" (Rüdersdorf at Berlin) and is designated "fast kahl" has anything to do with var. *glabrata* Fristedt.

Polygala vulgaris var. *Ballii* (Nym.) Ostf. A very much disputed variety (or rather group of races). All agree in the desire to distinguish a variety, but the material from the various regions does not prove to be alike; the Irish plant is different from that of the Faroes, and there also prove to be difficulties in referring all British var. *Ballii* to the same taxonomical unity (see PRAEGER, WILLIAMS). Plants which have been referred to this variety or allied varieties, are found from the Faroes (rather common), Scotland (DRUCE l. c. "a lowland limestone plant with profuse blue flowers") and Ireland (see PRAEGER, 93, 422), here they have from of old been identified on Ben Bulben, where at present we have the only habitat of *Arenaria ciliata* ssp. *hibernica* Ostf. & Dahl. The localities are all calcareous; (both on Ben Bulben and Glenade it grows together with *Dryas*). In the Faroes, on Kodlur at Ejde, I also saw it together with *Dryas*. In Norway the variety was first found by Ostenfeld on Mögsterøen (HOLMBOE Bergens Mus. Årbok. Naturvidsk. rekke. No 10, 1917—18); according to information given by Dr. KNUT FÆGRI it is furthermore to be found in four localities, all in Sunnhordland near Bergen and all calcareous. It really seems, as if we have a northern-Atlantic calciphilous group of races, the individual elements of which, owing to isolation (caused by the last glaciation?) are rather different. It should further be mentioned that more remotely allied types seem to occur in southern Europe (the eastern Pyrenees, BENNET 1878), San Sebastian (Herb. Cop.); see also WILLIAM'S remark on *P. vulgaris* var. *Pindicola* (p. 522).

Finally may be mentioned the *Geranium Robertianum* ssp. *celticum* which was set up by OSTENFELD (1919), and which has a particulary narrow area in western Ireland; also *Plantago lanceolata* var. *depressa* Rostr. which is to be found in the Faroes and the Shetlands (BEEBY 1907), and the var. *dubia* subvar. *eriophora* of *Plantago lanceolata* mentioned on p. 34. The latter should be sub-

[1] In the earlier editions of BABINGTON's Manual there was a var. *glabra* (st. l. & calyx smooth; on mountains) but subsequently this variety was abandoned (PUGSLEY in a letter).

jected to a closer examination as, with the exception of the Shetlands, it seems rare in the British Isles. Williams only mentions pronouncedly hairy plants from the Shetlands, but it is probable that Druce's plants from West Ross belong to this subvar. The hairiness can, to some extent, be modified by outward conditions glass house plants being less hairy than open air plants. This makes it difficult in herbaries to distinguish the otherwise sharply defined type (see figs. 5—6) cf. discussions on hairiness etc. of *Pl. lanceolata* plants from England in Rep. Bot. Exchange Club 1911, p. 115. Perhaps there is here a northern Atlantic stock of relics (Vestmannaeyr, the Faroes, the Shetlands, Scotland) of an otherwise Mediterranean-Azorian plant.

All in all, the total zoological-botanical material from Scandinavia, Ireland, the Faroes and Iceland as well as from Greenland, is large enough to set up a rather plausible supposition, *viz.* that a great part of the present flora in the said countries may have existed there since the last interglacial period. The quartary-geological material, however, puts certain obstacles in the way of this hypothesis. Scattered investigations of English bogs pointed rather in the direction of there having been an arctic flora in ice-free places during the last glacial epoch (see the Discussion in Weiss 1934); pollenstatistic investigations in Ireland (Jessen & Farrington 1938) show somewhat similar features. The species found are, however, all lowarctic-subarctic.

As neither the biogeographical nor the quartary-geological material can be overlooked, it follows that a synthesis of the material of the two groups must be attempted. But here we necessarily enter the fields of aut-ecological and race biological investigations, the latter to examine whether the presumed stocks of relics are genetically different from one another and from the species in its entirety (cf. my theory on biotype-elimination by migration across the Atlantic (*Eriocaulon septangulare, Anthoxanthum* et. al.)).

We must try to find out whether the stock of biotypes of *Eriocaulon*, which is to be found in Ireland, is at all able to live elsewhere in Europe in otherwise similar localities. If it thrives excellently and spreads locally when being transplanted to English bogs, this is an indication that the Irish occurrence is a result of the survival of the species from the glacial epoch in Ireland and the later local spreading there. If it cannot thrive outside Ireland, this may be due to the fact that during its migration there it has become hyper-oceanic; the distribution in Ireland then becomes climatically determined, but in this (and in several other cases) it is difficult to imagine a post-glacial immigration from America, and so the biotype elimination would take place in the interglacial period and later continued during the last glaciation. The species in that case, however, becomes a kind of relic[1].

Through the aut-ecological investigations we must try to understand how the Lusitanian and Atlantic species[2] could exist simultaneously with a glacial epoch. The studies of the Faroes heath which are mentioned on pp. 3—14 show that in any case the *Calluna-Erica cinerea* heath contains relatively southern species. It will then be of value to investigate what is the state of the latter when exposed to greater cold.

In studying the flora of the heath when approaching its vertical line of growth, it appears that a great number of the species here enters into other vegetational types than heath. In further illustration of this a few examples will be given:—

[1] Cf. furthermore the discussion in PRAEGER 1939.

[2] As to the Ecology of *Arbutus unedo* at Killarney in Ireland see TURNER & WATT (1939).

The Faroes. *Nardetum* at a height of 460 m., rich in species and probably covered by snow in the winter: *Blechnum*! *Potentilla erecta, Orchis maculatas, Galium saxatile, Calluna* (cf. Böcher 1937 b, p. 175).

South-facing herb-field, rich in species and 500 m. above the sea, probably covered by snow during the winter: *Galium saxatile, Anthoxanthum, Polygala serpyllacea, Brunella vulgaris, Hypericum pulchrum* v. *procumbens, Carex pilulifera, Veronica officinalis* v. *glabrata* (Böcher l. c., p. 179).

South-facing snow-patch, 670 m. above the sea: *Nardus, Veronica officinalis* v. *glabrata, Anthoxanthum, Galium saxatile, Blechnum, Potentilla erecta* (Böcher l. c., p. 182).

Grimmia heath, 360 m. above the sea: *Nardus, Anthoxanthum, Galium saxatile, Deschampsia flexuosa, Carex pilulifera, Luzula silvatica* (sterile). (Ostenfeld 1901 a, p. 32).

Grimmia heath 570 m. above the sea: *Deschampsia flexuosa, Galium saxatile, Veronica officinalis, Pinguicula, Anthoxanthum, Carex "flava"* (Ostenfeld l. c., p. 48).

Grimmia heath with *Nardus* and *Lycopodium alpinum* 360 m. above the sea: *Potentilla erecta, Galium saxatile, Anthoxanthum, Luzula silvatica* (sterile). (Ostenfeld l. c., p. 53).

Moist slope at a height of 400 m., the soil covered by *Anthelia julacea: Nardus* and *Juncus squarrosus* as dominants, besides i. a. *Narthecium* (sterile), *Potentilla erecta, Scirpus cæspitosus* (Ostenfeld l. c., p. 56),

The above-mentioned *Grimmia* heaths and more particularly the "moist slope" are surely in a large degree covered by snow during the winter.

In Norway I had in several localities an opportunity to observe how *Calluna*, the higher one gets, became more and more a snow-patch plant. As an example I may mention that it was observed on the top of Vaarstøl at Bykle in a south-facing *Vaccinium myrtillus* heath close to a typical snow-patch together with *Phyllodoce cærulea* and *Salix herbacea*, but was absent in the more snow-bare *Loiseleuria-Empetrum* heath rich in lichens.

There thus seems to be a connection between snow-patch (and herb-slope) and oceanic species (cf. Böcher 1938, pp. 286—287), which is easily understood, as the cover of snow causes the microclimate of the habitat to

become relatively oceanic (small temperature amplitude, moist soil etc.).

In this connection must also be mentioned a species like *Dabeocia polifolia*. According to PRAEGER this species reaches up to 600 m. above the sea at its northern limit in Ireland; in the Pyrenees *Dabeocia* is subalpine and covered by snow for five months. In the Azores it is according to GUPPY (l. c. p. 433) most common between a height of 1700 and 2500 m., but comes in scattered individuals down to about 800 m. above the sea. During the winter it is here exposed to frost, snow and wind, in the summer to drought and sun.

This discussion may be summarized as follows: The occurrence of low-subarctic species during the last glaciation and at the beginning of the post-glacial epoch need not exclude the occurrence of southern species. There may have been a low-arctic vegetation near the ice, whereas local, microclimatic oases existed on the south-facing slopes on the outermost capes or the freely situated islands, washed by the Golf Current, where the southern species could exist sheltered by snow against the winter cold. Along the Blosseville Coast the southern element is associated with the southern snow-covered herb slopes on capes and nunataks here, however, only slightly influenced by the Golf Current (the Irminger Current). Even though we imagine the temperature to fall several degrees, it is probable that snow-patches may have been able to form on the southern slopes of the lowland with a great deal of rather southern species, particularly those mentioned in the examples given above. Many of these have, however, now their optimum in heaths and related communities, i.e. pronouncedly temperate lowland communities. In the examples *Erica cinerea* and *Carex binervis* are lacking, the most southerly of the heath species

which on an average require 2° in January. It is therefore much more difficult to imagine a glacial survival of these species; however, it must be borne in mind how rough our thermic limitation of the plant areas is, and how little we know of the climate conditions resulting in a glacial epoch. As far as the Faroes are concerned *Erica cinerea* and similar species may perhaps at a rather early period have been able to immigrate postglacially from the south, but as to western Norway this is much more difficult, cf. Holmboe (1927), Degelius (l. c.).

The flora and vegetation of the Faroes must be examined once more from other points of view. Ecological and race-biological investigations are required on a large scale. As an example may be mentioned an interesting problem connected with *Pirola minor*. This temperate species is in the Faroes only found as alpine and most frequently sterile. What then is the relation of the Faroes biotypes to this species in its entirety? For all the problems which have been dealt with here, also other glaciological investigations will naturally be highly needed.

Dr. Knut Fægri has helped me with the planning of my voyage in Norway and later on with information as to the occurrence of the said varieties in that country. Dr. H. W. Pugsley, England, and Dr. Lloyd Praeger, Eire, have supplied me with information as to the occurrence of the species in Great Britain and Ireland, while Mr. Aug. Hesselbo has kindly determined a great part of the mosses mentioned in the treatise (more particularly liver mosses). To all of these I express my sincere thanks and I also beg to thank the Rask Ørsted Fond for defraying the expences of the translation of the treatise.

Copenhagen, October 1939.

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T. B. phot.

Fig. 1. Southwest-facing *Calluna-Erica cinerea* heath at Torvadalsá at Klaksvig (Bordö Vig), cf. Table 2, Nos. 4 and 6. My wife at the edge of a dense patch of heath on the border line of the grass-moss-community.

T. B. phot.

Fig. 2. South-facing slope with *Calluna-Erica cinerea* heath at Bordö Vig; in the foreground a transition vegetation very rich in grasses. The photograph taken on an unusually beautiful summer day, so hot that the evaporation of the earth caused the formation of misty clouds.

T. B. phot.

Fig. 1. *Calluna-Erica cinerea* heath at Haugesund in Norway (the shooting ranges at Kvala), cf. Table II, No. 8. Besides *Erica* and *Calluna*, *Juniperus communis* is seen.

Det Kgl. Danske Videnskabernes Selskab.
Biologiske Meddelelser. **XV**, 4.

SOME MARINE ALGAE FROM MAURITIUS

I. CHLOROPHYCEAE

BY

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KØBENHAVN
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The Danish zoologist Dr. TH. MORTENSEN, when he was staying at Mauritius in September—October 1929 for the purpose of studying Echinoderms from that island, at the same time collected and prepared with his usual energy and care a rather large number of algae. Since many of these were dredged in deep water, down to a depth of about 50 fathoms, it is no wonder that his collection contains much interesting material, and all the more so since Dr. MORTENSEN has prepared not only larger forms but also many of the small ones. On his return to Copenhagen Dr. MORTENSEN presented the Botanical Museum of the University with the collection. After having examined it superficially I immediately recognized its value and hoped one day to be able to study it more closely.

When therefore in September 1938 I received a letter from Dr. R. E. VAUGHAN, the Director of the Royal College, Curepipe, Mauritius, asking me to assist him in determining his collection of algae from Mauritius, I was very glad to agree to his proposal, and Dr. VAUGHAN has several times sent me rich gatherings of algae from the island, partly dried and partly preserved in formol and sea-water, and also slides with algae.

The algal flora of Mauritius is by no means unknown. Already from the year 1834 a small list comprising 24 species was published by the well known Irish algologist

W. H. Harvey in his paper: "Notice of a collection of Algae, communicated to Dr. Hooker by the late Mrs. Charles Telfair, from "Cap Malheureux", in the Mauritius. With descriptions of some new and little known species." It is evident from the title that this paper is based upon a collection of algae made by Mrs. Telfair.

At the end of the year 1873 G. Dickie published a paper: "On the Algae from Mauritius." It contains 155 species, some fresh water algae being included in the list. The algae were collected by Colonel Nicholas Pike who during a stay at Mauritius made considerable gatherings there. Like those of Mrs. Telfair the specimens of algae which Dickie determined are included in the Kew Herbarium. Dickie's paper is a mere enumeration of the species. Not even the localities where the algae were found are mentioned and the descriptions of the new species are rather poor.

More recently (1934) F. Jadin has published a fuller list of species comprising the algal flora of Mauritius as well as of Réunion, the other large island of the Mascarene Islands. Jadin gathered algae at the islands from February till the end of October 1890. The title of his paper is: "Algues des Iles de la Réunion et de Maurice". Although this list shows a great advance on the lists previously published, nevertheless several of the determinations are not quite modern. According to kind information from Dr. Feldmann, Algiers, this is no doubt due to the fact that Jadin made the determination of his collection several years ago but postponed the publication until 1934; a list of the *Nostocaceae* of the islands is thus published in Bulletin Soc. bot. France, t. XL, p. CXLVIII—CLXXIII, 1893.

Finally we may mention that Montagne and Millardet

in a treatise by L. MEILLARD, "Notes sur l'île de la Réunion (Bourbon)" have given a list of the algae from that island.

It was of course of importance to me to be able to examine these old collections of algae from Mauritius, and upon my application to Sir ARTHUR HILL, Director of the Royal Botanical Gardens, Kew, and to the Keeper of the Herbarium, Dr. A. D. COTTON, they were so kind as to let me have on loan in Copenhagen the *Chlorophyceae* from Mauritius kept in the Kew Herbarium, and after the return of these, the *Phaeophyceae*. While I still had the last-mentioned here the war broke out, and with Sir ARTHUR HILL's concurrence the algae will remain here until the end of the war.

But it is not only in London that collections of algae from Mauritius are found. In Paris also, in the Muséum d'Histoire naturelle, copious collections from the islands are kept, as I am informed by Dr. FELDMANN, who has taken a great interest in my intended work on the algae from Mauritius.

First of all JADIN's collection is found there. It is said to be very rich, and also contains a good many duplicates. Doctor FELDMANN has kindly let me know that upon his application Director ALLORGE had given permission for a collection as comprehensive as possible of JADIN's duplicates to be sent to me, and Dr. FELDMANN had just begun to take out these duplicates when the war broke out and unfortunately made this impossible.

Further another good collection, that of le Vicomte DE ROBILLARD, according to Dr. FELDMANN is likewise found in Paris. This collection has a special value because it has been determined by BORNET. But it is incorporated in the THURET-BORNET Herbarium, and for that reason it is not

permitted to send it away from the Museum. It is therefore very much to be regretted that the war has rendered impossible a visit to Paris and London planned for this spring when I had hoped to be able to study the collections of algae from Mauritius found in the museums there.

Miss Ingeborg Frederiksen has most kindly drawn some of the figures.

To the Trustees of the Carlsberg Foundation I am much indebted for a continued grant for algological researches.

CHLOROPHYCEAE

I. Chaetophorales.

Fam. 1. Chaetophoraceae.

Endoderma Lagerh.

1. Endoderma vagans Børgs.

Børgesen, F., Mar. Alg. D. W. I., vol. II, p. 418, fig. 400.

In the walls of a not yet determined *Griffithsia* an *Endoderma* was found which, as to size and shape of the cells, seems to agree entirely with the West Indian plant.

Mauritius: Pointe aux Roches in rock pools, R. E. V. no. 125.
Geogr. Distr.: West Indies.

Phaeophila Hauck.

1. Phaeophila dendroides (Crn.) Batters.

Batters, A., Catalogue, p. 13. — *Ochlochaete dendroides* Crn., Florule, 1867, p. 128. *Phaeophila Floridearum* Hauck, Meeresalgen, p. 464. Huber, J., Contrib. à la conn. des Chaetoph., p. 327, pl. 16, fig. 1—11. Schussnig, B., Alg. Abhandl., p. 16, pl. IV.

Found in the walls of *Hypnea* and *Griffithsia* from rock-pools.

Mauritius: Pointe aux Roches, R. E. V. (no. 125).
Geogr. Distr.: Widespread.

II. Ulvales.

Fam. 1. Ulvaceae.

Monostroma Thuret, Wittr.

1. Monostroma oxycoccum (Kütz.) Thur.

Thuret, G., Note sur la Synon. des Ulva, 1854, p. 16, emend. Hamel, Chlorophyc. Côtes Franç., p. 146. — *Ulva oxycocca* Kütz., Phycol. Germ., p. 244. *Monostroma latissimum* (Kütz.) Wittr., Monogr. Monostroma, p. 33, pl. 1, fig. 4. For more synonyms compare Hamel l. c.

A rather large specimen which has most probably been lying loose is found in Dr. Vaughan's collection. The thallus is about 23 μ thick and the cells are roundish polygonal and arranged without order. The specimen is 21 cm broad. Its colour is light olive-green and the surface is shining.

Mauritius: Ilôt Brocus, Aug. 1938 in tidal creek, R. E. V. no. 197.

Geogr. Distr.: Atlantic Ocean, Mediterranean Sea, New Zealand, Pacific Ocean etc.

Enteromorpha Link.

1. Enteromorpha compressa (L.) Grev.

Greville, R., Alg. Brit. p. 180. Hamel, Chlorophycées Côtes Franç., p. 156, where the literature is mentioned.

var. *prolifera* (Muell.) Hamel, l. c. p. 160.

A single specimen in Dr. Vaughan's collection may perhaps be referable to this variety. The plant grows gregariously in a dense clump upon a small stem of a sea-grass. In the tubular stem the cells are arranged in rows; they are roundish or square, about 20—25 μ in diameter. Up along the stem are found numerous monosiphonic thin proliferations.

It was found in brackish water in tidal creeks near the sea.

Mauritius: Ilôt Brocus. $^{12}/_{8}$ 33, R. E. V. no. 176.
Geogr. Distr.: Widespread.

2. Enteromorpha flexuosa (Wulfen) J. Ag.

J. AGARDH, Till Algern. System., III, VI Ulvaceæ, p. 126. BØRGESEN, Mar. Alg. D. W. I., vol. 1, p. 5. FELDMANN, Alg. mar. Côte des Albères, p. 52, where more literature is mentioned.

The thallus is as a rule simple or with a few branchlets or prolifications. It is tubular, somewhat inflated and flexuous above. The cells are arranged regularly in rows and are up to about 20 μ broad.

Dr. VAUGHAN writes about it that it is "very common in Barachois where it is collected for fishing bait".

Mauritius: Ilôt Brocus, Aug. 1938, R. E. V. no. 195.
Geogr. Distr.: Seems to occur in most temperate and warm seas.

3. Enteromorpha chlathrata (Roth) J. Ag.

J. AGARDH, Till Algernes Systematik, III, VI Ulvaceæ, p. 153.

var. *Hopkirkii* (M. Calla) HAMEL, l. c., p. 165.

A large much ramified specimen found in the Kew Herbarium seems to be referable to this species.

Mauritius: Flacq, Baie de 4 cows, Jan. 23th, 1870, Colonel PIKE.

var. *crinita* Hauck, Meeresalgen, p. 429. HAMEL l. c., p. 165, where the literature is mentioned.

Some material preserved in formol and about which Dr. VAUGHAN writes "Shallow water, sandy bottom, lagoon in Ilôt Brocus", seems to be referable to this variety.

The plant is very soft, much ramified and provided with numerous often monosiphonic and more or less curved

proliferations. The cells are placed in rows and are square, about 30 μ in diameter, and the chromatophores fill up almost the whole cell.

Mauritius: Ilôt Brocus, Aug. 1938. R. E. V. no. 206.
Geogr. Distr.: Most probably widely spread.

Ulva L.

1. Ulva Lactuca L.

Linné, Spec. plant, vol. II, 1753, p. 1163.

Some small specimens growing in a "tidal creek near Sea" are found in Dr. Vaughan's collection.

Mauritius: Ilôt Brocus, 12. March 33, R. E. V. no. 180.
Geogr. Distr.: Widespread.

2. Ulva fasciata Delile.

Delile, Flore d'Égypte, p. 153, tab. 58, fig. 5, J. Agardh, Till Algernes Systematik, VI, p. 173. Montagne. Fl. d'Algérie, p. 151, tab. 14, fig. 1—2. For more literature compare De-Toni, Syll. Alg., vol. I, p. 114.

A rather badly preserved specimen is found in Dr. Vaughan's collection. The cells in the thallus are roundish-polygonal and placed without order; they have a diameter of about 20 μ.

Mauritius: Pt. aux Roches, in pools, 3rd of April 1931, R. E. V. no. 138.
Geogr. Distr.: In most warm seas.

III. Siphonocladales.

Fam. 1. Valoniaceae.

Valonia Ginn.

1. Valonia ventricosa J. Ag.

Agardh, J., Till Algernes Systematik, 5te Afd. VIII, Siphoneæ 1886, p. 96. Murray, George, Halicystis and Valonia, p. 50. Kuckuck, P., Über den Bau und die Fortpflanzung von Halicystis Areschoug und Valonia Ginnani, p. 42. Vickers, Phycologia Barbad., p. 21, pl. XXIII A. Børgesen, Mar. Alg. D. W. I., vol. I, p. 27, fig. 16.

A single specimen of this species is found in Dr. Mortensen's collection, agreeing entirely with my description and figures. It was found near the shore in shallow water at low tide.

Mauritius: Flat Island, $^{17}/_{10}$ 29, leg. Th. M.

Geogr. Distr.: West Indies, Malayan Archipelago, American Samoa.

2. Valonia utricularis (Roth) C. Ag.

C. Agardh, Species Algarum, p. 431. J. Agardh, Till Algernes Systematik, 5te Afd., VIII, p. 98. Kuckuck, Über den Bau etc., p. 28.

Some well developed specimens preserved in spirit are found in Dr. Mortensen's collection. In the Kew Herbarium several specimens gathered by Colonel Pike are also referable to this species.

Mauritius: Between Gunners Quoin and Flat Island at a depth of about 30 fathoms on sandy bottom, $^{16}/_{10}$ 29, Th. M.

Geogr. Distr.: Widespread in warmer seas.

3. Valonia Ægagropila C. Ag.

C. Agardh, Spec. Alg., p. 429. J. Agardh, Till Alg. System., 5te Afd. VIII. Siphoneæ, p. 99. Kuckuck, P., Über den Bau und die Fortpflanzung von Halicystis Aresch. und Valonia Ginn. p. 36.

A few thalli are found in one of Dr. Vaughan's tubes.

Mauritius: Tamarin Bay, R. E. V. no. 319.

Geogr. Distr.: Mediterranean, Indian and Pacific Oceans, West Indies.

4. Valonia fastigiata Harv.

Harvey, Alg. Ceylon, no. 74. J. Agardh, Till Algern. System. VIII, p. 101.

Some specimens are found in the Kew Herbarium. Two of them are rather well prepared and agree very well with a specimen from Harvey's Friendly Island Algae no. 100 found in the Herbarium of the Botanical Museum in Copenhagen. The vigorous hapters are found scattered over the surface of the thallus often also in small groups.

Mauritius: Colonel Pike.

Geogr. Distr.: Mauritius, Ceylon, Friendly Islands, Australia.

Dictyosphaeria Decsne.

1. Dictyosphaeria cavernosa (Forssk.) Børgs.

Børgesen, Revision of Forsskål's Algae, p. 2, pl. 1, fig. 1. — *Ulva cavernosa* Forssk., Flora Ægypt.-arabica, p. 187. *Dictyosphaeria favulosa* (Ag.) Decsne, Classific. des Algues, p. 32. Børgesen, Mar. Alg. D. W. I., vol. I, pp. 33—39, figs. 20—22.

A well developed specimen is found in Dr. Vaughan's collection. He writes about it: "Pale green flattish discs".

Mauritius: Without locality, R. E. V. no. 230. "Common everywhere". Flacq, Jadin no. 299, Juillet 1890.

Geogr. Distr.: West Indies, Indian and Pacific Oceans.

2. Dictyosphaeria Setchellii nov. spec.

Frons pulvinata, subplanata et irregulariter difformis, cartilaginea, solida, ad 1—2 cm alta et 2—3 cm et ultra lata, infimo rhizoideis curtis ad substratum adfixa. Spinae ex

parietibus cellularum projectae, erectae aut suberectae, ca. 100—120 μ longae et in parte basali ad 10—12 μ latae, superficie levi et plana numerosae adsunt. Fig. 1.

Mauritius: Flat Island in shallow water, $^{17}/_{10}$ 1929, Th. M. Without locality, Colonel Pike. R. E. V. no. 230. "Flattish pale green discs, warty surface, very common everywhere".

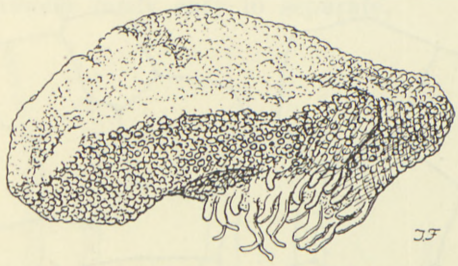

Fig. 1. *Dictyosphaeria Setchellii* nov. spec. Habit of a plant. × $1^1/_2$.

In Dr. Mortensen's collection some fine specimens of a *Dictyosphaeria* preserved in spirit are found. At first I thought I had to do with *Dictyosphaeria Versluysi* Web. v. B. but a comparison with this species and the other related species: *Dictyosphaeria van Bosseae* Børgs. and *Dict. australis* Setch. (the last-mentioned species I know only from the description of it) resulted in my finding that the plant from Mauritius does not exactly agree with any of the above-mentioned species, and therefore I prefer to make it a new species.

A comparison of the four species is given below:

	Diameter of cells	Size of spines: Length	Breadth at the base
D. Setchellii ...	800—1000—1500 μ	ca. 70—140 μ	ca. 9—15 μ
D. Versluysi ...	up to 2000 μ	ca. 130—150 μ	ca. 30 μ
D. Van Bosseae	500—700—1000 μ	up to 90 μ	ca. 8—12 μ
D. australis ...	500—1000 μ	60—88 μ	

It will appear from this survey, (compare also figs. 2—3), that *Dict. Versluysi* has by far the largest cells and spines and by these characters it is easily separable from the other 3 species. These have all cells of about the same size, *Dict. Setchellii* having the larger ones, while those of

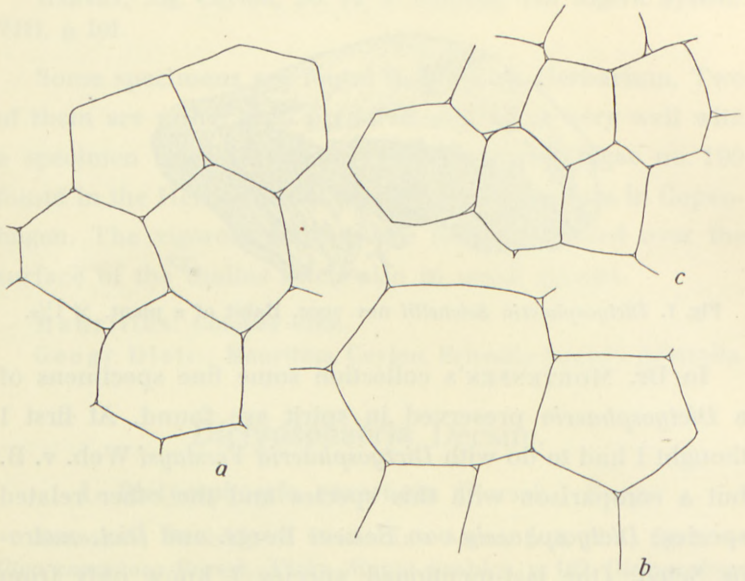

Fig. 2. Cells of: a, *Dictyosphaeria Setchellii* nov. spec.; b, *Dictyosphaeria Versluysi* Web. v. B.; c, *Dictyosphaeria van Bosseae* Børgs. (× ca. 20).

Dictyosphaeria van Bosseae are somewhat smaller (Fig. 2). And further the shape and size of the spines give some quite good characters. Thus in *Dict. Setchellii* (Fig. 3a) they are generally found in great numbers having as a rule a length of about 100—120 μ and a breadth of 10—11 μ at their base. They are slender, tapering evenly to the acute summits, and generally quite straight or sometimes bending a little, and they have smooth and even or sometimes a little waved surfaces. In *Dict. van Bosseae* the spines (Fig. 3c)

are less numerous, only found here and there in some of the cells. They are often rather bent and have more or less uneven, waved surfaces. As to the spines of *Dict. australis* they are said to be 60—88 μ long and further they are "sparsissimis et rarissimis". The 3 species in question are certainly closely related, but by means of the above-mentioned differences fairly easy to separate.

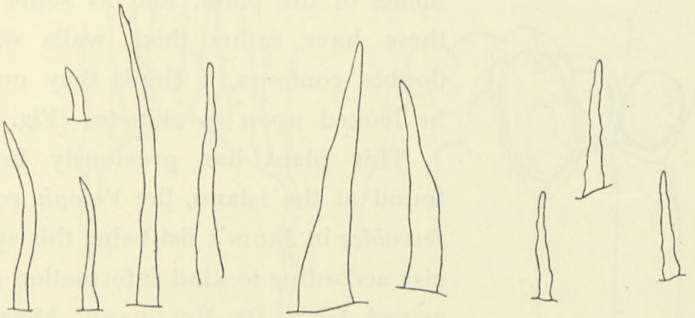

Fig. 3. Spines of: a, *Dictyosphaeria Setchellii* nov. spec; b, *Dictyosphaeria Versluysi* Web. v. Bosse; c, *Dictyosphaeria van Bosseae* Børgs. (× ca. 250).

In the Kew Herbarium a few dried specimens determined as *Dictyosphaeria sericea* Harv. by DICKIE are referable to this species.

Fam. 2. Siphonocladaceae.

Ernodesmis Børgs.

1. Ernodesmis verticillata (Kütz.) Børgs.

BØRGESEN, Some Chlorophyceae from the Dan. West Indies, II, 1912, p. 259. Mar. Alg. D. W. I., vol. I, p. 66, figs. 51—54. — *Valonia verticillata* Kütz., Spec. Alg., p. 508; Tab. Phycol. VI, tab. 88.

The discovery of this species in Dr. VAUGHAN'S collection so far from its former known area of distribution is of much

interest. The plant from Mauritius in all respects agrees with the West-Indian one and I can therefore refer the reader to my description of it.

As was the case in the West-Indian plant the cell-content in the plant from Mauritius is often contracted into balls. The balls are found in the upper young segments of the plant, and as some of these have rather thick walls with double contours, I think they must be looked upon as akinetes (Fig. 4).

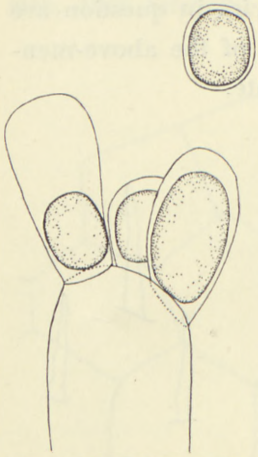

Fig. 4. *Ernodesmis verticillata* (Kütz.) Børgs. Tips of segments with akinete-formation. (× 30).

This plant has previously been found at the island, the *Valonia confervoides* in Jadin's list being this species according to kind information received from Dr. Feldmann, Algiers.

Mauritius: In a lagoon at Ilôt Brocus, R. E. V. Aug. 1938.

Geogr. Distr.: The West Indies, Brazil.

Chamaedoris Mont.

1. Chamaedoris Delphinii (Hariot) Feldm. et Børgs.

Feldmann, J., Sur la classification de l'ordre des Siphonocladales, p. 18. — *Siphonocladus Delphinii* Hariot, Quelques Algues de Madagascar, p. 470. *Chamaedoris annulata.* Areschoug, Phyceae Capensis, p. 14. Dickie, G., Algae of Mauritius, p. 198.

Feldmann, as mentioned in the above-quoted treatise, made the interesting discovery, when studying the type specimen of Hariot's *Siphonocladus Delphinii* in the Museum in Paris, that in reality this plant is a *Chamaedoris.* But due to deficiency of material he does not say anything

about its specific relationship, neither does he mention that it should be specifically different from the West Indian species. The examination of specimens from Mauritius, which I consider the same as those from Madagascar, has proved this to be the case.

In spite of the rather close resemblance of the plant

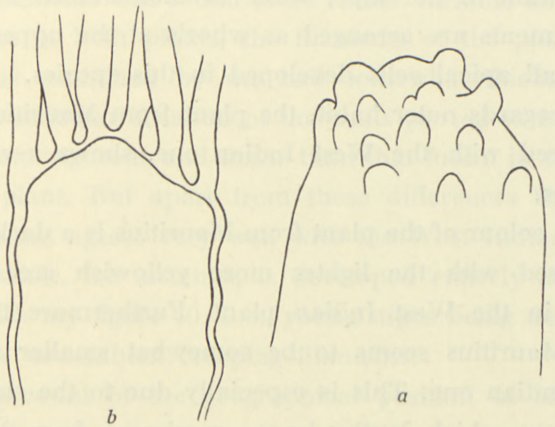

Fig. 5. *Chamaedoris Delphinii* (Hariot) Feldmann. a, apex of young stipe showing development of the filaments of the capitulum. b, longitudinal section through the upper end of a young stipe. (× 30).

from Mauritius (Plate I), of which I have had only some dried material, to the West Indian *Ch. Peniculum* (Sol.) Kuntze (compare my figure 42 in Mar. Alg. D. W. I. p. 59), several features in its outer habit aroused my suspicion as to its real identity. So I commenced to study it more carefully, with the result that I found the plant from Mauritius fundamentally different from the West Indian one.

Above all this species differs from *Ch. Peniculum* by the fact that the small apical cells in the stipe, from which the filaments of the capitulum in *Ch. Peniculum* are developed (compare my figure 41, l. c., p. 58), are missing.

Fig. 5b shows a longitudinal median section of the upper end of the stipe of the plant from Mauritius. No traces of transverse walls are found. The bases of the filaments forming the capitulum are seen to be developed from the upper end of the stipe. And fig. 5a shows the development of the filaments. They are seen to grow without order from the summit of the stipe, while in *Chamaedoris Peniculum* the filaments are arranged as whorls at the upper end of the small apical cells developed in this species.

As regards outer habit, the plant from Mauritius, when compared with the West Indian one, shows several differences.

The colour of the plant from Mauritius is a darker green compared with the lighter more yellowish green colour found in the West Indian plant. Furthermore the plant from Mauritius seems to be somewhat smaller than the West Indian one. This is especially due to the size of the capitulum which in the larger specimens from Mauritius attains only about $1^1/_2$ cm, whereas the breadth of the capitulum in the West Indian plant from shallow water was about 3 cm, and in plants from deep water even up to 10 cm. Then the shape of the capitulum seems to be ball-like in the plant from Mauritius, that is to say, judging from dried material, whereas in the West Indian one it is more or less cup-shaped, even if ball-shaped ones also occur. The tendency to proliferations from the stem was found in several of the specimens from Mauritius; this is a rather rare feature in the West Indian plant. At first I also had the impression that the annulation of the stipe in the plant from Mauritius was more narrow than that of the West Indian plant, but after having measured the annulations of several plants from both localities I found

that rather large variations occur even in the same stipe, and that a distance of about 470 μ is the most common in both plants.

Finally the filaments of the capitulum in the plant from Mauritius show some smaller differences when compared with those of the West Indian plant. While the dimension of the filaments was found to be rather variable and about the same in both plants, the filaments of the plant from Mauritius examined by me had somewhat thicker walls and the cuticular striation of the walls proved to be somewhat more vigorous and dense than that found in the West Indian plant. But apart from these differences the habit of the plant agrees very well with the West Indian plant, and its base, for instance, is developed entirely in agreement with my figure 40, l. c., young stipes being developed from the decumbent creeping filaments.

On account of the geographical position of Mauritius and Madagascar, so close to each other, it seems natural to regard the plants from these islands as the same species. It was therefore of great interest to me that by the courtesy of Monsieur le directeur P. Allorge, Laboratoire de Cryptogamie du Muséum National d'Histoire Naturelle, Paris, and Monsieur le Docteur J. Feldmann I had the privilege of borrowing the type-specimen from Madagascar, so that I could study it in Copenhagen. It consists of two capitulums, a larger one about 3 cm broad, thus the same size as that found by me in the West Indian specimens, and a smaller one only about half the size, in which a small piece of the stipe is also preserved. The colour of the filaments in the capitulum of these specimens was the same dark green as that found in the plant from Mauritius, and the shape of the capitulum was likewise the same, and

an examination of the filaments showed that these agreed very well with those of the plant from Mauritius, having thick walls and a well developed striation. For this reason I do not hesitate to regard the two plants as identical. Of course an examination of the building up of the capitulum would have been desirable, but as I feared that an examination of these authentic specimens would have been fruitless, I refrained from spoiling them.

The *Chamaedoris annulata* mentioned by Areschoug in "Phyceae Capensis", p. 14, is also undoubtedly the same as the plant from Mauritius. Areschoug says: "Planta Natalensis et Brasiliensis in omnibus congruunt, quamquam illa plerumque minor haec robustior est", and this agrees with my observations.

The Indian species *Chamaedoris auriculata* Børgs. from Dwarka in the Arabian Sea differs from the plant from Mauritius in having, like the West Indian plant, apical cells in the upper part of the stipe, and by its more or less excentric flat capitulum.

The interesting species *Chamaedoris orientalis* Okam. and Higashi (On the Marine Algae from Kotosho (Botel Tobago), p. 98, pl. 10) differs essentially by its oblong capitulum, the filaments of which it is composed being developed annularly from about 13 articulations in the upper end of the stipe[1].

Mauritius: Off Flat Island, Oct. 1929, Th. Mortensen.
Geogr. Distr.: Fort Dauphin, Madagascar; Port Natal.

[1] After this paper had been sent to the press the South-African algologist, Dr. G. Papenfuss, Cape Town, paid a visit to the Botanical Museum, Copenhagen, and among some algae which he brought with him was a *Chamaedoris* from Port Natal. This I immediately recognised as the same as that from Mauritius. Dr. Papenfuss, who is at present working with his collection of marine algae from the Cape at the Botanical Museum in Lund, has most kindly sent me some young stipes

Fam. 3. ***Boodleaceae.***

Cladophoropsis Børgs.

1. Cladophoropsis Sundanensis Reinb.

Reinbold, Th., Chlorophyceen Ind. Ocean in Nuov. Notarisia, 16, 1905, p. 3; Algues Siboga, p. 77, fig. 18.

In the Kew Herbarium some specimens (no. 36) are found that form dark-green roundish *Chladophoropsis*-like clumps. Dickie has called the plant *Valonia Cladophora.*

After an examination of a slide made of one of the specimens it seems to agree fairly well with *Cladophoropsis Sundanensis* according to Reinbold's description. The thicker filaments have a diameter of about 80—100 μ, the thinner ones are about 40—50 μ.

From the basal filaments rhizoids are given off which fasten themselves to the substratum.

Undoubtedly related to this species is *Cladophoropsis limicola* Setchell (American Samoa, p. 176, fig. 40), but its thallus is thicker.

Mauritius: Cassis, Aug. 2, 1869, Colonel Pike.
Geogr. Distr.: Malayan Archipelago.

Boodlea Murray et De-Toni.

1. Boodlea composita (Harv.) Brand.

Brand, F., Ueber die Anheftung der Cladophoraceen etc. p. 187. Reinbold in Weber van Bosse, Algues Siboga, p. 70. — *Cladophora composita* Harv. in Hooker. Journ. of Bot., vol. I, 1834, p. 157.

of this plant preserved in formol and seawater, by means of which I have been able to ascertain that my description given above, which was based upon dried material only, is correct. I wish to thank Dr. Papenfuss most heartily for his kindness.

In one of the tubes sent by Dr. Vaughan (no. 311) I was fortunate enough to find a well conserved lump of *Boodlea composita*. This species has been described by Harvey upon a dried specimen from Mauritius, and as such material of this plant is rather unfit for examination a renewed investigation of fresh material was most desirable. To be sure, Brand has given a fairly detailed description of a closely related form, but his material was not from Mauritius but from the Sandwich Islands. Brand was of the opinion that the plant had originally come from these Islands but, as pointed out by Setchell, Tahitian Algae, p. 77, this is incorrect, the plant being first described upon material from Mauritius. Kützing's figures in Tab. Phycologicae also are drawn from a plant from the Mascarene Islands.

Dr. Vaughan's specimen forms a small low cushion, a few cm high as well as broad. It is of a very loose and soft consistency, and this is most probably to some extent due to the fact that the peripheric young branch-systems have no tenacula at all. Only once and in a not quite young filament have I found a rhizoid (Fig. 6d) in the summit of a branchlet and this was not separated from the mother cell by a transverse wall as is usually the case with *Boodlea*. On the other hand, Brand, in the plant examined by him, found tenacula to be fairly common but not in all parts of the plant.

The peripheric branch-systems are generally branched rather regularly, in a featherlike way (Fig. 6a, b), as also shown by Kützing's figures, and in agreement with forma *elongata* mentioned by Brand. The branches are as a rule opposite, but sometimes 3 or, in rare cases, 4 branches issue from the nodes. The formation of the branches (Fig. 6a, b, c) reminds one very much of *Struvea* and most

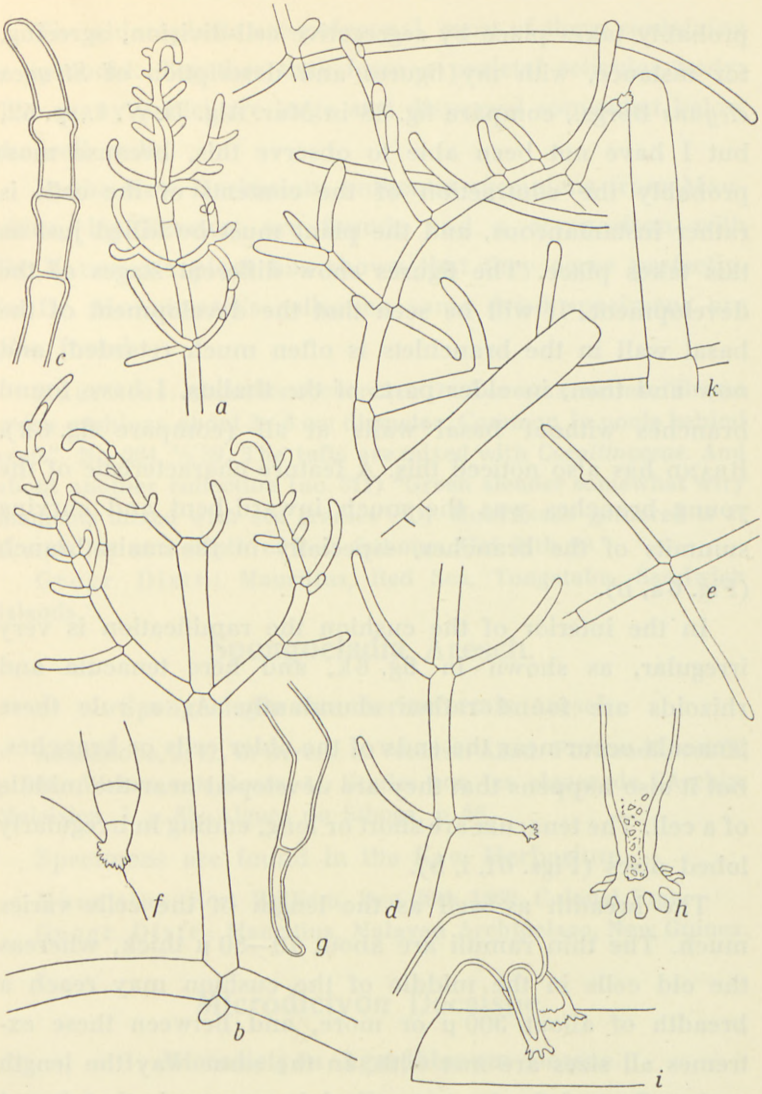

Fig. 6. *Boodlea composita* (Harv.) Brand. a, b, summits of the peripheric branches; c, tip of a filament showing the growing out of the side branches; e, a rhizoid begins to grow from the side of a branch; d, f, h, i, tenacula in summits of the branches; g, a rhizoid growing out from the tip of a branch; k, part of the thallus from the interior of the thallus. (a, b, d, e, k = × ca. 20; c, f, g, h, i = × ca. 50).

probably takes place by segregative cell-division, agreeing, for instance, with my figures and description of *Struvea elegans* Børgs., compare fig. 38 in Mar. Alg. D. W. I., p. 52, but I have not been able to observe this, because most probably the contraction of the contents of the cells is rather instantaneous, and the plant must be killed just as this takes place. The figures show different stages of the development. It will be seen that the development of the basal wall in the branchlets is often much retarded, and now and then, in older parts of the thallus, I have found branches without basal walls at all (compare fig. 6k). Brand has also noticed this. A feature characteristic of the young branches was the much inward-bent and curving summits of the branches, especially in the main branch (Fig. 6a, b).

In the interior of the cushion the ramification is very irregular, as shown in fig. 6k, and here tenacula and rhizoids are found rather abundantly. As a rule these tenacula occur near the ends of the older cells or branches, but it also happens that they are developed near the middle of a cell. The tenacula are short or long, ending in irregularly lobed discs (Figs. 6f, i, h).

The breadth as well as the length of the cells varies much. The thin ramuli are about 70—80 μ thick, whereas the old cells in the middle of the cushion may reach a breadth of about 300 μ or more, and between these extremes all sizes are met with. In the same way the length varies. One of the longest cells I have seen is that found uppermost in fig. 6k. It was 3 mm long, and as the breadth was 80 μ, it was thus 37 times as long as broad. Kützing in his somewhat schematic figures has also drawn some long cells.

The chloroplasts are polygonal, most of them containing a pyrenoid. Together they form a parietal reticular body. The many nuclei are large and dispersed somewhat below the cell walls.

In the Kew Herbarium authentic specimens from Mauritius, leg. TELFAIR are found; and a comparison with Dr. VAUGHAN'S plant has shown that they agree perfectly. In Dr. MORTENSEN'S collection some dried specimens are also found.

Mauritius: About its occurrence Dr. VAUGHAN writes: "Bright green cushions about 3—6 cm diameter. Common in pools behind reefs". No. 264, 7/2 39. The tufts are mixed with *Corallinaceae*. And about another collection (no. 311) "Green slender somewhat wiry filaments mixed with sea grasses". Dr. MORTENSEN gathered it at Grand Bay at a depth of 2—3 fathoms, Oct. 24th 29.

Geogr. Distr.: Mauritius, Red Sea, Tongatabu, Sandwich Islands.

Spongocladia Aresch.

Spongocladia vaucheriaeformis Aresch.

ARESCHOUG, J. E., in Öfvers. k. Vetensk. Akad. Forhandl., vol. X, p. 201. WEBER VAN BOSSE, A., Etudes sur les algues de l'Archip. Malaisien, I, p. 85; Algues du Siboga, p. 86.

Specimens are found in the Kew Herbarium.

Mauritius: Fort William, Dec. 26th 1869, Colonel PIKE.

Geogr. Distr.: Mauritius, Malayan Archipelago, New Guinea.

Microdictyon Decaisne.

1. Microdictyon Agardhianum Decsne.

DECAISNE, J., Plantes de l'Arabie Heureuse, p. 115. SETCHELL, W. A., The genus Microdictyon, p. 479. — *Microdictyon tenue* Gray, On Anadyomene and Microdictyon, p. 291.

In Dr. MORTENSEN'S collection two well prepared specimens of a *Microdictyon* are found, the colour of which in

the dried condition is greyish-greenish. The plant adheres more or less strongly to paper. The specimens are fastened to the substratum by means of a broad not coherent base, small pieces of coral etc. being found attached round about the middle of the plant over a rather large area. The plant belongs to the group *Annuliferae*, and to Section *Eumi-*

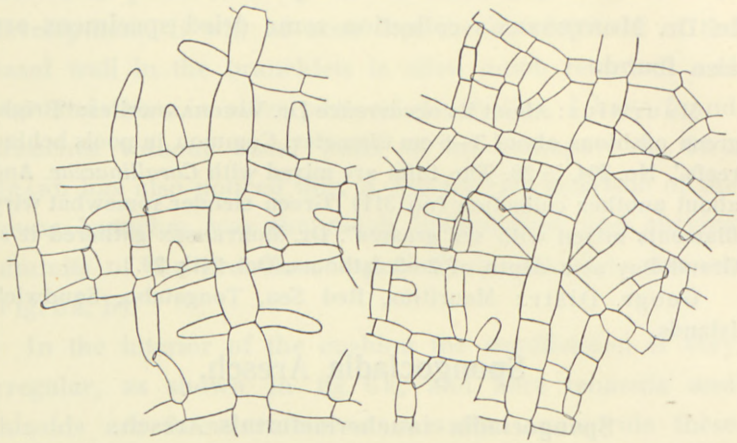

Fig. 7. *Microdictyon Agardhianum* Decsne. Parts of the thallus. (× ca. 18).

crodictyon, according to the division in Setchell's monograph. As mentioned here the geographical distribution of this group is from the Red Sea westward through the Mediterranean Sea and the eastern part of the Atlantic Ocean to the Caribbean Sea. The occurrence of this plant at Mauritius is therefore most interesting and still more so because it agrees as well with *M. Agardhianum* from the Red Sea that I do not hesitate to refer it to that species.

Plate II, fig. 1 shows a photograph of one of the plants. It is seen to be roundish with an irregularly waved outline, about 5—6 cm broad, and, as mentioned above, it is fixed to the substratum by means of a broad base. Fig. 7 shows

a drawing of some parts of the plant. The primary filaments are composed of cells which are about $1^1/_2$ times longer than broad, their breadth being about 130—50 μ. They form a marked venation and are branched as a rule at right angles. The secondary branches are about 90—100 μ thick and consist of cells about twice as long as broad. These give off other branchlets which are about 60 μ thick, having segments about $1^1/_2$ times longer than broad. The branchlets also generally issue at right angles; they are either straight, giving rise to rectangular meshes, or curved, forming triangular meshes. If this description is compared with SETCHELL's, the resemblance of the two plants will be obvious.

Professor SETCHELL once in a letter informed me that Dr. A. H. NASR, Biological Station Ghardaqa, Red Sea, had succeeded in finding DECAISNE's old species. I therefore wrote to Mr. NASR and asked him to send me, if possible, a little material of the plant so that I could compare it with the plant from Mauritius, and Mr. NASR was so kind as to do so; I received some well preserved material. After having made drawings of both plants and compared these, it was obvious that the mode of ramification etc. was quite the same in both plants, the only observable difference being that the plant from the Red Sea was in all respects a little smaller than the plant from Mauritius, but this may be due to accidental circumstances. Further, the plant from the Red Sea has a somewhat lighter green colour than the plant from Mauritius, but as the two plants otherwise entirely agree I do not hesitate to refer the plant from Mauritius to DECAISNE's species.

From the western part of the Indian Ocean two species are previously recorded: *Microdictyon pseudohapteron* Gepp

and Gepp and *Microdictyon Krausii* Gray; the latter is known also from Mauritius. But both these species are entirely different from *M. Agardhianum* and belong to quite other groups of *Microdictyon*.

It must further be pointed out that Dickie in his "Algae of Mauritius", p. 198, mentions *Microdictyon Agardhianum*, but this observation has not been stated by Setchell in his treatise on *Microdictyon*. In the Kew Herbarium I have examined a specimen (no. 148) from Barkly Isl., Jan. 9, 70, leg. Pike, determined by Dickie as *Microdictyon Agardhianum*. According to my notes it is like a large clump and I have also determined it as a *Boodlea*.

Mauritius: Flat Island, $^{17}/_{10}$ 1929. Th. M.
Geogr. Distr.: Red Sea.

2. Microdictyon Kraussii Gray.

Gray, J. E., On Anadyomene and Microdictyon in Journ. of Bot. IV, 1866, p. 69. Setchell, W. A., The genus Microdictyon, p. 519, figs. 39—43.

Some specimens preserved in formol are found in Dr. Vaughan's collection. They agree very well with Setchell's description and figures.

From the base, which is composed of a plexus of rhizoids, the primary filaments are given off, spreading flabellately in all directions. The lowermost cells in these filaments are elongated clavate, up to about 165 μ thick and 5—7 times as long, the cells becoming gradually shorter upwards. From the lowermost ends of the clavate cells, especially in the basal part of the thallus but higher up as well, though to a less degree, rhizoids are given off. The rhizoids grow downwards along the walls of the cells, thus strengthening the basal part.

The ramification is flabellate throughout, reminding one very much of that of *Anadyomene*. Besides the cells continuing the veins, up to 3 cells issue in descending order from the apical ends of the cells in the veins. In the upper part of the thallus the cells in the veins become nearly cylindrical and about as long as broad. While the thallus in the lower part is rather open, with long interstices between the long cells found there, the meshes higher up in the thallus become quite small, and in parts the thallus is here almost compact. The wall is yellow and keeps its colour well in formol. I found it was up to 10 μ thick, thus much thicker than the size given by SETCHELL, but most probably the formol influences the thickness of the wall.

The basal part of this and related species reminds one very much of *Rhipidiphyllon*; compare my figures of this plant in Mar. Alg. Easter Island p. 252, figs. 3 and 4b with SETCHELL's figures 41, 46 and 51. *Rhipidiphyllon* may in a way be said to represent an everlasting infantile stage of development of this and related species of *Microdictyon*; on p. 526 SETCHELL discusses this fact.

Microdictyon Kraussii has hitherto been recorded from the coast of Natal only.

Mauritius: Ilôt Brocus, in lagoon, R. E. V. no. 211.
Geogr. Distr.: Coast of Natal.

Microdictyon(?) spec.

Dr. MORTENSEN's collection contains some quite small dried lumps of a green alga which I first thought was a *Boodlea*, but after examining it more thoroughly I have found that it cannot be referred to that genus owing to its ramification and to its want of tenacula etc.

The specimens form small irregularly shaped lumps, the larger ones about 1 cm broad. Their colour is a dirty olive-green. The filaments of which the thallus is composed

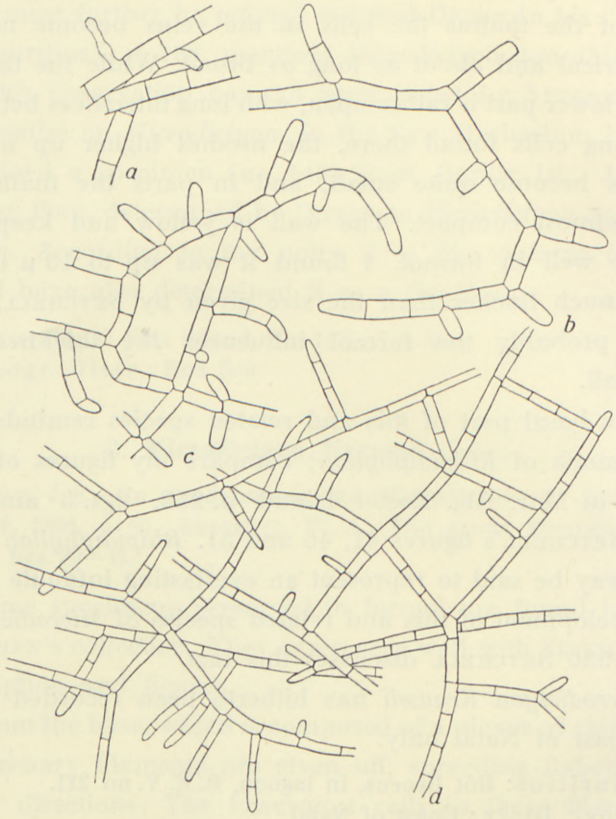

Fig. 8. *Microdictyon* (?) spec. a, part of the thallus with a rhizoid. b, c, parts of the young thallus. d, older thallus. a, × ca. 50; b, c, × ca. 40; d, × 25).

vary as to size, the thicker ones being about 75 μ broad, while the thinner ones and the ramuli are from 30—50 μ thick. The length of the cells varies from $1^1/_2$—2 up to 3 times or sometimes, though rarely, 4 times that of the breadth (Fig. 8). Marked main branches are not present,

and the ramification is very irregular in all directions. The branches are generally given off rectangularly, or nearly so, from the mother branch. As a rule only a single branch issues from each joint, sometimes two and then they are opposite or almost so. When a third one is given off, it is often vertically placed in proportion to the other ones. The branches are often short with obtuse apices. When in touch with other branches, the summits of the branches adhere to them, in this way forming a cushion-like network. At the same time the branches stop growing in length. Some small pieces of coral etc. are found in the cushion, and once I found a short rhizoid (Fig. 8a).

The chromatophores are roundish-polygonal discs and form together a more or less perforated parietal plate. In most of the chromatophores a pyrenoid is present. I have already mentioned that this plant shows much likeness to *Boodlea*. It also shows some likeness to *Cladophora boodleoides* Børgs., Mar. Alg. Canary Islands, I Chlorophyceae, p. 56, compare for instance figures 21 and 22c.

But the genus with which the specimens from Mauritius seem to agree most is *Microdictyon*; it is reminiscent of it not only by its rectangular ramification, but also by the way the tips of the branchlets adhere to neighbouring filaments. I have not, however, found annular thickenings. In this connection attention must especially be directed towards the plant which BERTHOLD has described as *Microdictyon Spongiola*. The description BERTHOLD gives of this plant is: "Bildet lockere, kuglige, schwammähnliche Räschen auf *Peyssonnelia squamaria* und *P. rubra*. Die Zweige stehen allseitig von den Achsen ab und sind nicht so regelmässig mit einander verwachsen wie bei *M. umbilicatum*". BERTHOLD found the plant in November on the north side of

Capri near the surface of the sea. This description must be said to correspond in an astonishing way to the plant from Mauritius as well. And it is especially interesting that Georg Bitter (in his paper: Zur Morphologie und Physiologie von Microdictyon umbilicatum), during a stay at the Zoological Station, Naples, points out that he has succeeded in developing experimentally similar cushion-like specimens of normal *Microdictyon* specimens. According to Bitter strong light in connection with the movement of the waves should develop a tendency to ramification in all directions.

Whether the plant from Mauritius is the result of such a development it is of course impossible to ascertain. It is more likely that it is to be regarded as the representative of a new genus. But in order to decide this, better and especially better conserved material in alcohol is needed.

Setchell, l. c. pp. 576—79, has discussed the similar problem concerning the more or less spongiose thallus of *Microdictyon Montagnei* Harv.

Mauritius: Flat Island, 17. Oct. 1929, leg. Th. M.

Fam. 4. Anadyomenaceae.

Anadyomene Lamour.

1. Anadyomene Wrightii Gray.

Gray, J. E., On Anadyome and Microdictyon, p. 48, tab. 44, fig. 5. J. Agardh, Till Algernes Systematik, VIII, Siphoneæ, p. 124. Okamura, K., Icones Jap. Alg., Vol. I, p. 198, tab. 40, figs. 1—6. Weber, A., Alg. Siboga, p. 73, figs. 14—15.

In Dr. Mortensen's collection a few small specimens are found which agree very well with J. Agardh's description. The most characteristic feature of this species (Fig. 9)

is the strong development of one or two of the up to 7 daughter veins radiating from the summit of the mother vein, while the rest of the veins remain short. Furthermore these vigorously developed veins are nearly cylindrical, about 150 μ thick, and often reach a length of 2 mm or more before they become branched again, and these long parts of the veins become divided by transverse walls into 2—3 cells according to their length. The small cells filling up the interstices between the veins vary as to size and are roundish polygonal or elongated of shape. Transverse sections show that these parts of the thallus are more or less composed of two layers of cells, compare OKAMURA's figures 5—6.

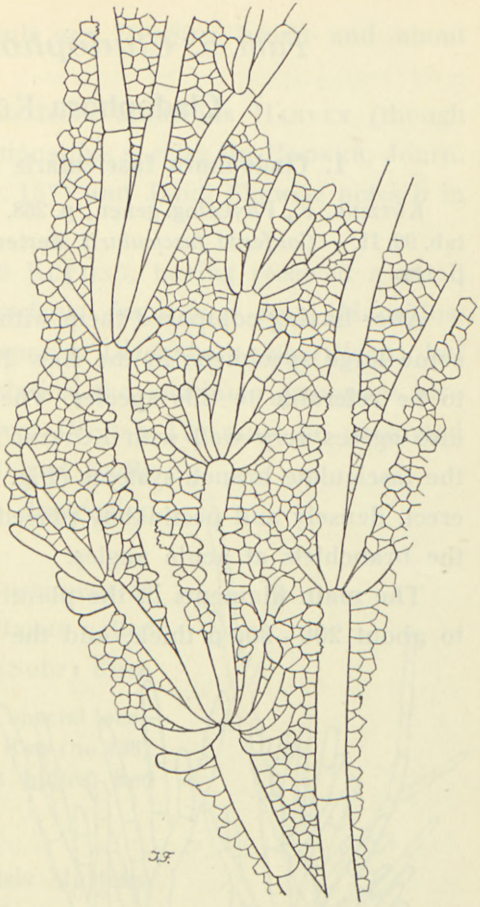

Fig. 9. *Anadyomene Wrightii* Gray. Part of the thallus. (× 30).

Mauritius: Between Gunners Quoin and Flat Island at a depth of 25 fathoms, TH. M. $^{15}/_{10}$ 1929.

Geogr. Distr.: West Indies, Loochoo Islands, Malayan Archipelago, Japan, etc.

Fam. 5. Cladophoraceae.

Cladophora Kütz.

1. Cladophora fascicularis (Mert.) Kütz.

KÜTZING, F., Phycolog. gener., p. 268. Tabulæ Phycol., vol. III. tab. 90, II. — *Conferva fascicularis* Mertens in C. AGARDH, Systema, p. 114.

After having compared them with West Indian material, some large specimens in the Kew Herbarium seem to me to be referable to this species. The plant from Mauritius also agrees very well with KÜTZING's figure quoted above, the fasciculate branch-systems (Fig. 10) being composed of erect, densely and pectinately placed ramuli given off from the branchlets at acute angles.

The main filaments in the plant from Mauritius are up to about 200—300 μ thick, and the cells about 3 times as

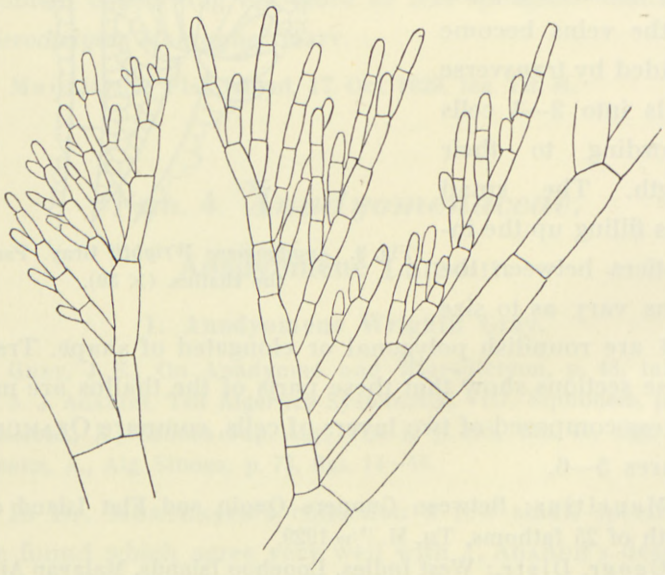

Fig. 10. *Cladophora fascicularis* (Mert.) Kütz. Two branch-systems. (× 30).

long. The upper ramuli are 70—80 μ broad and about 2—4 times as long.

In his list of Algae from Mauritius HARVEY (though with a ?) already mentions this species (in HOOKER, Journ. of Bot., vol. I, 1834, p. 157) and JADIN likewise notes it in his list, p. 156.

In Spec. Alg. p. 399 KÜTZING, having received a small piece of HARVEY's *Conferva fascicularis* from Mauritius, refers it to his new species *Cladophora mauritiana*; but according to his description the specimen (compare also his figure in Tab. Phycol. IV, tab. 12) must be presumed to have been a small and badly developed one, as his description and figure also shows.

According to the specimens in the Kew Herbarium, DICKIE called it *Cladophora Eckloni* (Suhr) Kütz.

Mauritius: Without special locality, leg. Colon. PIKE, Herb. Kew (no. 188).

Geogr. Distr.: West Indies, Red Sea, Mauritius.

2. Cladophora timorensis Martens.

MARTENS, G., Preuss. Expedition nach Ostasien, Die Tange, p. 22, tab. 2, fig. 6.

A dried specimen together with a slide in Dr. VAUGHAN's collection seem to agree rather well with MARTENS' description and figure.

The thicker filaments were about 125—150 μ, the ramuli were about 40—50 μ thick and the apical cells

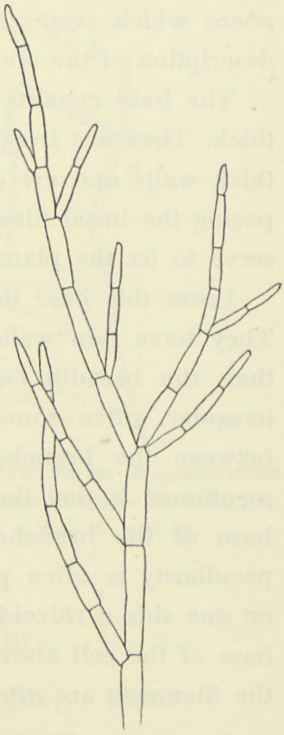

Fig. 11. *Cladophora timorensis* Martens. Part of the thallus (× 30).

decreased upwards more or less to about 25—30 μ. No base was present in the specimen (Fig. 11).

Mauritius: Ilôt Brocus, 12. March 1933, R. E. V. no. 178 in a "tidal creek near sea".

Geogr. Distr.: Timor, New Guinea, Samoa.

3. Cladophora patentiramea (Mont.) Kütz.

KÜTZING, F., Spec. Alg., p. 416; Tab. Phycol., vol. IV, pl. 69. — *Conferva patentiramea* Mont., Prodr. Phyc. antarct., p. 15 (not seen).

forma *longiarticulata* Reinbold in WEBER, Alg. Siboga, p. 84.

Two tubes of Dr. VAUGHAN's collections contain a *Cladophora* which seems to agree fairly well with REINBOLD's description of the above mentioned form (Fig. 12).

The base consists of decumbent filaments about 100 μ thick. They are irregularly bent and ramified. They have thick walls and are dark green of colour. The cells composing the basal filaments are short or long, and rhizoids serve to fix the plant to the substratum (Fig. 12 e).

From this base the erect filaments issue (Fig. 12 a, b). They have thin walls and long cells, many times longer than the breadth (about 60—70 μ). The ramification is irregular, often somewhat unilateral with long distances between the branches. Another feature which must be mentioned is that the formation of the cross walls at the base of the branches is often much retarded. Another peculiarity is often present: When a branch is given off on one side a rhizoid issues on the opposite side from the base of the cell above (Fig. 12 d). The uppermost ends of the filaments are often a little swelled.

Mauritius: Ilôt Brocus. "One of the commonest species in the lagoon". R. E. V. no. 207. Bel Air. "Rock pools near reef". Aug. 1939. R. E. V. no. 323.

Geogr. Distr.: Malayan Archipelago.

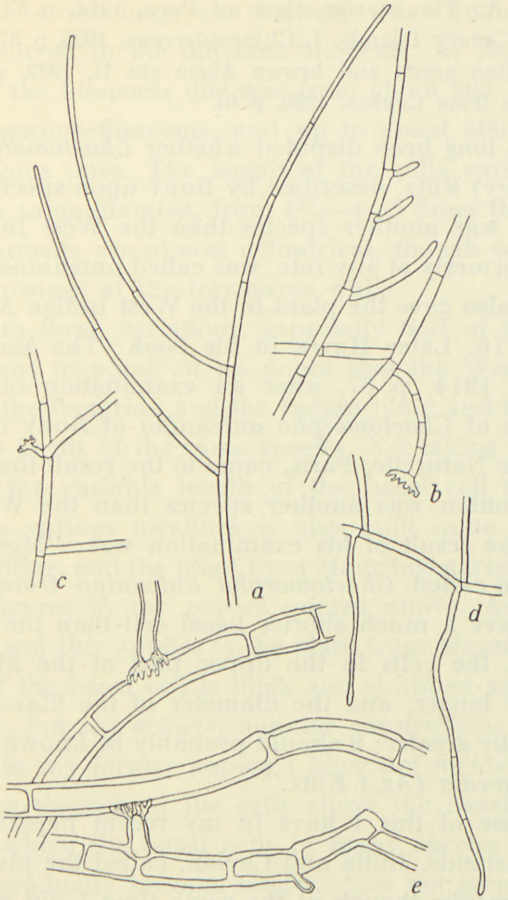

Fig. 12. *Cladophora patentiramea* (Mont.) Kütz. forma *longiarticulata* Reinb. a, upper ends of branches; b, c, d, parts of filaments with rhizoids; e, parts of basal filaments. (a, b, c, d × 25; e × 50).

Chaetomorpha Kütz.

1. Chaetomorpha antennina (Bory) Kütz.

Kützing, F., Spec. alg., 1849, p. 379. Vickers, A., Phycologia Barbadensis, 1908, p. 19, pl. VIII; Børgesen, F., Mar. Alg. D. W. I., vol. I, 1913, p. 16, figs. 4—5. — *Conferva antennina* Bory, Voyage, t. II, 1804, p. 161. *Chaetomorpha media* (Ag.) Kütz., l. c., p. 380.

Howe, M. A., The marine Algae of Peru, 1914, p. 37. Børgesen, Mar. Alg. Canary Islands, I, Chlorophyceae, 1925, p. 37, figs. 9—10; Some Indian green and brown Algae etc. II, 1932, p. 51; Some Mar. Algae from Ceylon, 1936, p. 64.

It has long been disputed whether *Chaetomorpha antennina* (Bory) Kütz. described by Bory upon specimens from Réunion was another species than the West Indian plant which, formerly at any rate, was called *antennina*, the name which I also gave the plant in the West Indian Algal Flora, 1913, p. 16. Later Howe in his book: The Marine Algae of Peru, 1914, p. 37, after an examination of the type-specimen of *Chaetomorpha antennina* of Bory in Muséum d'Histoire Naturelle, Paris, came to the result that the plant from Réunion was another species than the West Indian plant. The result of his examination was: "Most, at least, of the so-called *Chaetomorpha antennina* from the West Indies have a much shorter basal cell than the type from Réunion, the cells in the upper part of the filament are relatively longer, and the diameter of the filaments above is generally greater; it should probably be known as *Chaetomorpha media* (Ag.) Kütz."

Because of this I have in my recent papers from the Canary Islands, India and Ceylon, called the plant *Chaetomorpha media*, though at the same time I did not conceal my doubts as to whether it really, owing to great variation of the basal cell, was specifically different from the plant from Réunion.

It was therefore most interesting to find, in a tube in Dr. Vaughan's collection, several well conserved tufts of a *Chaetomorpha* in which many filaments occurred which had basal cells up to a length of 15—16 mm, thus quite the same as found in the plant from Réunion. But this length was not found in all the filaments, in some the length of the

cells was 3—4 mm only, and between these limits various lengths occurred in the different filaments. As regards the breadth of the filaments this was from about 200—400 μ in the less vigorous filaments, and up to about 500 μ in the more vigorous ones. The length of the cells varies much even in the same filament, from $1^1/_2$—4—5 times the length.

The filaments are almost cylindrical, though with quite small narrowings at the transverse walls.

Owing to these variations, especially that of the basal cell, it seems to admit of no doubt that the West Indian as well as the Canarian and the Indian plant and that from Ceylon are forms of the same species. Not taking into consideration the variable length of the basal cell the plant from these various localities is also built quite like that from Mauritius, and the plant from Mauritius agrees entirely with the figures in my papers quoted above. As pointed out there, and this applies to the plant from Mauritius, too, the wall of the basal cell is thick and stratified and has in most cases, but not always, annular constrictions near the base. But in my former papers I have not mentioned that the growing through of the cells above the basal one, by means of which the basal cells in many species of *Chaetomorpha* gradually become longer, does not seem to take place in this species; at least I have not found this to be the case in the specimens examined by me.

The Kew Herbarium contains a specimen collected at Mauritius by Colonel Pike.

In agreement with the occurrence of this species in other places the plant at Mauritius was found in exposed localities.

Mauritius: Salvinia, R. E. V. no. 254. Cane Point, May 1870, Colonel Pike.

Geogr. Distr.: Réunion, West Indies, Brazil, Canary Islands, India, Ceylon, Pacific coast of Mexico etc.

2. Chaetomorpha aerea (Dillwyn) Kütz.

KÜTZING, F., Spec. Alg., p. 379. HAUCK, F., Meeresalgen, p. 438. HAMEL, G., Chlorophyc. des côtes franç., p. 123, figs. 38 A, B, C, D. — *Conferva aerea* Dillwyn, Brit. Conf., tab. 80.

In Dr. VAUGHAN's collection some specimens (no. 204) are found, the filaments of which have a breadth of about 300 μ.

In Dr. MORTENSEN's collection some thinner specimens are found which I think are also referable to this species. The specimens have filaments 120—160 μ broad, with thick and stratified walls and cells from $^1/_2$—2 times as long, which are not constricted at the transverse walls. The filaments taper below to about 88 μ and are fixed to the substratum by means of a disc which is broadened. As is commonly the case in *Chaetomorpha* the basal cells become lengthened and strengthened by the growing through of the cells above the basal one. The plant very much resembles KÜTZING's figures in Tab. Phycol., tab. 55, figs. II and III, which he calls *Ch. variabilis* and *Ch. Linum* respectively. The former is by HAUCK in "Meeresalgen", p. 438, referred to *Ch. aerea* as a synonym, the latter is not mentioned in HAUCK's list of synonyms, neither as synonym of *Ch. aerea* nor of *Ch. Linum*.

Mauritius: Barachois, Ilôt Brocus, R. E. V. (no. 204). Cannonier's Point, Oct. 29. TH. M.

Geogr. Distr.: Widely spread in temperate and warm seas.

3. Chaetomorpha linoides (Ag.) Kütz.

KÜTZING in Bot. Zeit. 1847, p. 167; Spec. Alg., p. 377. — *Conferva linoides* Ag., Syst. p. 98.

The Kew Herbarium contains a specimen of a plant determined by DICKIE as *Chaetomorpha javanica* Kütz. After

having examined this plant, it appears to me that it does not agree very well with KÜTZING's description and figures of this species.

It seems to agree better with *Chaetomorpha linoides* (Ag.) Kütz.

The plant from Mauritius forms an erect, dense and broad tuft about 8 cm high. The filaments (Fig. 13) are about 70—110 μ broad and the cells from $^3/_4$—2 times as long as the breadth. The filaments are cylindrical, not constricted at the transverse walls, and the walls are rather thick 3—4 μ. The filaments are fastened to the substratum by small discs which gradually grow together, and since new filaments are most probably also developed from them during the growth the result is a dense tuft of filaments. The cells above the discs become successively longer and strengthened, the walls becoming thick and stratified because the lowermost cells in the filaments above the basal one gradually grow downwards into the latter (Fig. 13b).

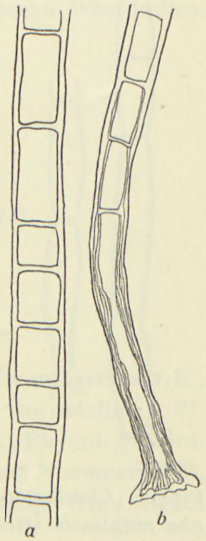

Fig. 13. *Chaetomorpha linoides* (Ag.) Kütz. a, part of a filament; b, basal part of a filament. (× 55).

When the plant from Mauritius is compared with KÜTZING's short description and his figure of *Chaetomorpha javanica* (I have not seen any authentic specimen) this latter shows much thinner filaments and much shorter cells.

Another species having almost the same size is *Chaetomorpha indica* Kütz., Spec. Alg., p. 376 and Tab. Phyc., vol. III, pl. 52, fig. III, which I likewise only know from the short description and figure of it; it

differs essentially by thinner and shorter cells. In my paper "A List of Marine Algae from Bombay", p. 12, I have referred a *Chaetomorpha* to *Ch. indica*. The Indian plant likewise has shorter cells and the filaments are a little narrowed at the transverse walls.

Mauritius: Grand River, May 1879, Colonel Pike in Herb. Kew.

Geogr. Distr.: Pacific and Atlantic Oceans.

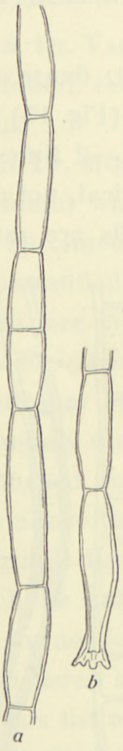

Fig. 14. *Chaetomorpha natalensis* (Hering) De-Toni. a, part of a filament; b, base of a filament. (× 55).

4. Chaetomorpha natalensis (Hering) De-Toni.

De-Toni, J. B., Sylloge Algarum, Vol. I, p. 275. Levring, T., Verzeichnis, p. 7, fig. 3 F, G, H, I. — *Conferva natalensis* Hering in Flora, 1846, p. 215. *Teresias natalensis* (Her.) Aresch., Phyceae capensis, p. 7.

Mingled with *Cladophora patentiramea* there occurred a few filaments of a slender *Chaetomorpha* which seem to agree very well with the description of this species, but I have had no authentic material with which to compare it. The filaments (Fig. 14a) are composed of cells from about 2 up to 4—5 times as long as broad. The cells are cylindrical-subelliptical and narrowed at the transverse walls, where they are 55 μ broad, whereas in the middle of the cells their diameter is about 66 μ. The basal cell is fastened to the substratum by means of an irregularly shaped disc (Fig. 14b).

Mauritius: Bel Air in rock pools near reef. Aug. 1939, R. E. V. no. 323.

Geogr. Distr.: Port Natal.

Rhizoclonium Kütz.

1. Rhizoclonium Kerneri Stockm.

Stockmayer, S., Algengattung *Rhizoclonium*, p. 852. Børgesen, F., Mar. Alg. D. W. I., p. 20, fig. 8. Feldmann, J., Algues Côte des Albères, p. 73, fig. 20 A, B, C, D, E.

The specimens had a breadth of about 15 μ and the length of the cells was about 3—4 times the breadth.

It was found intertwined with *Cladophora*.

Mauritius: In the lagoon at Ilôt Brocus, R. E. V. no. 207.

Geogr. Distr.: Mediterranean Sea, Atlantic Ocean, West Indies.

IV. Dasycladales.

Fam. 1. Dasycladaceae.

Neomeris Lamouroux.

1. Neomeris annulata Dickie.

Dickie, G., On the Algae of Mauritius, p. 198. Solms-Laubach, H., Über die Algengen. Cymopolia, Neomeris und Bornetella, p. 61. Børgesen, F., Mar. Alg. D. W. I., p. 71. Howe, M. A., Phycol. Studies, IV, p. 87. Svedelius, N., Zur Kenntnis der Gattung Neomeris 1923, 449. Børgesen, Marine Algae in Ostenfeld, Plants from Beata Island, St. Domingo, p. 17.

The few specimens from Mauritius upon which Dickie based the species are found in the Kew Herbarium.

Mauritius: Colonel Pike.

Geogr. Distr.: Fairly widespread in the Indian and Pacific Ocean, West Indies etc. Compare Svedelius' map with its distribution, l. c. p. 467.

Acetabularia Lamouroux.

1. Acetabularia Moebii Solms.

Solms-Laubach, H., Monograph of the Acetabularieae p. 30, pl. IV, fig. 1.

In the Kew Herbarium only the discs of several specimens are present, but nevertheless they are easily recognisable. The disc examined by me had 15 rays laterally strongly calcified and with broadly rounded ends. The coronal knobs are roundish and have as a rule 5 hair-scars, sometimes 4 and in one case only 3.

This small species is described from specimens from Mauritius, and as far as I know it has not been found anywhere else. But in a collection of algae from Ghardaqa, the Red Sea, sent to me for identification a few years ago by Dr. A. A. Nayal, the Egyptian University, Kairo, some specimens of this plant are found mounted together with a specimen of *Udotea javensis* Gepp, for which species too the Red Sea is a new locality.

According to the specimens in Herb. Kew *Acetabularia Caliculus* in Dickie's list is this species.

Mauritius: Colonel Pike.
Geogr. Distr.: Mauritius, Ghardaqa (Red Sea).

V. Siphonales.

Fam. 1. Bryopsidaceae.

Bryopsis Lamour.

1. Bryopsis indica A. & E. S. Gepp.

A. and E. S. Gepp., Mar. Alg. "Sealark" Expedition, p. 379, pl. 47, figs. 10, 11.

Some small specimens in Dr. Mortensen's collection are referable to this species. The Kew Herbarium contains

several specimens gathered at Mauritius by Colonel PIKE; they have been referred to this species by Mrs. & Mr. GEPP; compare their remarks, l. c. pp. 380. In DICKIE's list the plant is called *Bryopsis caespitosa* Suhr.

Mauritius: Flat Island, 16. Oct. 1929, TH. M. Gabriel Is. Colonel PIKE $^{22}/_{5}$ 1871.

Geogr. Distr.: Indian Ocean.

Fam. 2. Caulerpaceae.

Caulerpa Lamouroux.

1. Caulerpa Webbiana Mont.

MONTAGNE in Ann. sc. nat., 2. sér., t. 9. bot. 1838. WEBER v. BOSSE, A., Monographie, p. 269.

Dr. MORTENSEN's collection contains a single specimen of the typical form. It had been dredged at a depth of 60 meters.

Mauritius: Flat Island, $^{16}/_{10}$ 1929. TH. M.

Geogr. Dist.: Seems to occur in most warm seas.

2. Caulerpa Mauritiana nov. spec.

Caulerpa mediocris, a surculo terete, ca. $1^{1}/_{2}$—2 mm lato, in arena repente et rhizoideis robustis adfixo et a frondibus erectis composita. Frondes planae, glabrae, lineari-spathulatae, apice basique late obtuso, ad 7 cm longae et 7 mm latae, marginibus integerrimis, rectis, parallelibus, pedicellatae sunt. Stipites cylindici teretiusculi, longitudine variabile, ad 1 cm 2 mm longi et $1^{1}/_{2}$ mm lati. Frondes simplices aut interdum prolificationibus paucis ex margine aut pagina plana emergentibus instructae (Plate III).

Mauritius: Grand Bay at a depth of 2—3 fathoms of water in a rather sheltered locality, growing on sandy bottom with *Halophila*, $^{24}/_{10}$ 1929. Dr. TH. MORTENSEN legit.

It is not without hesitation that I proceed to describe a new species of *Caulerpa* knowing by experience that these plants show a great power of accommodation and therefore often a very deviating appearance according to their different external conditions of life. But with the specific interpretation of *Caulerpa* now prevalent, it seems to me most correct to consider it a new species.

Two larger specimens and a smaller one are found in Dr. Mortensen's collection. In the two larger specimens (Plate III) the prostrate horizontal creeping rhizome is about 31 cm long, provided below with vigorous rhizoids and upward erect assimilating shoots. The vigorous rhizoids issue at a mutual distance of about 2—3 cm, whereas the distance between the assimilators varies from one to seven cm. The assimilators have below a short cylindrical stem of variable length, from a few mm up to about 1 cm and 2 mm. They are leaflike structures, spathulate and linear of shape with entire, straight, parallele margins, without anything like teeth or serratures, and with broadly rounded upper ends and bases. Most of the leaflike shoots are simple but a few are provided with a single or a few proliferations issuing from the upper ends of the shoots. In one case a proliferation is given off from the edge of the thallus near its middle; in another case one issues from the flat side of it. The larger simple assimilators are $6^1/_2$ cm long and 7 mm broad and the most proliferous assimilator reaches a height of 9 cm. It is only one of the specimens that has proliferous assimilators, in the other two these are quite simple. While the two larger specimens are quite alike as to size and appearance the third specimen is in all respects smaller. The largest erect assimilator in this specimen is only $3^3/_4$ cm long and at its broadest $4^1/_2$ mm broad. Most of the erect

assimilators in the specimen taper a little upwards from the broadest part somewhat above the base. Furthermore all the pedicels are short and of almost the same length, about 2 mm.

The Mauritius plant seems to hold an intermediate position between *Caulerpa ligulata* (Harv.) J. Ag. (= *Caulerpa flagelliformis* f. *ligulata* Web. v. Bosse) on the one side, and *Caulerpa brachypus* Harv. on the other side. Of the first-mentioned species I have had for comparison with the Mauritius plant some fine specimens from False Bay, collected 19th October 1929 by Dr. Th. Mortensen, besides specimens from the herbarium of the Botanical Museum, Copenhagen, and from my own collection. At a cursory glance they seem to be much alike, but a more thorough examination soon shows considerable differences. Thus the annulation of the stipes, of the basal ones as well as those of the proliferations, and the marked tendency to dichotomy of the assimilators found in *Caulerpa ligulata* is not present in the Mauritius plant. Further the gradual narrowing towards the base of the assimilators in *Caulerpa ligulata* is not found in the Mauritius plant, in which the assimilators above the stipe quickly reach their normal breadth and keep this up to the broadly rounded apex. In addition *Caulerpa ligulata* is much more proliferous and a taller plant. Owing to these differences the Mauritius plant must be said to be well separated from *Caulerpa ligulata*.

In a paper: "On *Caulerpa anceps* Harv." Yendo[1] has

[1] When the specific name *brachypus* is used and not *anceps* it is because Yendo in this paper has made an exhaustive comparison of *Caulerpa anceps* and the related species *Caulerpa brachypus* and *Caulerpa Stahlii* and arrives at the result that they are scarcely specifically different, basing his examinations upon material which he collected on a reef somewhat below the surface of the sea at low tide near the

given an exhaustive description of this plant (as stated in the note below = *Caulerpa brachypus*) and I refer the reader to this treatise. As the most essential characters by which *Caulerpa brachypus* and related forms differ from *Caulerpa Mauritiana* must be mentioned that the assimilators are much more irregularly shaped and often richly provided with proliferations, that the margins of the assimilators are serrated and provided with teeth, that the short pedicels, when present, are ball- or clubshaped and finally that the plant, of which the late Professor YENDO as well as Professor YAMADA most kindly sent me specimens, as a rule is only half as large as the plant from Mauritius. YENDO, however, points out that the winter-form may reach a height of about 6—7 cm, thus about the same size as the plant from Mauritius.

A comparison between the plant from Mauritius and *Caulerpa prolifera* shows that the latter plant has a much larger obovate-lanceolate leaf tapering evenly upwards and downwards with even transitions in the stipe, and although narrowleafed forms, for instance one gathered by me near Las Palmas at the Canary Islands, may show some resemblance to the plant from Mauritius, the assimilators always taper upwards and downwards from their middle. And when we add to this the geographical distribution, *Caulerpa*

biological station at Misaki. After having collected material at different seasons of the year he found that the summer forms ressembled *Caulerpa anceps*, whereas the more robust *Caulerpa brachypus* was the winter form. It is evident from his description that this species is to a great extent polymorphic. In this respect compare also OKAMURA's fine figures in "Icones", pl. 125 and M^me^ WEBER's remarks in "Algues Îles Kei", p. 89. In this paper M^me^ WEBER also pointed out that the consequence of YENDO's discovery must be that the specific name of this species must be *Caulerpa brachypus* as the oldest name (1859), whereas *Caulerpa anceps* dates from 1872.

prolifera never having been found outside the Atlantic Ocean and the Mediterranean Sea, it seems to me most appropriate to consider the plant from Mauritius as specifically different from *Caulerpa prolifera*.

In this comparison I have taken into consideration only the two large specimens. The small specimen I presume to be a young and less developed plant, but this cannot be decided with so little material.

3. Caulerpa scalpelliformis (Rr. Br.) Web. v. Bosse.

Weber van Bosse, A., Monographie des Caulerpes, p. 286.

f. *denticulata* (Decsne) Web. v. Bosse.

A single specimen is found in Dr. Mortensen's collection. The assimilators are short and broad and the upper ends of the lobes are broadly rounded and denticulate.

The Kew Herbarium contains one specimen closely resembling the one mentioned above and another very much ramified specimen with quite short ($1^1/_2$—2—3 cm) assimilators only. Furthermore a specimen with long narrow assimilators resembling the forma *Dwarkensis* from the northern part of the Arabian Sea is found there; compare Børgesen, Some Indian green and brown Algae etc. II, p. 56—57, pl. I.

Two specimens are present in Dr. Vaughan's collection.

Mauritius: Cannonier's Point, Oct. 1929, Th. M. Grand River, Dec. 67, Colonel Pike. Black River Bay, R. E. V. no. 130.

Geogr. Distr.: Indian Ocean, Red Sea, Brazil etc.

4. Caulerpa sertularioides (Gmel.) Howe.

Howe, M. A., Phycological Studies. II, p. 576. — *Fucus sertularioides* Gmelin, Historia Fucorum 1768, p. 151.

Several specimens are found in Dr. Vaughan's collection.

A single specimen was collected by Dr. Mortensen at a depth of about 2—3 fathoms.

forma *Farlowi* Web. v. Bosse, l. c. p. 295.

Dr. Mortensen's collection contains some quite small specimens, 1—$1^1/_2$ cm high, in which the pinnules are placed round the axis in all directions.

Mauritius: Grand Bay, $^{25}/_{10}$ 1929, Th. M. Cannoniers Point, $^6/_5$ 71, Colonel Pike. Port Louis harbour, R. E. Vaughan no. 129.

forma *Farlowi*: Flat Island, 16. Oct. 1929, Th. M.

Geogr. Distr.: Most warm seas.

5. Caulerpa serrulata (Forssk.) J. Ag., emd. Børgs.

Børgesen, F., Revision, p. 5. — *Caulerpa Freycinetii* Ag., Spec. Alg., p. 446. Weber van Bosse, A., Monographie, p. 310, where the literature is quoted.

Dr. Vaughan's collection contains a large specimen no. 246 but without any indication of locality.

A fine specimen is found in the Kew Herbarium. It is mentioned in Dickie's list as *Caulerpa najadiformis* Bory.

Mauritius: On the reefs, Barkley Isl., Aug. 70, Colonel Pike.

Geogr. Distr.: Most warm seas.

6. Caulerpa cupressoides (Vahl) Ag.

C. Agardh, Spec. Alg., p. 441. Weber van Bosse, A., Monographie, p. 323. — *Fucus cupressoides* Vahl, En deel kryptogamiske Planter fra St. Croix, p. 29.

A single dried specimen without locality or other data is found in Dr. Vaughan's collection (no. 244). The branches in the assimilators are slender and covered with ramuli from near their bases. The short naviculoid ramuli are

placed in 3 rows. It seems to have an intermediate position between f. *typica* and f. *mamillosa*.

Mauritius: Fr. Neyrolles (the specimen without locality).
Geogr. Distr.: West Indies, Indian and Pacific Oceans.

7. **Caulerpa racemosa** (Forssk.) Web. van Bosse.

Weber van Bosse, A., Monographie, p. 357. — *Fucus racemosus* Forssk., Flora Ægypt. — Arab., p. 191.

A specimen with small vesicles and rather short assimilators coming near to var. *uvifera* is found in Dr. Vaughan's collection (no. 36).

Mauritius: Point aux Roches, in sandy pools, R. E. V. no. 36.
Geogr. Distr.: Tropical seas.

8. **Caulerpa peltata** Lamour.

Lamouroux in Journ. de Bot. II, 1809, tab. 3, fig. 2. Agardh, J., Till Algernes System. I, p. 37. Svedelius, N., Ceylon species of Caulerpa, p. 131. Børgesen, F., Some Indian green and brown Algae, II, p. 62, fig. 5.

var. *typica* Web. v. Bosse, Monographie, p. 373.

Some small specimens have been gathered by Dr. Th. Mortensen. Each of the stems carried mostly only one or two discs. A similar form is found in the Kew Herbarium gathered by Colonel Pike.

Mauritius: Flat Island, $^{16}/_{10}$ 1929, Th. M.
Geogr. Distr.: Indian and Pacific Oceans, Red Sea, Canary Islands, West Indies.

Fam. 3. Codiaceae.

Avrainvillea Decaisne.

1. Avrainvillea gracillima nov. spec.

Frons sordide brunneo-viridis, solitaria, pusilla, ca. 2 cm alta, flabelliformis, e stipite et flabello composita.

Stipes cylindricus, ca. 7—8 mm longus et $^3/_4$ mm latus, superne cuneatim applanatus in flabellum transiens.

Flabellum tenue, membranaceum, oblonge-reniforme aut subcordatum, margine superiore late rotundato, subintegro vel parum lacerato, inconspicue zonatum.

Filamenta frondis subcylindrica, torulosa aut submoniliformia, brunneo-flava, 6—30 μ crassa (Plate II, fig. 2).

Mauritius: Between Gunner's Quoin and Flat Island, at a depth of 25 fathoms, $^{15}/_{10}$ 29, Th. M.

This elegant small species differs from all known species of *Avrainvillea* because of its small size, the whole plant attaining a height of 2 cm only.

The slender cylindrical terete stipe has a length of 7—8 mm and is about $^3/_4$ mm thick. Below the stipe broadens out into a small disc attached to pieces of calcareous algae. The stipe is of firm consistency and passes evenly into the cuneate base of the flabellum, which is oblong, reniform, or subcordate, with broadly subentire or fibrillous upper margin, about $1^1/_2$ cm broad and $1—1^1/_4$ cm high. The flabellum is thin and membranaceous, rather firm below, of a more loose consistency above. A not very distinct zonation is found near the upper margin of the frond. The colour is brownish to olive-green and dull. The surface is a little gritty.

The filaments of the flabellum (Fig. 15) are more or

less firmly interwoven and vary as to size, being from about 6 μ to 27 μ thick, rarely up to 30 μ below the dichotomies. The thin and the thick filaments are mixed together. The filaments are subcylindrical, often tortuous, moniliform and torulose. The colour is yellowish-brownish to

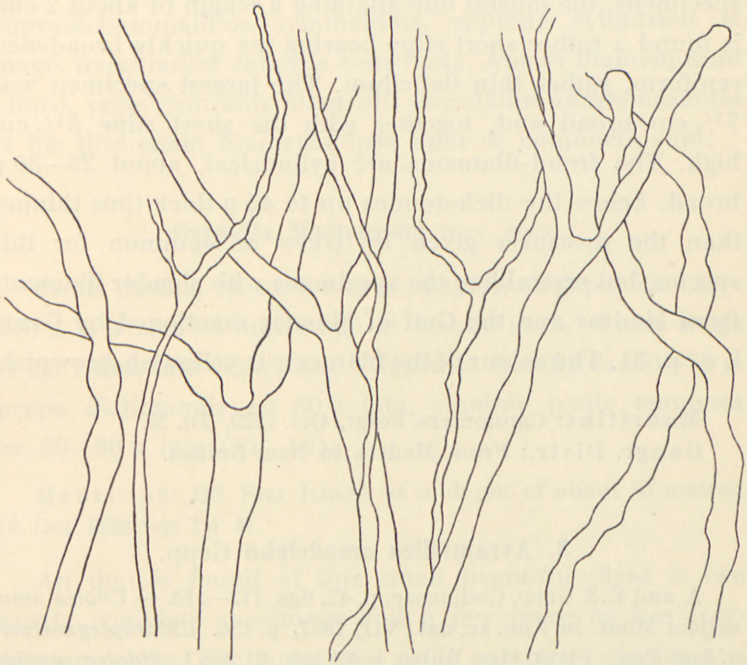

Fig. 15. *Avrainvillea gracillima* nov. spec. Filaments of the flabellum. (× 150).

dark brown. The occurrence of the plant is most certainly solitary. Only two specimens, both figured on Plate II, fig. 2, are found in the collection.

2. Avrainvillea erecta (Berkel.) Gepp.

A. and E. S. Gepp, The Codiaceae of the Siboga Expedition, p. 29, figs. 84—89. — *Dichonema erectum* Berkeley in Hooker's London Journal of Botany, I, p. 157, tab. VII, fig. 11. *Avrainvillea papuana*

Murray and Boodle in Journ. of Botany, XXVII, 1889, p. 71, tab. 289. For more synonyms compare A. and E. S. Gepp's monograph.

The specimens in Dr. Mortensen's collection seem to agree very well with A. and E. S. Gepp's description. Above the root-mass which is not very much developed in these specimens, the longest one attaining a length of about 2 cm, is found a rather short stipe bearing the quickly broadened reniform, rather thin flabellum. The largest specimen was $7^1/_2$ cm broad and, together with the short stipe $5^1/_2$ cm high. The frond-filaments are cylindrical, about 25—30 μ broad, below the dichotomies up to 45 μ thick thus thinner than the measures given by Gepp as common for this species, but resembling the specimens with slender filaments from Madras and the Gulf of Manaar mentioned by Gepp, l. c., p. 31. The colour of the filaments is yellowish-brownish.

Mauritius: Cannoniers Point, Oct. 1929, Th. M.
Geogr. Distr.: From Madras to New Guinea.

3. Avrainvillea amadelpha Gepp.

A. and E. S. Gepp, Codiaceae, p. 42, figs. 112—115. — *Udotea amadelpha* Mont. in Ann. sc. nat., VII, 1857, p. 136. *Chloroplegma sordidum* Zan., Plant. Mar. Rubr., p. 82, tab. XI, fig. 1. *Udotea sordida* Mont., Dickie, Alg. Mauritius, p. 198.

The Kew Herbarium contains fine specimens gathered by Colonel Pike and, as mentioned above, determined by Dickie. The habit of the specimens agrees very well with Gepp's figure 112 of forma *Montagneana*. As has been pointed out by Gepp, l. c. p. 43 the pseudo-cortex in the plant from Mauritius is not so well developed.

Mauritius: Colonel Pike, May 1870.
Geogr. Distr.: Indian Ocean, Red Sea.

Geppella nov. gen.[1]

Thallus viridis, non incrustatus, verisimiliter cyathiformis, stipitatus. Stipes monosiphonius, tenuis, superne in frondem leniter transiens. Frons submonostromatica et reticulata ex filamentis dichotome divisis, constrictionibus supradichotomalibus symmetricis, superne cylindricis et magis irregulariter ramosis constructa. Apices filamentorum obtusi, sæpe cum aliis filamentis annulatim anastomosantes et hic illic etiam filamenta ipsa inter se conjuncta sunt.

Geppella Mortensenii nov. spec.

Frons pusilla, ad 1 cm alta et ultra(?), ex stipite ca. 130—140 μ lata et 3—4 mm longa et fronde ca. 5—6 mm longa et ca. 7 mm lata composita. Filamenta frondis ca. 60—70 μ, prope dichotomiis ad 80 μ lata, superne paulo tenuioria ca. 50—60 μ lata (Fig. 16).

Mauritius: Off Flat Island at a depth of about 60 metres, 16. Oct. 1929 leg. Th. M.

All that is found of this small beautiful plant is two nearly complete specimens and a few more or less fragmentary parts. The larger, not quite complete, specimen is about 1 cm high and its upper part is about 7 mm broad. The stipe in this specimen is 3 mm long and 130—140 μ broad. Below, the stipe is divided into a few rhizoidlike branchlets by means of which it is fastened to the substratum. Above, as Fig. 17 a shows, the monosiphonous stipe becomes furcated into two branches which quickly by repeated furcations (Fig. 17 b) are divided into a number

[1] Named in honour of Dr. A. Gepp and the late, much regretted Mrs. Ethel S. Gepp, authors of the excellent monograph of the *Codiaceae*.

of filaments gradually increasing upwards and forming the most certainly cyathiform part of the thallus. The material being so scarce, and all dried on a small piece of paper together with pieces of *Microdictyon*, I dare not deny the possibility that the frond may be flabellate, but I do not

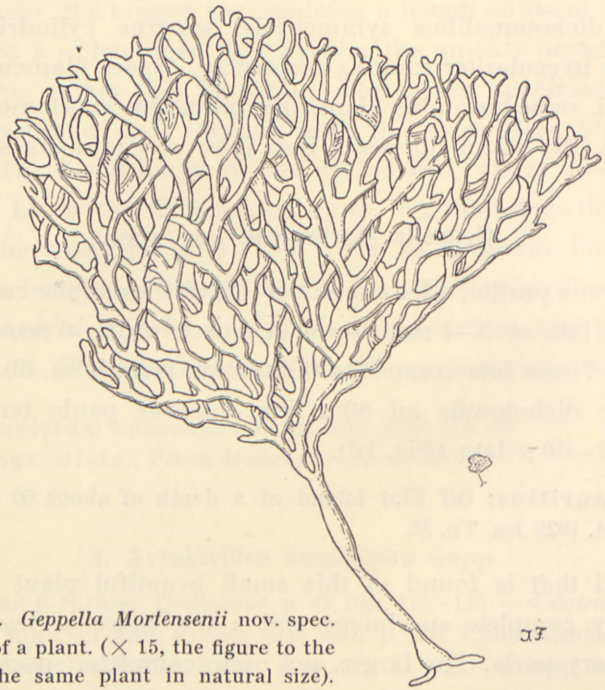

Fig. 16. *Geppella Mortensenii* nov. spec. Habit of a plant. (× 15, the figure to the right the same plant in natural size).

think so. The frond has a cuneate base which is quickly broadened. The frond is composed of a reticular tissue with numerous open interstices between the filaments. It consists of a single layer of filaments with the exception that now and then one filament may cross another. The filaments in the lower part of the flabellum are rather regularly dichotomously divided and above the dichotomies they are not a little narrowed (Fig. 17 b). Higher up the filaments become nearly cylindrical, and the ramification

is more irregular, in the upper parts of the frond antler-like, with lateral branchlets sometimes ramified several times (Fig. 18). In the lower part of the frond the filaments are about 60—70 μ, and below the dichotomies up to 80 μ thick, tapering a little upwards to about 50—60 μ. The upper ends of the filaments are obtuse and broadly rounded.

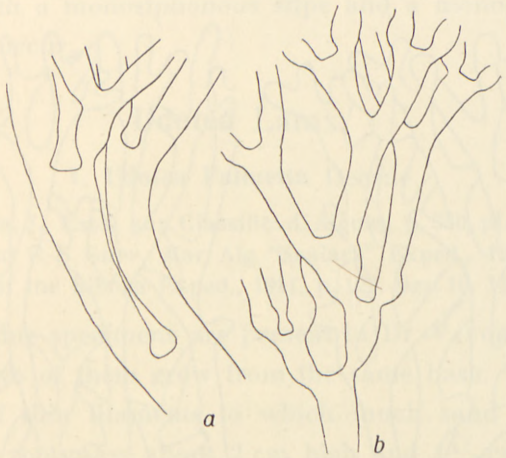

Fig. 17. *Geppella Mortensenii* nov. spec. a, upper end of the stipe with the first divisions; b, filaments shoving divisions in the basal part of of the front. (× ca. 50).

It is especially characteristic of this plant that all the summits of the filaments as well as the filaments themselves when they come in contact with neighbouring filaments anastomose with these in a way very similar to that found in the group *Annuliferae* of *Microdictyon*, that is to say, by annular swellings on the tips of the filaments (Fig. 18b). And not only the tips of the filaments attach themselves to other filaments, but neighbouring filaments sometimes too become attached to each other by means of kneelike swellings; compare Fig. 18b in the middle to the right.

The colour of the plant is green. Chalk incrustations are not present. The discoid chromatophores are roundish-

polygonal without pyrenoids. Large oblong to reniform grains of starch are present in great numbers. No transverse walls are found in the thallus.

In a family composed of so many closely related forms all consisting of dichotomously divided filaments it is of course necessary to take into consideration even rather

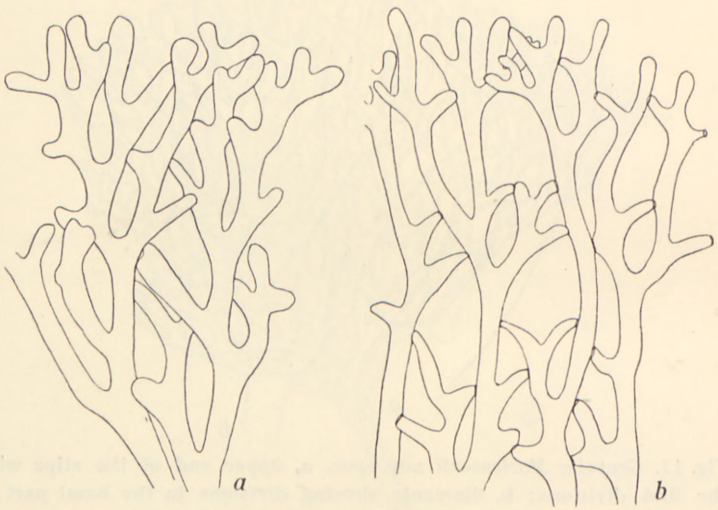

Fig. 18. *Geppella Mortensenii* nov. spec. a, part of young thallus near the edge; b, more developed thallus, nearly all the apices of the filaments have become attached to neighbour-filaments (× ca. 50).

insignificant features to get sufficient characters to separate the forms. Besides the outer habit it is especially the various shapes and ways of ramification of the filaments which afford the essential characters on which to rely. It is of course also upon such characters that A. and E. S. Gepp have based the various genera in their most useful monograph, and upon which I too base this new genus. The antlerlike ramification and notably the fastening of the tips of the lateral branchlets to neighbouring filaments by means

of annular thickenings are not known in other forms of the *Codiaceae*. The kneelike anastomosing of the filaments found now and then reminds one of that found in *Rhipiliopsis*. As regards the general habit of *Geppella* its division into a stipe and a cyathiform upper part agrees with some of the genera, for instance *Udotea*, in which genus also species with a monosiphonous stipe and a monostromatic flabellum occur.

Udotea Lamx.

1. Udotea Palmetta Decsne.

Decaisne, J., Essai sur Classific. d. Algues, p. 380, pl. 17, fig. 15. Gepp, A. and E. S. Gepp, Mar. Alg. "Sealark" Exped., 1909, p. 385; Codiaceae of the Siboga Exped., 1911, p. 122, figs. 10, 11, 54.

Three fine specimens are present in Dr. Vaughan's collection. Two of them grow from the same base. The base consists of thin filaments to which much sand adheres, forming a root-mass about 2 cm high and $1^1/_2$ cm broad. The stipes are $1^1/_2$—2 cm long, and about 2 mm broad. The flabellum in the largest specimen is $2^1/_2$ cm high and 4 cm broad. The zonation is not very highly developed and the margin is lacerate. The specimens are rather bleached as most probably they have been cast ashore.

The discovery of this species at Mauritius is of interest, for according to Gepp's monograph the species is known only from a single locality, namely Cargados Carajos, where it was gathered at a depth of about 45 fathoms. The type-specimen in Herb. Paris, upon which Decaisne based the species, is without any information as to locality.

Mauritius: Black River Bay, G. Antelme in Herb. R. E. Vaughan no. 223.

Geogr. Distr.: Cargados Carajos.

2. Udotea argentea Zan.

ZANARDINI, J., Plant. Mar. Rubr., p. 82, pl. X, fig. 1. GEPP, A. and E. S., The Codiaceae, p. 125, figs. 15, 21, 22 c, 22 d, 25 a, 57—62.

The specimens from Mauritius belong to the forma *typica*, the filaments of the frond bearing lateral appendages with lobately divided heads. As to habit two of the specimens agreed with GEPP's fig. 57 of a plant from Cargados Carajos. Another specimen, R.E.V. no. 276, was very proliferous, resembling var. *spumosa* Gepp, but the appendages of the filaments had heads which were lobately divided.

Mauritius: Point aux Roches in sandy pools, R. E. V. no. 139, $^{3}/_{4}$ 31.

forma ad var. *spumosum* vertens: Flic en Flacq lagoon. Growing in sand behind reef. R. E. V. no. 276, $^{17}/_{1}$ 39.

Geogr. Distr.: Red Sea, Seychelles, Malayan Archipelago, Queensland.

Halimeda Lamx.

1. Halimeda Tuna (Ellis et Sol.) Lamx.

LAMOUROUX, J. V., Sur la Classif. d. Polypiers corall. etc. p. 186. BARTON, The genus Halimeda, p. 11, where the literature is mentioned.

Several specimens are found in Dr. VAUGHAN's collection, most of them belonging to forma *typica* and forma *platydisca*. A single specimen with elongate-cuneate joints resembled var. *Albertisii* Picc.

According to Dr. VAUGHAN this species is very common in sandy shallow pools. Dr. MORTENSEN has dredged a specimen at a depth of about 50 meters.

Mauritius: Cannoniers Point, Point aux Roches etc.

forma *platydisca* (Decsne) Barton, The genus Halimeda, p. 11, where literature is mentioned.

A small piece of this form is found in Dr. MORTENSEN's collection. It was gathered at a depth of about 50 meters.

Mauritius: Between Gunner's Quoin and Flat Island, $^{15}/_{10}$ 29, TH. M.

Geogr. Distr.: Widely distributed in temperate and warm seas.

2. Halimeda Opuntia Lamx.

LAMOUROUX, J. V., l. c. p. 186. BARTON, l. c. p. 18, where the literature is mentioned.

forma *typica* Barton, l. c., p. 20, pl. II, fig. 19.

Mauritius: Off Flat Island, Oct. 29, TH. M. According to Dr. VAUGHAN the plant is common in lagoons and pools.

Geogr. Distr.: Widely distributed in temperate and warm seas.

3. Halimeda incrassata (Ell. et Sol.) Lamx.

LAMOUROUX, J. V., l. c., p. 186. BARTON, l. c., p. 25 where the literature is cited.

Only a single specimen is found in Dr. VAUGHAN's collection. It is a strongly calcified plant showing much likeness to forma *robusta* Børgs., Mar. Alg. D. W. I., p. 112, fig. 90.

Mauritius: Without locality, Father NEYROLLES. Herb. R. E. V. no. 245.

Geogr. Distr.: Warm seas.

Codium Stakh.

1. Codium arabicum Kütz.

KÜTZING, F., Tabulae Phycologicae, vol. VI, pl. 100, fig. II. SCHMIDT, O. CHR., Beiträge, p. 30, fig. 11.

Some specimens in the Kew Herbarium collected by Colonel PIKE at Rochetois, Aug. 29th 1869 and at Port Louis Harbour, Dec. 28th 1869 seem, especially as regards the last mentioned plant from Port Louis Harbour, to agree

fairly well with Schmidt's description of this species. I wish, however, to point out that I have not been able to examine any type-specimen of Kützing's.

The accompanying figures (Fig. 19) show some utricles drawn from a slide of the plant from Port Louis Harbour.

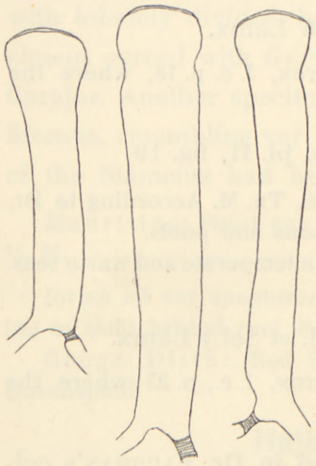

Fig. 19. *Codium arabicum* Kütz. Utricles from the thallus. (× ca. 150).

The utricles are as a rule slender, cylindrical or, more rarely, somewhat clavate, about 53—77 μ broad or now and then a little more, and about 300—400 μ long. They generally have a roundish or somewhat flattened summit and are often narrowed near their upper ends, thus forming a "Blasenkopf". The apical wall is not thickened, or only slightly so, up to about 4 μ. In the specimen from Rochetois the vesicles are often more clavate and somewhat broader, up to about 132 μ. The specimen from December has gametangia (Fig. 20).

Schmidt gives a description of a plant from Madagascar, with which especially the plant from Port Louis Harbour agrees very well. According to the dimensions of the utricles mentioned by Schmidt in his description of *C. arabicum* the plant from Mauritius is within the limits given in this diagnosis, but the dimensions of the utricles of the plant from Mauritius, in the small preparations examined by me neither reach the breadth nor the length which they are said to attain in Schmidt's diagnosis.

Professor Setchell who has likewise taken proofs of these specimens in the Kew Herbarium, and with whom

I have discussed the problem, writes about the specimens from Port Louis Harbour that they are somewhat nearer to *C. arabicum* but do not exactly correspond with KÜTZING's type specimen. Professor SETCHELL points out that he is more inclined to refer them to the West indian species *C. intertextum* Collins & Herv. After an examination of typical material of this species (Phycotheca Bor. Am. no. 2018) and according to COLLIN's and HERVEY's somewhat defective description (The Algae of Bermuda p. 58) and the good description and figures given by TAYLOR, The Mar. Algae of Florida, p. 79, pl. 6, figs. 11—12, pl. 7, fig. 5, whose figures show, besides cylindrical utricles, very broadly clavate ones, up to 195 μ or rarely even 294 μ, I prefer to do as SCHMIDT did with the plant from Madagascar, and refer them to *Codium arabicum*.

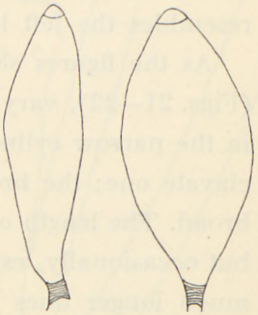

Fig. 20. *Codium arabicum* Kütz. Two gametangia. (× ca. 150).

Mauritius: Rochetois, Aug. 29th and Port Louis Harbour, Dec. 28th 1869, Colonel PIKE.

Geogr. Distr.: Indian and Pacific Oceans.

2. **Codium coronatum** Setch.

SETCHELL, W. A., Tahitian Algae, p. 82, pl. 10, figs. 2—5; pl. 11, figs. 2—3; pl. 12, figs. 1, 5.

var. *aggregata* nov. var.

a forma *typica* præcipue differt utriculis angustioribus et latioribus in aggregatis minoribus separatis, apicibus utriculorum sæpe depressis et parietibus internis superne foveolatis.

Mauritius: Tamarin Bay. R. E. V. no. 317.

Some material preserved in formol and seawater in Dr. Vaughan's collection seems to show so much likeness to *Codium coronatum* Setch. that I think it can be considered a variety of this species.

The material consists of a small lump with a somewhat waved surface and a narrower prolongation; it very much resembles the left half part of Setchell's fig. 2, pl. 10.

As the figures show, the shape and size of the utricles (Figs. 21—22), vary much, their breadth ranging from 55 μ in the narrow cylindrical form up to 300 μ or more in the clavate one; the broadest one examined by me was 407 μ broad. The length of the utricles was as a rule about 700 μ, but occasionally, especially amongst the clavate ones, some much longer ones occurred which were about 1200 μ or even more. However, such anomalous utricles often occur in specimens of *Codium* and are most probably due to abnormal growth, caused for instance by the danger of overlapping from adjacent utricles. Vouk in "Studien über adriatischen Codiaceen", p. 10 proposes to call them sacculi in order to distinguish them from the normal utricles.

As regards the plant from Mauritius it is worth noting that it is easy to divide the utricles into three groups: the thin cylindrical ones, those of medium size, cylindrical-clavate of shape, and the broad clavate ones. Furthermore, the first and last mentioned as a rule occurred in small groups, either of the one or of the other kind, while those of medium size were found mingled with thin ones. The thin cylindrical utricles (Figs. 21 a, b, c) are from about 55—100 μ broad. Their summits vary as to shape, being roundish-cupola-like to almost flat or often depressed in the middle. Many are capitate, being narrowed somewhat below the apex. The wall of the apical end is thickened

and has a distinctly dotted inner wall. The thin utricles carry no hairs.

The utricles of medium size (Figs. 21 a, b, d, e, f) are from about 80 to 170 μ broad and carry a whorl or sometimes irregularly arranged hairs or scars of these. Their

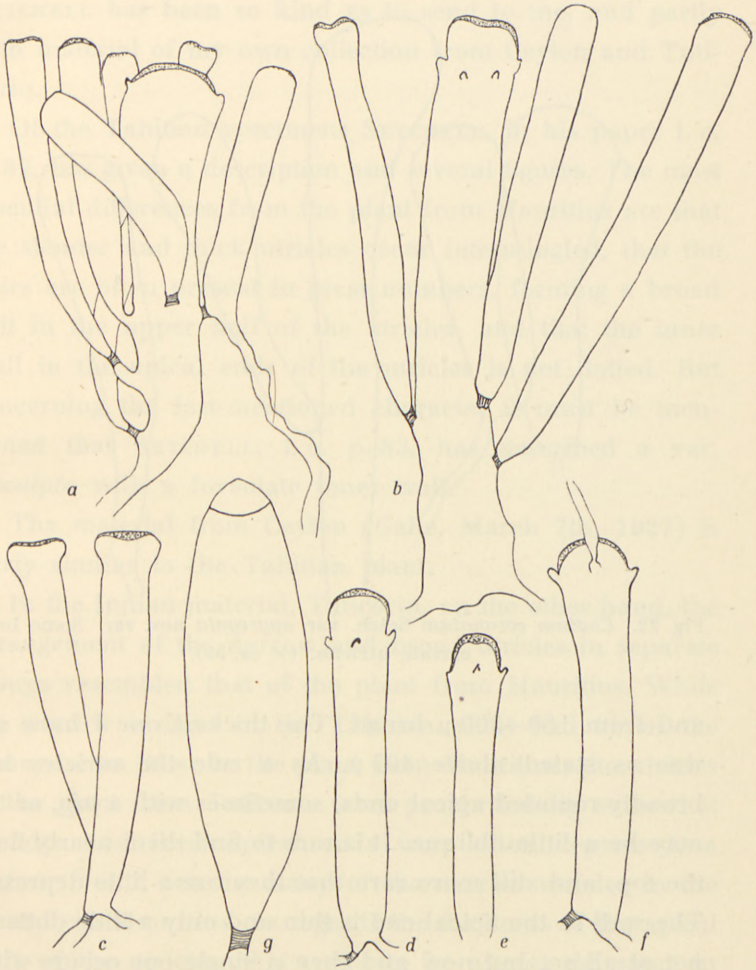

Fig. 21. *Codium coronatum* Setch. var. *aggregata* nov. var. a, b, two utricles of medium size surrounded by thin utricles; c, two thin utricles; d, e, f, utricles of medium size; g, a gametangium (a—f × ca. 75; g × ca. 200).

apical ends are roundish cupola-like and have as a rule thick walls with a dotted inner wall. Sometimes thickenings are found up to 15—16 μ. They generally occur scattered between the thin ones, but also now and then several together.

The third kind of utricles (Figs. 22) are clavate of shape

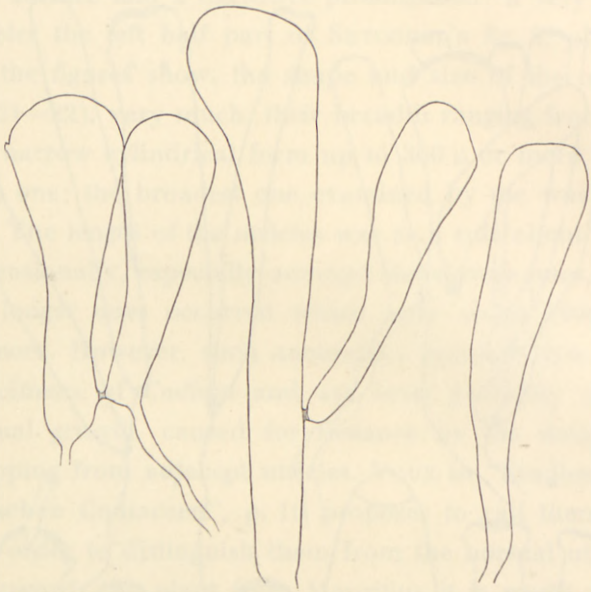

Fig. 22. *Codium coronatum* Setch. var. *aggregata* nov. var. Some broad, clavate utricles. (× ca. 55).

and from 180—300 μ broad. The thickest one I have seen was as stated above 407 μ. As a rule the utricles have broadly rounded apical ends, sometimes with a top, or they may be a little oblique. It is rare to find them nearly flat at the top, and still more rare that they are a little depressed. The wall in the apical end is thin and only a little dotted or not at all so, but now and then a single one occurs with a little thickening. Hairs are found in some of them but more than half of them have none. Generally they form lumps.

Only once have I come across a gametangium (Fig. 21 g). It was spindle-shaped, 260 μ long and 83 μ broad.

I have compared the plant from Mauritius partly with a typical specimen of *Codium coronatum* Setch. from Tahiti: Reef at Tahara Mountain, July 9, no. 5240, which Professor SETCHELL has been so kind as to send to me, and partly with material of my own collection from Ceylon and Tuticorin.

Of the Tahitian specimens SETCHELL, in his paper l. c. p. 82, has given a description and several figures. The most essential differences from the plant from Mauritius are that the slender and thick utricles occur intermingled, that the hairs are often present in great numbers, forming a broad belt in the upper half of the utricles, and that the inner wall in the apical ends of the utricles is not dotted. But concerning the last-mentioned character it must be mentioned that SETCHELL, l. c. p. 83, has described a var. *insculpta* with a foveolate inner wall.

The material from Ceylon (Galle, March 7th, 1927) is fairly similar to the Tahitian plant.

In the Indian material, Tuticorin, on the other hand, the arrangement of the narrow and broad utricles in separate groups resembled that of the plant from Mauritius. While the narrow utricles in the Tahitian plant had about the same size as those in the plant from Mauritius, the utricles in the plant from Tuticorin were proportionally somewhat broader and their apical ends were often depressed, a feature found, too, in many of the narrow utricles in the plant from Mauritius. As regards the thickness of the apical ends of the utricles, this seems to be almost the same in the specimens examined, but the plant from Mauritius differs from the other ones by its dotted inner wall.

It remains to be mentioned that the only gametangium I have succeeded in finding in the plant from Mauritius is a good deal larger, and especially longer, than the measures given by Setchell for the gametangia of the plant from Tahiti.

As will be seen from this comparison, the plant from Mauritius, though in many respects showing much likeness to *Codium coronatum* differs from it also in several respects, but as I have only had a single small specimen to examine, I prefer to consider it a variety of this species which, by the way, seems also to be a rather variable plant.

3. Codium spongiosum Harv.

Harvey, W. H., in Transact. Royal Irish Acad. 33 (1854) p. 565; Phycologia austral., tab. 55. Schmidt, Beitr. z. Kenntn. d. Gattung Codium, p. 32, figs. 14—15.

var. *Mauritiana* nov. var.

A forma *typica* praecipue differt utriculis tenuioribus et brevioribus.

Dr. Vaughan's collection contains a specimen preserved in formalin which I have referred as a variety to this species. It has a very soft mucilaginous flattened thallus with roundish foldings, the utricles being imbedded in mucilage and very loosely connected. When compared with Harvey's and Schmidt's descriptions (I have not seen any original material) the plant from Mauritius seems to agree quite well with these as to habit and consistency. On the other hand, the shapes and sizes of the utricles (Fig. 23) differ somewhat, the utricles in the plant from Mauritius being somewhat more slender than those in the typical form. The breadth given by Schmidt is 200—800 μ (rarely ± 1000 μ) whereas in the plant from Mauritius the breadth

was 200—400 μ, in rare cases more; once I have found a vesicle 628 μ broad. As to the length of the utricles it is as a rule about 2 mm but a length of 3 mm is often met with

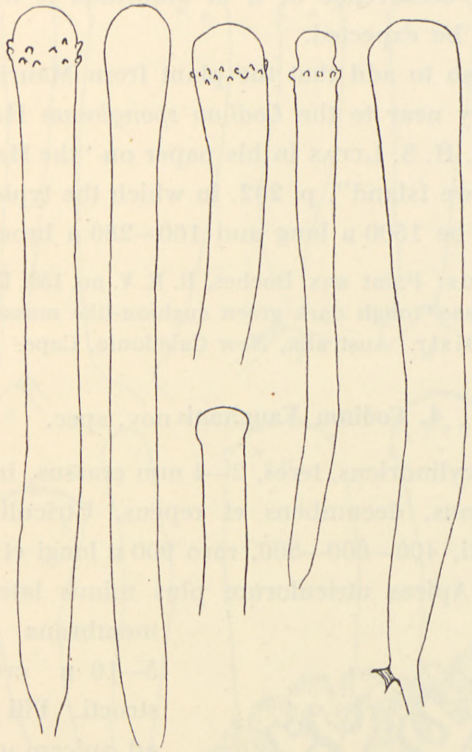

Fig. 23. *Codium spongiosum* Harv. var. *Mauritiana* nov. var. Utricles and uppermost parts of some. (× ca. 30).

and some attain almost 4 mm. But otherwise the shape of the sporangia agreed well with SCHMIDT's figures and description. The wall at the summit of the utricles was thin, about 2—3 μ thick. Curiously enough, I have not seen any hairs, but near the apices of the utricles an often broad ring of scars from hairs is frequently found, though this is not always the case; in some utricles no scars whatever are present.

Because of these differences I consider the plant from Mauritius a variety of *C. spongiosum*. As Schmidt has stated that a specimen from Cape in Herb. Hamburg is this species, the occurrence of it at Mauritius is nothing but what might be expected.

Yet I wish to add that the plant from Mauritius seems to come very near to the *Codium spongiosum* Harv., mentioned by A. H. S. Lucas in his paper on "the Marine Algae of Lord Howe Island", p. 202, in which the typical utricles are said to be 1500 μ long and 160—280 μ broad.

Mauritius: Point aux Roches, R. E. V. no. 159. Dr. Vaughan describes it as: "tough dark green cushion-like masses".

Geogr. Distr.: Australia, New Caledonia, Cape.

4. Codium Vaughani nov. spec.

Thallus cylindricus, teres, 2—4 mm crassus, irregulariter subdichotomus, decumbens et repens. Utriculi subcylindrici-obovati, 400—600—800, raro 900 μ longi et 44—100—250 μ lati. Apices utriculorum plus minus late fornicati, membrana incrassata, 5—10 μ crassa constructi. Pili numerosi ad apicem utriculorum adsunt (Fig. 24).

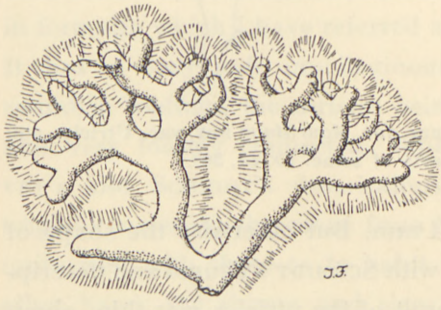

Fig. 24. *Codium Vaughani* nov. spec. Part of the thallus with hairs. (× 1½).

Mauritius: Ilôt Brocus, R. E. Vaughan no. 163, without locality or date but with the remark: "Common everywhere, deep olive green slimy dichotomous thallus".

This plant (Fig. 24) is, I am sure, closely related to *Codium repens* (Crouan) Vickers, having utricles of nearly

the same shape and size, and were it not that *C. repens* is only known from the West Indies I think I should have

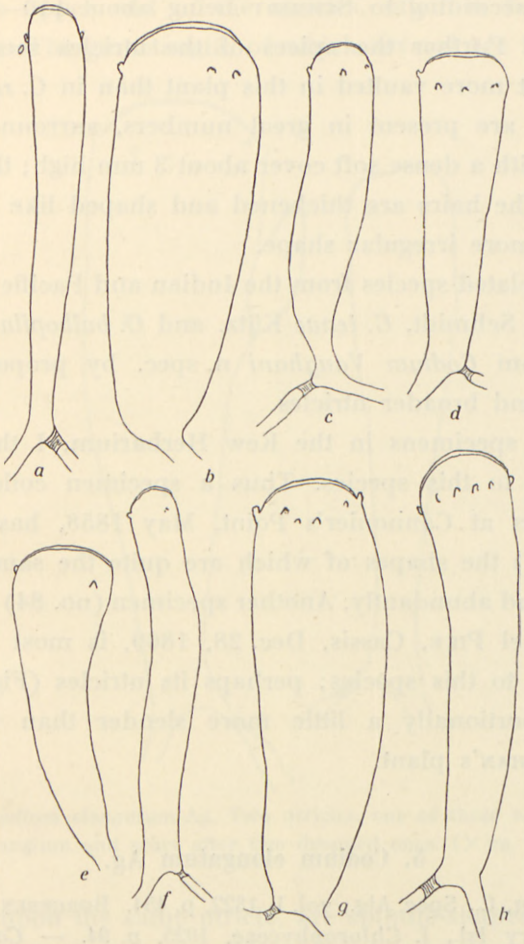

Fig. 25. *Codium Vaughani* nov. spec. a, b, c, d, utricles of R. E. V. no. 163; e, f, utricles of Pike's plant, Dec. 29, 1869; g, h, utricles of Ayre's plant, May 1858. (× ca. 80).

referred the plant from Mauritius to it as a variety. But as differences due to size and shape of the utricles (Fig. 25) were found as well, I prefer to give it specific rank. The

length of the utricles is almost the same in both plants but they are not so broad in the plant from Mauritius, the breadth according to Schmidt being about 110—450 μ in *C. repens*. Further the apices of the utricles seem to be somewhat more vaulted in this plant than in *C. repens*.

Hairs are present in great numbers, surrounding the thallus with a dense soft cover about 3 mm high; the upper ends of the hairs are thickened and shaped like sausages or of a more irregular shape.

The related species from the Indian and Pacific Oceans: *C. Geppei* Schmidt, *C. tenue* Kütz. and *C. bulbopilum* Setch. differ from *Codium Vaughani* n. spec. by proportionally shorter and broader utricles.

Some specimens in the Kew Herbarium, I think, are referable to this species. Thus a specimen collected by Dr. Ayres at Cannonier's Point, May 1858, has utricles (Fig. 25 h) the shapes of which are quite the same. Hairs were found abundantly. Another specimen (no. 84) gathered by Colonel Pike, Cassis, Dec. 28, 1869, is most certainly referable to this species; perhaps its utricles (Fig. 25 e, f) are proportionally a little more slender than those in Dr. Vaughan's plant.

5. Codium elongatum Ag.

Agardh, C., Spec. Alg., vol. I, 1822, p. 454. Børgesen, F., Mar. Alg. Canary Isl., I. Chlorophyceae, 1925, p. 94. — *Codium decorticatum* (Woodw.) Howe, Phycological Studies, V, 1911, p. 494. O. C. Schmidt, Beiträge, p. 52? *Ulva decorticata* Woodw., Observations upon the generic Character of Ulva, p. 55.

Dr. Vaughan's collection contains a single fruiting specimen of this species. The utricles (Fig. 26) have a breadth of about 300 μ, in rare cases up to about 500 μ, and a

length of about 1100 μ. The wall of the apical ends of the utricles is thin, and hairs or scars from these are found near the upper end of the utricles, often in fairly great numbers. The gametangia, of which several are frequently

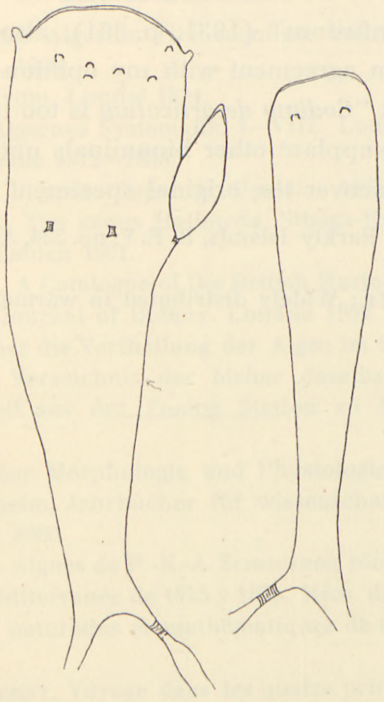

Fig. 26. *Codium elongatum* Ag. Two utricles, one of these with a gametangium and scars after two dropped ones. (× ca. 70).

present upon the same utricle, are spindle-shaped or ovate and about 100—140 μ broad and up to about 400 μ long.

This species, for which formerly AGARDH's name was used, has more recently been termed *Codium decorticatum* (Woodw.) Howe. This author, in the paper quoted above, pointed out that *Ulva decorticata* Woodw. was this species, and that WOODWARD's name as the oldest one ought to be

used. Against this I (1925, p. 95) have raised objections as, in my opinion, the description of Woodward cannot be said to correspond so very well to *Codium elongatum* Ag., and any reference to Woodward's plant is excluded as the material seems to be lost. Setchell, in his paper: "Some early algal Confusions" (1931, p. 361), also discusses the question and in agreement with my opinion arrives at the conclusion that "*Codium decorticatum* is too uncertain in its application to supplant other binominals until some happy chance may uncover the original specimen".

Mauritius: Barkly Islands, R. E. V. no. 334, August 1939, "cast up by waves".

Geogr. Distr.: Widely distributed in warmer seas.

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together with some more essential synonyms, the last mentioned printed in italics.

Indleveret til Selskabet den 19. Februar 1940.
Færdig fra Trykkeriet den 27. Maj 1940.

Chamaedoris Delphinii (Hariot) Feldm. et Børgs. (× 1).

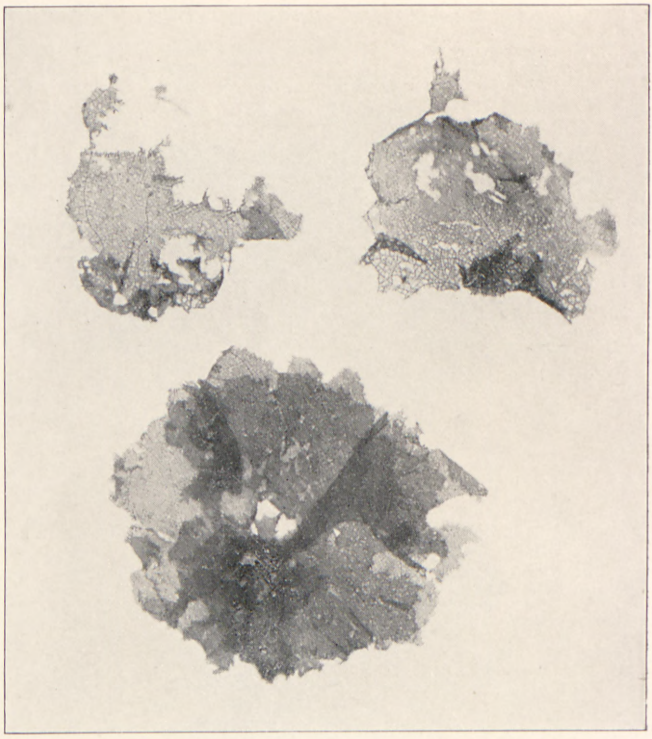
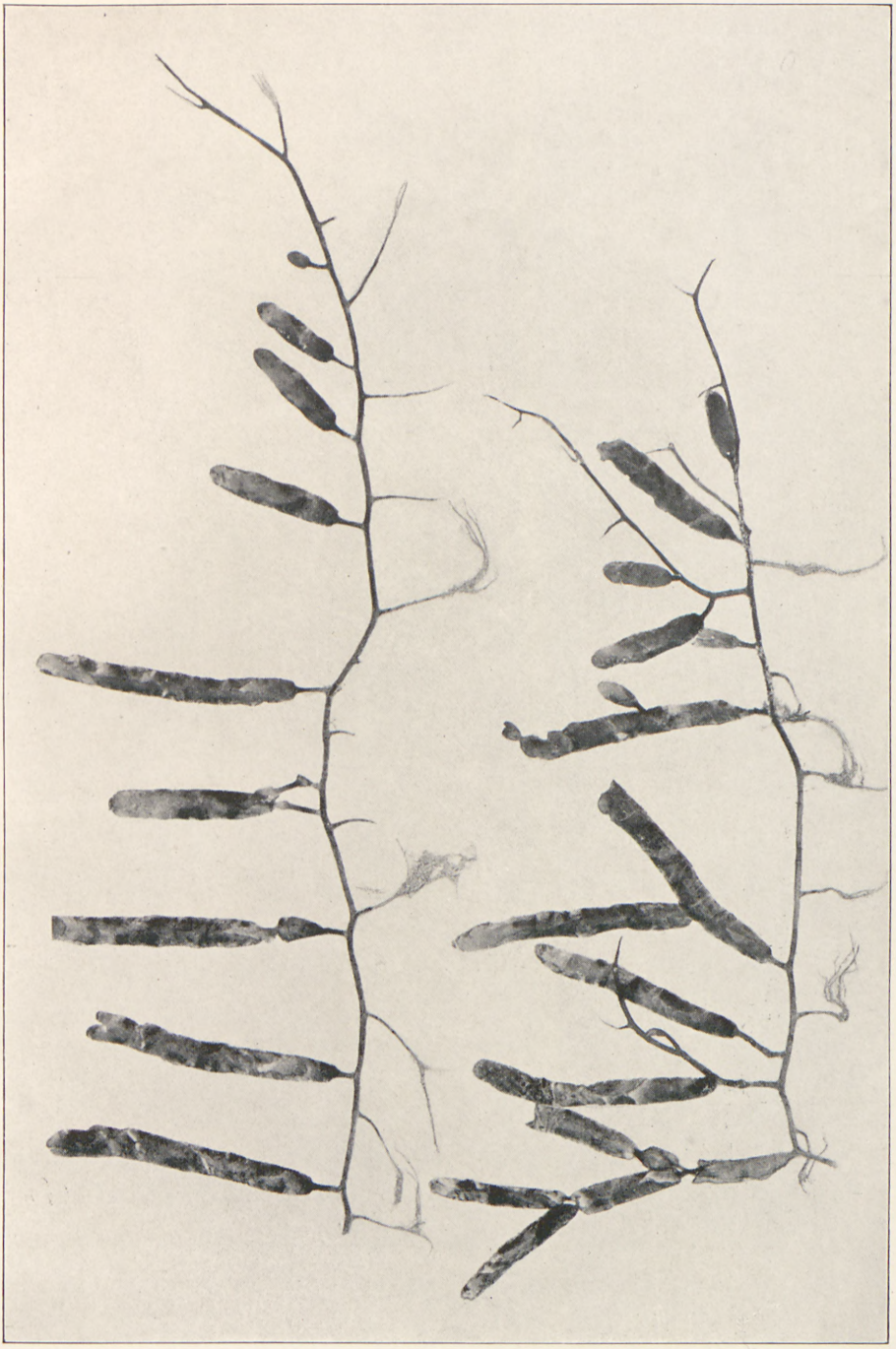


Fig. 1. *Microdictyon Agardhianum* Decsne. (× 1).

Fig. 2. *Avrainvillea gracillima* Børgs. (× 1).



Caulerpa Mauritiana Børgs. (ca. 1/2 size).

Det Kgl. Danske Videnskabernes Selskab.
Biologiske Meddelelser. **XV**, 5.

TURNOVER OF LECITHIN, CEPHALIN, AND SPHINGOMYELIN

BY

G. HEVESY AND L. HAHN

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Phosphatide molecules present in the body have been taken up with the food or have been built up in the organism. A spectacular proof of the synthesis of phosphatides in the body is given by the fact that ducks raised on diets containing phosphorus only in inorganic form laid 85—195 eggs during the summer[1]. These eggs contained 200—400 gm. phosphatides (corresponding to 8—16 gm. phosphatide P), and this very appreciable amount was synthesised by the organs of the ducks. On the other hand, phosphatides can enter the circulation from the intestine. The amount of phosphatide which is daily led by the intestinal lymph into the circulation of the rabbit[2] on normal diet was calculated to be about 50 mgm. This is only about 1/5 of the amount daily synthesised in the liver (comp. p. 24); one must further consider that at least an appreciable part of the above mentioned 50 mgm. was synthesised in the mucosa of the small intestine. Thus, the phosphatide molecules of the organs will be only to a small extent obtained directly from the food, the overwhelming majority being built up in the body.

Concept of turnover.

The ultimate aim of the investigation of the origin of the phosphatide molecules present in the body is to be able

[1] G. Fingerling, Biochem. Z. **38**, 448 (1911).

[2] H. Süllmann and W. Wilbrandt, Biochem. Z. **270**, 52 (1934).

to state in which form the hydrogen, carbon, nitrogen, oxygen, and phosphorus atoms present in the phosphatide molecules were taken up by the body and in what steps they were involved until ultimately incorporated into phosphatide molecules. This exacting task can hardly be solved at present, and we must content ourselves with the determination of the place and rate of formation of the phosphatide molecules in the body from glycerol, fatty acid, choline (or another organic base), and phosphate. We will denote, in what follows, as turnover rate the rate of synthesis of phosphatide molecules from inorganic phosphate and other components independent of the actual mechanisms involved, and we shall measure this rate by determining the extent to which labelled phosphate present in the cells of an organ is incorporated into these newly formed phosphatide molecules. As the phosphatide content of an organ is usually constant, we can follow that with the formation of new phosphatide molecules the decomposition of an equal or similar number of old molecules goes hand in hand. The possibility must also be envisaged that new formation and decomposition of phosphatides do not take place in the same organ, but that the newly formed molecules are synthesised in one organ and carried into the other by the circulation. This point will be discussed on p. 25.

The turnover rate can also be measured by following the rate of incorporation of fatty acids or of choline, for example, into the phosphatide molecule. The turnover rates measured by using different indicators need not necessarily be identical. It would be conceivable, for example, that the incorporation of the phosphate radical into the phosphatide molecules would be preceded by the formation of glycero-

phosphate and that this process would be a comparatively slow one in contrast to all other steps involved in the synthesis of the phosphatide molecule. In this case, the turnover rate measured, using labelled P as an indicator, would be slower than that found when using labelled fatty acids or labelled choline. The opposite would be the case if the reorganisation of the phosphate bond were to take place at a faster rate than the corresponding release and incorporation of fatty acids or choline into the phosphatide molecules.

The question if and to what extent the rate of phosphate incorporation into the phosphatide molecule differs, for example, from that of the fatty acid incorporation into the latter cannot be answered at the time being.

Feeding cats with mixed glyceride, the acids of which were composed to 85 per cent of elaidic acid, SINCLAIR[1] found 12 hours later the plasma phosphatide fatty acids to contain 19 per cent of elaidic acid. In our experiments we found (comp. p. 30) that, after the lapse of 16 hours, about 4 per cent of the phosphatides extracted from the plasma of rabbits contained labelled phosphate.

Indicators applied in turnover measurements.

a) Change of the degree of unsaturation of fatty acids.

Since the phosphatides contain both saturated and unsaturated fatty acids, the change of the composition of the fatty acids of the organ phosphatides after ingestion of cod liver oil, for example, can be utilised to get information

[1] R. G. SINCLAIR, J. Biol. Chem. **115**, 215 (1937).

on the rate of the phosphatide turnover in the organ in question. A change in the iodine number of the phospholipids extracted from the liver of dogs[1] and cats[2] after the ingestion of cod liver oil and the disappearance of the changes within 24 hours and 2 to 3 days, respectively, was observed at an early date.

b) Incorporation of iodized fatty acids into the phosphatide molecule.

Iodized fatty acids, whether injected intravenously or given by mouth, enter the phosphatides of the liver, the blood[3], and the milk[4], for example.

c) Incorporation of elaidic acid into the phosphatide molecule.

This method was repeatedly used in the investigation of the turnover of phosphatides. The rate of entrance of elaidic acid into and disappearance from the phosphatides was found to be rapid in the liver and the intestinal mucosa and comparatively slow in the muscle. The process was found to be essentially complete in the liver within a day, but in the muscle only after the period of many days[5].

[1] G. Ioannowics and E. P. Pick, Wien. Klin. Wochenschr. **23**, 573 (1910).

[2] R. G. Sinclair, J. Biol. Chem. **82**, 117 (1929). Comp. also R. G. Sinclair, Phys. Rev. **14**, 351 (1934).

[3] C. A. Artom, Arch. inter. Physiol. **36**, 191 (1933); C. A. Artom and G. Peretti, Arch. inter. Physiol. **36**, 351 (1933).

[4] F. X. Aylward, J. H. Blackwood and J. A. B. Smith, Biochem. J. **31**, 130 (1937).

[5] R. Sinclair, J. Biol. Chem. **111**, 270 (1935), and **121**, 161 (1937). M. F. Kohl, J. Biol. Chem. **126**, 709 (1938).

d) Incorporation of fatty acids, labelled by introduction of heavy hydrogen, into the phosphatide molecule.

Linseed oil was deuterated and the "heavy" fat obtained fed to rats. The investigation of the deuterium content of the phosphatides extracted from different organs gives information on the phosphatide turnover in the organ in question[1].

e) Incorporation of analogues of choline, in which arsenic replaces nitrogen, into the phosphatide molecule.

Arsenic can be detected in the lecithin fraction isolated from the liver and the brain of rats kept for 21 days on a diet containing arsenocholine chloride[2].

f) Incorporation of labelled phosphate into the phosphatide molecule.

This method will be discussed in detail.

Most of the methods outlined above were successfully applied to show that a marked turnover takes place in some of the organs, and the application of the methods a), c), and f) lead to the result that the rate of the phosphatide turnover is much faster in the intestinal mucosa and in the liver than in the other organs. None but the "phosphate method" was applied, however, to arrive at quantitative data as to the rate of rejuvenation of the phosphatide molecules present in the different organs.

[1] B. Cavanagh and H. S. Raper, Biochem. J. **33**, 17 (1939).
[2] A. Welch, Proc. Soc. Expertl. Biol. and Med. **35**, 107 (1937).

Quantitative determination of the turnover rate by using labelled phosphate.

The formation of phosphatide molecules containing ^{32}P inside the tissue cell can only take place when the process of phosphatide formation was preceded by a penetration of ^{32}P into the cell, and the same applies to all indicators used in turnover experiments. This point was hitherto not considered. Its great importance is best seen by the following.

Let us assume that labelled phosphate or elaidic acid cannot penetrate into the cells of an organ. In this case, no turnover could be ascertained, even if a very intense one were actually taking place. To arrive at a proper figure for the turnover rate we have to compare the percentage of ^{32}P in the total inorganic P of the cells with the percentage of ^{32}P in the total phosphatide P extracted from them. If these ratios, which correspond to those of the specific activities of the inorganic P and the phosphatide P, are found to be equal, we can conclude that all phosphatide molecules were renewed during the experiment. In this case, a proportional partition of ^{32}P between the inorganic P and the phosphatide P present in the cells took place. This is only possible if the phosphate radical of all the phosphatide molecules was split off in the course of the experiment, a process which was then followed by a synthesis of phosphatide molecules with incorporation of other phosphate radicals in which $^{32}PO_4$ was represented proportionally to its total number present. If the specific activity of the phosphatide P is found to be, for example, 10 per cent of that of the inorganic P, we can conclude that 10 per cent of the phosphatides were renewed during the experiment.

Due regard must, however, be given to the change of the specific activity of the cellular inorganic P in the course of the experiment. By administering the labelled phosphate in several portions of suitably varying quantities in the course of the experiment, we can maintain a constant specific activity of plasma and interspace phosphate. As to the cellular concentration of ^{32}P, which is nought at the start of the experiment and then gradually increases, we determine the change of concentration with time experimentally and compare the specific activity of the phosphatide P extracted at the end of the experiment with the average value of the specific activity of the inorganic P which prevailed during the experiment.

When determining the specific activity of the cellular inorganic P, due regard must be taken to the fact that a part of the tissue inorganic ^{32}P is of extracellular origin. As the extracellular volume of the tissue is known and the specific activity of the extracellular P does not differ much from that of the plasma P, we can easily correct for the presence of the extracellular P in the tissue inorganic P. Since the extracellular phosphate in the case of the muscle tissue, for example, amounts to only about 1/90 of the cellular inorganic P, the correction mentioned above becomes only significant in experiments of short duration. If the rate of penetration of the inorganic phosphate differs greatly in the cells of different tissues, as it actually does, for example, in the case of the liver and the muscle, we do not get proper information on the relative rate of turnover of the phosphatides in these organs by comparing the specific activity of the liver phosphatide P with that of the muscle phosphatide P. Conclusions based on such a comparison will greatly underestimate the relative rate of phosphatide

turnover going on in the muscle cells into which the inorganic P diffuses at a slow rate, in contrast to its penetration into the liver cells. We will arrive, however, at correct figures by comparing the ratio

$$\frac{\text{specific activity muscle phosphatide P}}{\text{specific activity muscle inorganic P}}$$

with the corresponding ratio of liver products.

If we wish to draw quantitative conclusions from experiments carried out with elaidic acid as an indicator, we have to compare the elaidic acid content of the organ phosphatides with that of the elaidic acid content of the fatty acid mixture present in the corresponding cells in freely disponible state. The latter magnitude is not known and the same consideration applies to the work with deuterated fat as an indicator. We may get some, though very restricted, information by comparing the heavy hydrogen (D) content of the organ phosphatides with that of the organ glycerides. After the lapse of 10 hours, the ratio

$$\frac{\text{liver phosphatide D}}{\text{liver glyceride D}} : \frac{\text{kidney phosphatide D}}{\text{kidney glyceride D}},$$

where D denotes the relative heavy content of the total "non-exchangeable" hydrogen, was found to be 1:2.

Experimental procedure.

The labelled phosphate of negligible weight, dissolved in physiological sodium chloride solution, was injected into the vena jugularis of the rabbit drop by drop during the experiment. Per hour 2,5 cc. were injected, the experi-

ment took usually 4 hours. By taking small samples from the ear vein at different intervals, the change in the activity of the plasma was followed. In several cases, we extracted the inorganic P of the plasma and measured its specific activity (activity per mgm. P), in others we con-

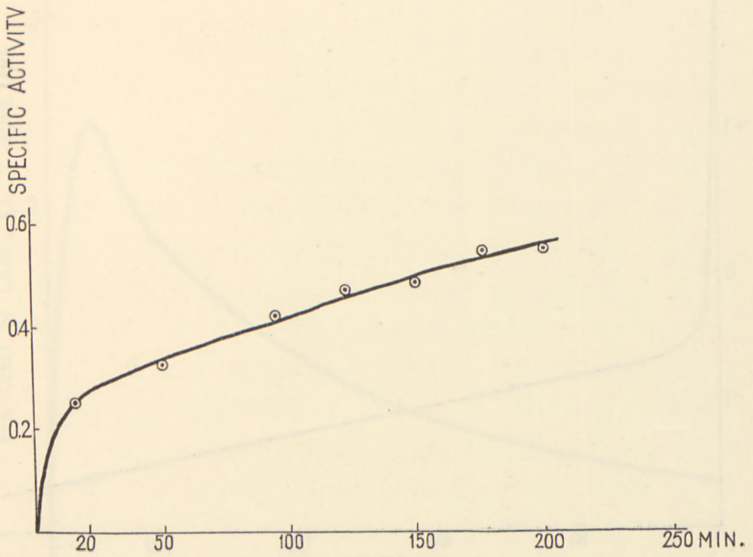

Fig. 1. Change of the specific activity of the plasma inorganic P during continuous intravenous injection of labelled phosphate to a rabbit. (Specific activity = per cent of the labelled P injected, found in 1 mgm. P).

tented ourselves with the measurement of the total activity of the plasma which, in experiments of short duration, is solely due to the inorganic phosphate present.

The labelled P was injected drop by drop into the vena jugularis in order to obtain a comparatively small and easily accountable change in the activity level of the plasma (see Fig. 1). If all the labelled P is injected at the start of the experiment, as in our early experiments and in all experiments carried out by other workers with labelled P,

the activity level of the plasma is very high at the beginning, and it is slow at the end of the experiment (see Fig. 2). If the labelled P is given by subcutaneous injection or by mouth, the activity of the plasma first increases with

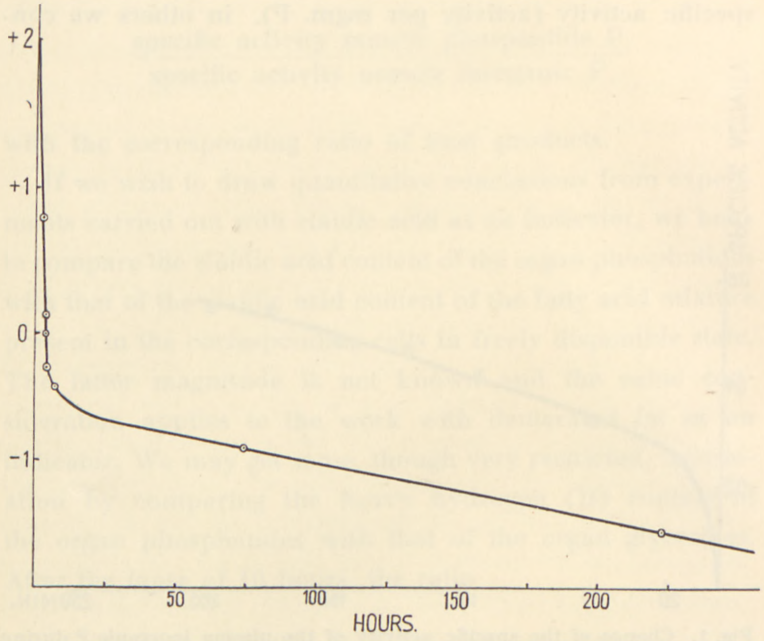

Fig. 2. Change of the logarithm of the labelled P content of the plasma with time after intravenous injection of labelled phosphate.

time and later decreases (see Fig. 3). The sensitiveness of the radioactive indicator, thus, changes very appreciably in the course of the experiment. If we are successful in keeping the activity level of the plasma constant during the experiment, we can eliminate great difficulties otherwise encountered when calculating the turnover rate of organic phosphorus compounds.

The changes in the activity of the plasma, shown in Fig. 1, can be further reduced by injecting amounts de-

creasing with time. In our later experiments we have chosen this procedure and varying amounts of labelled P were administered by subcutaneous injection. In an experiment

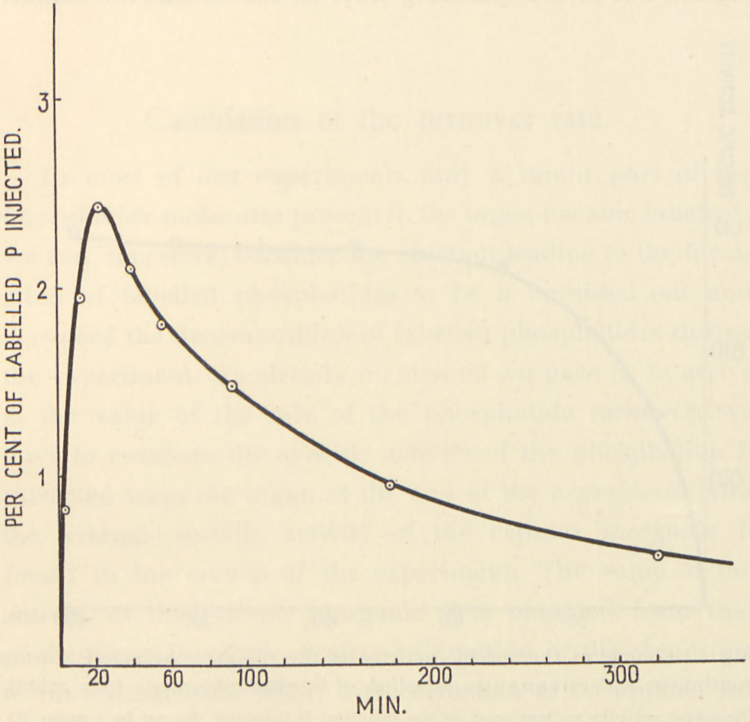

Fig. 3. Change of the specific activity of the plasma inorganic P after subcutaneous injection of labelled phosphate to a rabbit.

taking 12 hours, for example, labelled P was injected every 20 min. In experiments taking several weeks, in the later phases of the experiment injections were made twice a day. The change in the plasma activity in such an experiment taking 4 hours is seen in Fig. 4. In experiments taking several hours or days a constant activity level could be easily obtained.

The determination of the turnover rate of the phospha-

tides present in the different organs necessitates the determination of the specific activity of the inorganic P and phosphatide P extracted from the organ. This determination was carried out in the following way. At the end of the experi-

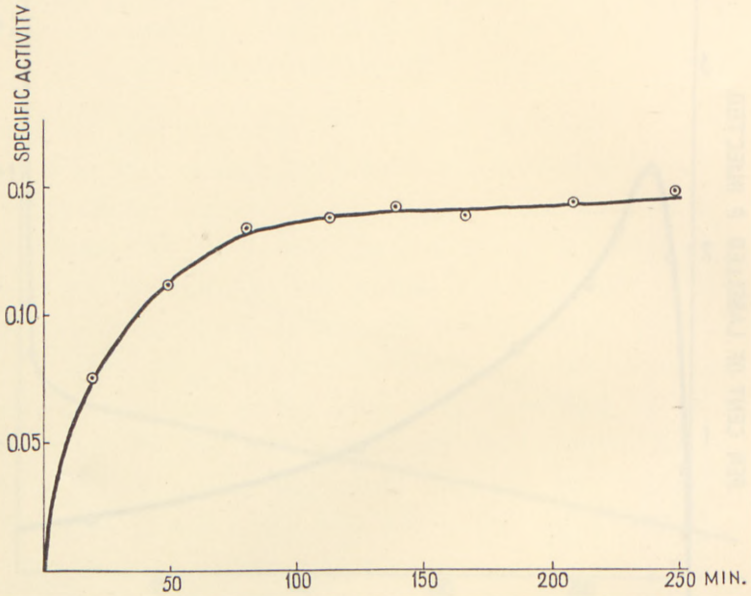

Fig. 4. Change of the specific activity of the plasma inorganic P during continuous subcutaneous injection of labelled phosphate to a rabbit. (Specific activity = per cent of the labelled P injected, found in 1 mgm. P).

ment the animal was killed by bleading. The organs were at once placed in liquid air, minced, and extracted with cold 10 per cent trichloroacetic acid. The inorganic phosphate present was precipitated as ammonium magnesium phosphate at 0°. Muscle samples were taken before death. To secure the phosphatide present in the organs, these were first dried with cold acetone and then treated with ether, later with boiling alcohol. The ether-alcohol extracts were evaporated in vacuo and taken up several times with

petrol-ether; the phosphatides were then converted into phosphate by wet ashing. The procedure applied when isolating lecithin, cephalin, and sphingomyelin will be discussed on page 36.

Calculation of the turnover rate.

In most of our experiments only a minor part of the phosphatide molecules present in the organ became labelled; we can, therefore, consider the reaction leading to the formation of labelled phosphatides to be a onesided one and disregard the decomposition of labelled phosphatides during the experiment. As already mentioned on page 8, to arrive at the value of the rate of the phosphatide turnover, we have to compare the specific activity of the phosphatide P extracted from the organ at the end of the experiment with the average specific activity of the cellular inorganic P found in the course of the experiment. The value of the activity of the cellular inorganic P is obtained from that of the tissue inorganic P after subtraction of the share due to the extracellular fluid. The correction to be applied for the presence of extracellular P in the tissue inorganic P is, in most cases, a small one. In the liver of the rabbit, for example, out of 30 mgm. inorganic P only about 0.6 mgm. is located in the interspaces. We arrive at this figure by assuming that the interspaces make out[1] 22 per cent of the weight of the liver and the inorganic P content of the interspaces is 3 mgm. per cent. The specific activity of the liver extracellular P is, after 4 hours, 2.5 times higher than the specific activity of the tissue inorganic P; correspondingly,

[1] J. F. Manery and B. Hastings, J. Biol. Chem. **127**, 657 (1939).

5 per cent of the total inorganic P activity of the liver is due to extracellular P.

In the case of the muscle, we arrive by an analogous consideration at the result that 25 per cent of the activity of the tissue inorganic P is of extracellular origin. The extent of the correction to be applied increases with decreasing length of the experiment, since in experiments of short duration only a small amount of labelled P penetrates into the cells.

With regard to the considerations stated above, one must recognise the possibility that some of the phosphorus which one identifies, even after the most careful experimental procedure, as inorganic P, was in fact present in the tissue in the form of very labile, not yet known, organic phosphorus compounds. Very labile P compounds of that kind, if present, would probably be in fast exchange equilibrium with the inorganic P present, and their presence would therefore not much influence the calculation given above. The labile P of adenyltriphosphoric acid comes, for example, very quickly into exchange equilibrium with the inorganic P of the tissues or the corpuscles; it is often permissible to replace the specific activity of the inorganic P by that of the above mentioned labile P. The behaviour of creatinephosphoric acid is discussed on page 28.

When calculating the turnover rate of phosphatides, we must consider the average specific activity of the cellular inorganic P prevailing during the experiment. This value is obtained by determining the specific activity of the tissue inorganic P and the plasma inorganic P at different intervals. The change of the specific activity of the tissue inorganic P is seen in Table 1, that of the plasma inorganic P is discussed on page 11.

Table 1.

Specific activity of the organ inorganic P as percentage of that of the plasma inorganic P.

Organ	100 min.	240 min.
Liver	12.7	42.1
Muscles	0.8[1]	4.6
Intestinal mucosa	14.7	42.8
Brain[2]	0.32	1.4
Kidneys	85	90

[1] In spite of all precaution taken, some creatine P may have been split off before the extraction of the inorganic P. The creatine P being, in experiments of short duration, less active than the inorganic P, such a decomposition may partly be responsible for the low value obtained in the experiment taking 100 min. only.

[2] Comp. p. 45.

It is of interest to remark that, in the case of the kidneys, after the lapse of 100 min. an almost proportional partition of ^{32}P between plasma and cellular P is reached. When investigating, after 4 hours, the inorganic P of the marrow of the kidney, which makes out only a minor part of the total inorganic P of the kidney, the specific activity was found to be only 48 per cent of that of the plasma.

Cellular and non-cellular formation of phosphatides.

The turnover rates recorded in the fourth column of Tables 3 to 9 are calculated on the assumption that the formation of phosphatide molecules takes place inside the cells with participation of cellular inorganic P. Let us assume for a moment that the formation of phosphatide molecules takes place on the cell wall facing the inter-

spaces. Then, not the cellular but the extracellular phosphate radicals[1] would enter the newly formed phosphatide molecules. As the specific activity of the extracellular inorganic P is often much higher than that of the cellular inorganic P, in the last mentioned case more active P atoms would take part in the synthetic process than in the first mentioned one. A high activity of the newly formed phosphatide would then not indicate such a high turnover as it would if the formation of the phosphatide molecules took place with participation of the less active cellular P. It is obvious that the sensitivity of our radioactive indicator will be very different in the two cases mentioned above. Though it is much more probable that the turnover of the phosphatide molecules takes place inside the cells we have also recorded, in the fifth column of the above mentioned tables, the turnover rates calculated on the assumption of an extracellular formation of the phosphatide molecules. The values thus obtained give the lower limit of the turnover rate, while those obtained in column 4 give the upper limit. It is conceivable that some of the phosphatide molecules are renewed inside the cell wall. In that case the inorganic P entering the newly formed phosphatide molecules will have a specific activity being intermediary between that of the extracellular and the cellular P. A continuous drop of the specific activity of the inorganic P in the cell wall may namely take place while the phosphate penetrates from the interspaces into the cells.

In the corpuscles the phosphatides are known to be

[1] From this view-point, it is without any significance whether the phosphate radical is directly incorporated into the phosphatide molecule or through intermediary stages.

practically concentrated in the stroma[1], and the thickness[2] of the latter to correspond to that of very few molecular layers. It is, therefore, quite conceivable that in the outer layer of the stroma a slow rejuvenation of the phosphatide molecules takes place with incorporation of plasma P. Should we find an organic P fraction extracted from the cells or the corpuscles to show a higher specific activity than the cellular, respectively corpuscular inorganic P, in this case we would be justified to conclude that the synthesis of the organic compound in question did not take place inside the cells, respectively the corpuscles. Investigations in the above mentioned direction may bring forward results of histochemical interest.

[1] B. N. Erickson, H. H. Williams, S. S. Bernstein, J. Arvin, R. L. Jones and J. G. Macy, J. Biol. Chem. **122**, 515 (1938).

[2] Danielli, J. Cell. Comp. Physiol. **7**, 393 (1936).

Results of experiments.

PART I

Investigation of the total petrol-ether soluble phosphatide mixture.

Experiments with rabbits.

Table 2.

Specific activity of the inorganic P and phosphatide P extracted from the organs.

Rabbit I. — Weight: 2.4 kg.

Intravenous injection during 4 hours.

Fraction	Specific activity in relative units
Plasma inorganic P	100
Liver tissue inorganic P at the end of the experiment	36.2
Liver tissue inorganic P corrected for the change in plasma activity during the experiment	44
Liver cellular inorganic P at the end of the experiment corrected as above	40.8
Liver cellular inorganic P average value during the experiment	20.4
Liver phosphatide P	3.0
Kidney tissue inorganic P at the end of the experiment	67.7
Kidney tissue inorganic P corrected for the change in plasma activity during the experiment	82.3
Kidney cellular inorganic P at the end of the experiment corrected as above	82.0
Kidney cellular inorganic P average value during the experiment	73.5
Kidney phosphatide P	5.5

Table 3.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit II. — Weight: 2.6 kg.

Intravenous injection during 215 min.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	19.0	19.0	3.86
Kidney	382	18.3	4.8	3.7
Small intestine	111	7.9	7.1	1.61
Stomach	58	4.46	7.7	0.91
Heart	57.2	1.53	2.7	0.31
Lungs	66.3	4.04	6.1	0.82
Spleen	70.2	3.65	5.2	0.74
Marrow[3]	40.8	1.63	4.0	0.33
Brain	..	0.06	..	..

[1] Calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

[3] In several experiments the specific activity of the marrow inorganic P was found to be surprisingly low, even lower than that of the ester P. These low values were presumably due to the presence of traces of only slightly active bone P in the marrow sample.

Table 4.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit III. — Weight: 2.3 kg.

Intravenous injection during 234 min.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	16.3	16.3	3.2
Muscles	7.8	0.56	7.2	0.11

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 5.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit IV. — Weight: 2.5 kg.

Intravenous injection during 215 min.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	14.8	14.8	2.9
Kidney	374	23.2	6.2	4.6
Small intestine (mucosa)	107	20.0	18.7	3.9
Heart	64.6	3.47	5.37	0.68
Lungs	76.1	7.67	10.1	1.51
Brain	..	0.175	..	..

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 6.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit V. — Weight: 2.1 kg.

Intravenous injection during 250 min.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	18.6	18.6	2.76
Kidney	364	22.8	6.3	3.58
Small intestine (mucosa)	115	23.6	20.5	3.54
Muscle	12.0	0.87	7.3	0.11

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 7.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit VI. — Weight: 2.6 kg.

Subcutaneous injection during 255 min.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	14.8	14.8	3.2
Corpuscles	29.0	1.51	5.2	0.33

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 8.

Specific activity of the cellular inorganic P and phosphatide P extracted from the organs.

Rabbit VII. — Weight: 2.4 kg.

Subcutaneous injection during 11.5 hours.

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	25.2	25.2	14.9
Corpuscles	25.5	4.03	15.8	2.39
Muscles	14.7	1.31	8.9	0.78
Brain	—	0.55	—	—
Marrow	36.5[3]	31.8	87.0	18.8

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

[3] As the presence of traces only of bone P in the marrow sample investigated lowers the specific activity of the marrow inorganic P, the recorded figure for the inorganic P of the marrow may be too low and that recorded for the rate of renewal of the phosphatide P of the marrow, correspondingly, too high.

Table 9.

Extent of renewal of phosphatides.

Rabbit IX. — Weight: 2.5 kg.

Subcutaneous injection during 50 days.

Organ	Percentage of phosphatides not renewed	
	A[1]	B[2]
Liver	0	0
Muscle	73	64
Marrow	0	0
Corpuscles	3	3

[1] Rate of renewal calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Rate of renewal calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Critical remarks.

In Tables 2—9, data were given for the turnover rate of phosphatides in different organs of the rabbit. When calculating those values we assumed that the labelled phosphatides present in the organs were synthesised in situ. In what follows, we will discuss how far this assumption is justified.

Liver phosphatides.

Let us first consider the liver phosphatides. Apart from the liver, a very intense turnover is going on in the intestinal mucosa, and the possibility must be envisaged that the labelled phosphatides were carried into the liver from the intestine by the plasma. The plasma was found to contain only small amounts of labelled phosphatides, the specific activity of the plasma phosphatide P being, after the lapse of 4 hours, only 1/7 of that of the liver phosphatide P. This fact excludes the possibility that a substantial part of the labelled liver phosphatides was led from the intestine or any other organ into the liver. Large amounts of water can be led from one pond into the other by a narrow channel; salt water, however, (salt corresponding to labelled phosphatides in our case) cannot pass the channel without the water of the channel becoming salt as well. The concept of "specific activity" proves, thus, to be of great use when putting forward considerations such as those discussed above.

One may say, in respect of these considerations, that, while the specific activity of the average plasma phosphatides is low, one of the phosphatide fractions (phosphatides represent a mixture of numerous compounds)

might be synthesised at a very fast rate in the intestinal mucosa, and the labelled molecules formed in this process might have rushed through the plasma at a fast rate into the liver without much raising the specific activity of the average plasma phosphatide P. As shown on page 51, the specific activity of the phosphorus present in different phosphatide fractions can differ substantially, but, in spite of exhaustive fractionation processes no fraction of extremely high or extremely low specific activity was found. Furthermore, the total amount of labelled phosphatides formed in the intestinal mucosa in the course of 4 hours amounts to only 1/5 of that formed in the liver during the same time.

In this connection it is of interest to remark that, according to the results obtained by Süllmann and Wilbrandt which are discussed on page 3, the intestinal lymph carries up to 0.1 mgm. phosphatide P[1] per hour; but, even if this amount of newly formed phosphatides is quantitatively led from the intestine into the liver, it would not suffice to account for the presence of the amount of newly formed phosphatides found in the latter which corresponds to more than 0.5 mgm. phosphatide P per hour.

An entirely different argument against the intestinal origin of the labelled phosphatides found in the liver is the following. The labelled phosphatides present in the plasma

[1] When oil is fed to the rabbit twice that amount was found to be carried by the intestinal lymph. The feeding of oil raises the rate of turnover in the intestinal mucosa and the liver as well, as shown in experiments on rats (C. Artom, G. Sarzana and E. Segrè, Arch. Intern. Physiol. **47**, 245, 1938; B. A. Fries, S. Ruben, J. Perlman and J. L. Chaikoff, J. Biol. Chem. **123**, 587, 1938) and also on isolated perfused cat liver, where the turnover rate was found to be about twice as high as in experiments in which non-lipemic (normal) blood was used (L. Hahn and G. Hevesy, Biochem. J. **32**, 342, 1938).

were not found to leave the blood stream at a very fast rate, half of the labelled phosphatides present leaving the plasma in the course of an hour, 30 per cent of these phosphatides being found in the liver[1]; thus, a rapid rush of labelled phosphatides through the plasma does not take place.

That the labelled phosphatides found in the liver are, at least to a large extent, formed in situ, was also shown in experiments on isolated perfused liver. Such investigations were formerly[2] carried out by us on isolated cat livers in which, after the lapse of 2.5 hours, the specific activity of the liver phosphatide P was found to be about 1.5 per cent of that of the liver inorganic P. A further proof that the phosphatides present in the liver were formed there was brought about by CHAIKOFF and his colleagues[3] who found that, in experiments on rats, the removal of tissues very active in phospholipid turnover, namely the gastrointestinal tract and the kidneys, does not markedly influence the phospholipid turnover in the liver.

Muscle phosphatides.

After discussing the origin of the labelled liver phosphatides we shall put forward similar arguments as to the origin of the labelled muscle phosphatides. The specific activity of the plasma phosphatides is found to be about 3 times higher after the lapse of four hours than that of the muscle phosphatides. Considerations based on the comparison of the specific activity of the plasma phosphatides and the muscle phosphatides do not, therefore, exclude

[1] L. HAHN and G. HEVESY, Nature **164**, 72 (1939).

[2] L. HAHN and G. HEVESY, Biochem. J. **32**, 342 (1938).

[3] B. A. FRIES, S. RUBEN, J. PERLMAN and J. L. CHAIKOFF, J. Biol. Chem. **123**, 567 (1938).

the possibility that the labelled phosphatides present in the muscles were carried into them from other organs. This possibility is, however, excluded by the result of experiments based on the rate of entrance of labelled phosphatides into the muscles[1]. While, in the course of 4 hours, phosphatides showing a relative activity of 0.54 units pass from the plasma into the muscles, phosphatides having an activity of 160 units were found to be present in the muscles after the lapse of the same time.

In experiments of short duration the creatine P of the muscles gets only partly labelled and, therefore, a decomposition of creatinephosphoric acid prior to the extraction of the inorganic P will lead to a "dilution" of the activity of the inorganic P present as such in the muscle tissue. We entertain some doubts as to the possibility of preventing the decomposition of some of the creatinephosphoric acid present even if great precautions are taken. The possibility that in our experiments taking only a few hours too low values are obtained for the specific activity of the muscle inorganic P cannot, therefore, be entirely discarded. As the extent of the newformation of the muscle phosphatides is calculated by comparing the specific activity of the phosphatide P with that of the inorganic P, a too low value of the specific activity of the inorganic P will manifestly lead to a too high value of the rate of newformation of the phosphatides.

Kidney phosphatides.

Kidney phosphatide P is found in experiments of short duration to be more active than the phosphatide P ex-

[1] L. Hahn and G. Hevesy, Nature **144**, 204 (1939); D. Kgl. Danske Vidensk. Selskab, Biol. Medd. XV, 6 (1940).

tracted from all other organs. From this fact we may, however, not follow that the kidney phosphatides are renewed at a faster rate than the phosphatides in the liver or the intestinal mucosa. The labelled inorganic P of the plasma diffuses with a remarkable speed into the kidney cells (see Table 1). This is in no way surprising in view of the role of the kidney cells as to excretion and re-absorption of phosphate. A result of this fast penetration of active phosphate into the kidney cells will be a formation of active phosphatide molecules already in the earliest stages of the experiment. This is not the case in the cells of other organs into which the labelled phosphate diffuses at a slower rate.

Labelled phosphatides of the plasma.

The renewal of phosphatides in the plasma can only be determined in experiments in vitro; in such experiments[1], taking 4.5 hours, the specific activity of the plasma phosphatide P was found to be smaller than 1/1000 of that of the inorganic P.

In experiments in vivo, an exchange between plasma phosphatides and organ phosphatides takes place and, as in some of the organs labelled phosphatides are formed at a fast rate, we will soon after the administration of labelled phosphate find labelled phosphatide molecules in the plasma, which were released from the organs. In fact, almost all phosphatide molecules found in the plasma were synthesised in the organs. The labelled phosphatide content of the plasma, at different times, is seen in Table 10. In this experiment, the labelled inorganic P content of the plasma was kept constant during 9 days.

[1] L. Hahn and G. Hevesy, Mem. Carlsberg **22**, 190 (1937).

Table 10.

Specific activity of phosphatide P and inorganic P of the plasma.

Time	Relative specific activity	
	Inorganic P	Phosphatide P
4 hours	100	0.53
16 hours	100	3.8
25 hours	100	8.1
37 hours	100	15.0
45 hours	100	22.0
55 hours	100	27 5
9 days	100	81.6

Three consecutive processes have to precede the appearance of labelled phosphatides in the plasma. Labelled inorganic P has to diffuse into the cells of the liver and other organs in which the plasma phosphatides are formed. The building up of the labelled phosphatide molecules represents the second process, their release into the plasma the third. In view of the time taken by these processes, it is easy to understand that in the early stages of the experiment the change of the labelled phosphatide content of the plasma has a more rapid than linear dependence with time.

Since a large part of the phosphatide molecules found in the plasma originated from the liver, it is of interest to compare the amount of the active phosphatides found in the plasma with that present in the liver at the end of the experiment.

As seen in column 3 of Table 11, after the lapse of 12 hours, the activity of the plasma phosphatides reached 3/4 of that of the liver phosphatides. A large part of the liver

Table 11.

Active phosphatide content of the liver and the plasma of rabbits.

Duration of the experiment	Ratio of active phosphatide content of liver and plasma	Extent of partition of labelled phosphatides between liver phosphatides and plasma phosphatides
4 hours	94	0.16
12 hours	18	0.76
9 days	14	1.0

phosphatides is, however, not yet renewed and a further substantial increase of the activity of the plasma phosphatides can only be expected by a corresponding increase in the active phosphatide content of the liver and other organs.

Phosphatide turnover in the corpuscles.

Compared with the phosphatide turnover going on in the organs the phosphatide turnover taking place in the corpuscles is but little. This is also shown by results obtained when investigating the origin of the yolk phosphatides[1]. In these experiments, 28 hours after administration of the labelled phosphate, the specific activity of the corpuscle phosphatides was found to be only 1/3 of that of the plasma phosphatides; showing the corpuscles to be, so-to-say, a by-path of the liver and other organ phosphatides on the way through the plasma into the yolk.

The labelled phosphatide molecules of the corpuscles

[1] G. Hevesy and L. Hahn, D. Kgl. Danske Vidensk. Selskab, Biol. Medd. XIV, 2 (1938).

Table 12.

Extent of partition of labelled phosphatides, originally present in the plasma, between the phosphatides of the corpuscles and of the plasma in experiments *in vitro*.

(Plasma of a rabbit containing labelled phosphatides shaken with corpuscles of another rabbit).

Animal	Time in hours	Extent of partition (Percentage)
Rabbit	0.5	1.8
	1.5	3.6
	3.0	4.0
	4.5	5.0
Hen	1.5	1.5
	2.0	2.0
	3.0	1.5

have various origins. Some of them were incorporated in the course of the red cell formation into a tissue containing labelled phosphatides. Some of the labelled phosphatide molecules came into the corpuscles after they reached the circulation. As seen in Table 12, in which the results of some experiments in vitro are recorded, a part of the phosphatide molecules of the corpuscles exchanges easily with those of the plasma. Presumably those situated in the outermost layer of the stroma take part in this exchange process. It is, however, rather difficult to interpret the comparatively high specific activity of the phosphatide P extracted from the corpuscles in experiments in vivo without assuming that a phosphatide turnover takes place in the corpuscles, though the rate of this turnover is small compared with that of most of the acid-soluble P compounds present in the corpuscles (see Table 14).

Table 13.

Extent of partition of labelled phosphatides, originally present in the plasma, between the phosphatides of the corpuscles and of the plasma in experiments *in vivo*.

Animal	Time in hours	Extent of partition (Percentage)
Rabbit (2—2.5 kg.)	24	16
	24	18
	24	17
	25	16
	42	34
Chicken (100—150 gm.)	18	6.0
	22.5	8.1

In experiments in vivo with rabbits (see Table 13), in the course of a day, the activity of the corpuscle phosphatide P was found to be only about 1/6 of that of the plasma phosphatide P. A still greater difference was found when investigating chickens blood.

Table 14.

Specific activity of phosphatide P and acid soluble P of the corpuscles.

Fraction	Relative specific activity after	
	4 hours	12 hours
Phosphatide P[1]	2.6	9.6
Inorganic P	100	100
Pyrophosphate P	99.5	100
Hydrolyzed by 1 n H_2SO_4 in 7 to 100 min.	100	100
Hydrolyzed in 100 min. to 12 hours	100	
Non-hydrolyzed	87	

[1] The active phosphatide molecules are partly such ones which were taken up from the plasma by an exchange process.

Using elaidic acid as an indicator, Sinclair[1] found, 8 hours after ingestion of the elaidic acid, 15 per cent of the fatty acids extracted from the plasma phosphatides to be composed of this distinctive fatty acid, while the corpuscles contained no more than traces of the indicator.

When iodised fatty acid was used as an indicator, it was found[2] not only in the phosphatides of the plasma but also in those of the corpuscles. In the latter, the concentration of iodised fat was even higher (3.3 per cent of the total fatty acids) than in the former (2.0 per cent). The application of iodised fatty acids leads, thus, to a result which is in contradiction to that obtained by using labelled phosphate or elaidic acid as indicators. Phosphatides containing iodised fatty acids are possibly selectively taken up by the corpuscles, another explanation being that the molecules of these compounds present in the plasma were decomposed at a faster rate than those incorporated into the stroma. Phosphatides containing iodized fatty acids represent non-physiological compounds and, as shown by the above example, the results obtained by using such indicators must be interpreted very cautiously.

In this connection, the observation[3] should be also mentioned that in lactating cows during fasting a marked decrease in the concentration of plasma P lipids takes place which persists for several weeks after realimentation, but there is no significant change in the amount of red cell phosphatides. This result also shows the absence of an intense interaction between plasma phosphatides and phosphatides present in the corpuscles.

[1] R. G. Sinclair, J. Biol. Chem. **115**, 211 (1936).
[2] C. Artom, Arch. Intern. Physiol. **36**, 101 (1933).
[3] J. A. Smith, Biochem. J. **32**, 1856 (1938).

PART II

Investigation of lecithin, cephalin, and sphingomyelin.

We discussed above the rate of renewal of the average petrol-ether soluble phosphatide molecules; in what follows, we wish to describe some experiments in which lecithin, cephalin, and sphingomyelin were separately investigated and their turnover rate determined. Chemically, cephalin differs from lecithin by containing aminoethanol instead of choline. The biological consequence of this replacement is very significant[1]. Cephalin is highly active in accelerating blood clothing, whereas lecithin is not. It was even reported[2] that cephalin prepared from cattle blood or cattle brain enhances, while lecithin inhibits the clotting of rabbits blood. The role of the phospholipids as transport agents of fats was much discussed, this role being often ascribed to lecithin alone.

In our first experiments, we determined the turnover rate of lecithin and cephalin in the organs of rabbits 4 hours after intravenous injection of labelled phosphate. We found the turnover rate of cephalin extracted from the liver, the intestinal mucosa and other organs to be pronouncedly faster than that of lecithin. Simultaneously, CHARGAFF[3] found the rate of rejuvenation of cephalin extracted from the liver and the intestinal tract of rats to be slower than that of lecithin. We were first inclined to explain this difference in the findings of CHARGAFF and ourselves by the fact that the former investigated the turnover process, in contradistinction to us, in carnivorous

[1] Comp. E. CHARGAFF, M. ZIFF and B. M. HOGG, J. Biol. Chem. **131**, 35 (1939).

[2] Y. OKARMURA, Mitt. med. Ges. Okoyama **48**, 1585 (1936).

[3] E. CHARGAFF, J. Biol. Chem. **128**, 592 (1939).

animals. We soon found, however, that it is the duration of the experiment which is decisive for the higher or lower rate found for the cephalin turnover. We will, in what follows, first describe the experimental procedure used.

Experimental procedure.

The tissue is dried with cold acetone and extracted first with ether and then with boiling alcohol. The second process is repeated several times. The solutions obtained were evaporated to dryness and taken up by petrol-ether in the presence of pulverised dry sodium phosphate. The latter was added in order to remove traces of active phosphate possibly present. The process was then repeated in the absence of phosphate and the dry residue taken up in ether. The next step was to precipitate the cephalin from the solution by adding 96 per cent alcohol. The filtrate obtained was evaporated and the residue containing lecithin extracted with ice-cold alcohol. This procedure was repeated and the purified lecithin obtained precipitated as chlorocadmium-lecithin. The compound obtained was thoroughly washed with ether in order to remove traces of chlorocadmium-cephalin possibly present.

The cephalin was prepared from the alcoholic precipitate obtained in the early treatment of the phosphatide mixture. To obtain pure cephalin the precipitate was repeatedly dissolved in ether and precipitated with alcohol.

To secure sphingomyelin the fraction insoluble in petrol-ether was collected and treated alternatively with ether and ice-cold alcohol. The last residue thus obtained was dissolved in a mixture of methyl alcohol and chloroform. By adding ether to this solution purified sphingomyelin was

precipitated. A further purification of this product was obtained by repeating the procedure described above. When sufficient amounts were available, the sphingomyelin was recrystallised from pyridin.

Experiments with rabbits.

In the experiments, the results of which are given in Tables 15 and 16, all the labelled phosphate was administered at the start of the experiment. In all later experiments, labelled phosphate was administered all through the

Table 15.

Specific activity of inorganic P and P of different phosphatide fractions.

Rabbit X. — Weight: 2.9 kg.

All labelled phosphate was administered at the start of the experiment by stomach tube. — The animal was killed after 19 hours.

Fraction	Specific activity relative to the	
	Plasma inorg. P	Inorg. P of the organ
	found at the end of the experiment	
Plasma lecithin P	39.1	..
Liver inorganic P	89.7	100
Liver lecithin P	46.3	51.6
Liver cephalin P	35.4	39.5
Liver sphingomyelin P	28.2	31.2
Brain inorganic P	4.67	100
Brain lecithin P	0.40	8.6
Brain cephalin P	1.04	22.4

Table 16.

Specific activity of inorganic P and P of different phosphatide fractions.

Rabbit XI. — Weight: 2.2 kg.

Labelled phosphate administered to the rabbit by subcutaneous injection at the start of the experiment. — The animal was killed after the lapse of 7 days.

Fraction	Relative specific activity
Plasma lecithin P	100
Plasma cephalin P	48.1
Plasma sphingomyelin P	74.5
Corpuscles lecithin P	88.5
Corpuscles cephalin P	73.1
Brain inorganic P	26.6
Brain lecithin P	14.1
Brain cephalin P	20.9

experiment to keep the specific activity of the plasma inorganic P at a constant level. In the experiments of short duration taking only 4 hours, the cephalin extracted from all the organs investigated was found to be much more active than the lecithin. While the sphingomyelin extracted from the liver did not much differ in its specific activity from that of the lecithin of this organ, in the muscle the sphingomyelin was found to be much more active than the lecithin but less active than the cephalin.

In experiments taking 12 hours, lecithin and cephalin were renewed in the liver to about the same rate while sphingomyelin was found to show a slower turnover rate. The relative activity of lecithin and cephalin was, thus, very materially different in the experiment taking 12 hours

Table 17.

Renewal of lecithin and cephalin.

Rabbit III. — Weight: 2.3 kg.

Intravenous injection during 234 min.

Fraction	Percentage of phosphatide renewed during the experiment	
	A[1]	B[2]
Liver lecithin	10.9	2.1
Liver cephalin	27.9	5.5

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 18.

Renewal of lecithin, cephalin and sphingomyelin.

Rabbit IV. — Weight: 2.5 kg.

Intravenous injection during 215 min.

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingomyelin	Lecithin	Cephalin	Sphingomyelin
Liver	4.38[3] / 3.67[4]	26.5	4.4	0.86[3] / 0.72[4]	5.2	0.86
Small intestine (mucosa)	17.0	40.6	—	3.55	8.47	—

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

[3] Fraction extracted with cold ether (not protein-bound lecithin?).

[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol (protein-bound?).

from that found in experiments of only 4 hours duration. This is not the case with the different phosphatide fractions secured from the muscles. In these fractions, both after 4 and after 12 hours cephalin and sphingomyelin are more active than lecithin.

When looking at the results of the experiments taking one day or more (see Tables 15, 16 and 22) we notice that the lecithin extracted from the liver is more active than the cephalin, while the opposite was found to be the case for the fractions secured from the brain.

Table 19.

Renewal of lecithin, cephalin and sphingomyelin.

Rabbit V. — Weight: 2.1 kg.

Intravenous injection during 250 min.

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingomyelin	Lecithin	Cephalin	Sphingomyelin
Liver	12.4	24.6	8.95	1.86	3.68	1.34
Kidney	3.7	13.5	—	2.10	7.7	—
Muscle	2.9[3] / 1.6[4]	21.7	15.1	0.044[3] / 0.024[4]	0.33	0.23
Small intestine (mucosa) ...	15.6	33.4	—	2.69	5.77	—

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

[3] Fraction extracted with cold ether.

[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol.

Table 20.

Renewal of lecithin and cephalin.

Rabbit VI. — Weight: 2.6 kg.

Subcutaneous injection during 255 min.

Fraction	Percentage of phosphatides renewed during the experiment	
	A[1]	B[2]
Liver lecithin	9.9	2.2
Liver cephalin	35.4	7.9

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Table 21.

Renewal of lecithin, cephalin and sphingomyelin.

Rabbit VII. — Weight: 2.4 kg.

Subcutaneous injection during 11.5 hours.

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingomyelin	Lecithin	Cephalin	Sphingomyelin
Liver	27.5[3] / 22.0[4]	25.9	14.8	16.3[3] / 13.0[4]	15.3	8.8
Muscle	5.6	20.6	17.2	0.49	1.81	1.51

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

[3] Fraction extracted with cold ether.

[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol.

Table 22.

Renewal of lecithin, cephalin and sphingomyelin.

Rabbit VIII. — Weight: 2.0 kg.

Subcutaneous injection during 9 days.

Fraction	Specific activity at the end of the experiment	Lower limit of percentage renewed
Liver inorganic P	100	..
Liver lecithin P	84.0	84.0
Liver cephalin P	84.8	84.8
Muscle inorganic + creatine P	40.3	..
Muscle ester P	18.7	46.4
Muscle lecithin P	12.5	31.0
Muscle cephalin P	11	27
Muscle sphingomyelin P	16.1	40.0
Brain inorganic P	18.8	..
Brain ester P	17.3	92
Brain lecithin P	5.3	28
Brain cephalin P	5.6	30
Plasma inorganic P	100	..
Plasma phosphatide P	82	..
Corpuscle acid soluble P	94	..
Corpuscle phosphatide P	61.7	65.6

Table 23.

Renewal of lecithin and cephalin.

Rabbit IX. — Weight: 2.5 kg.

Subcutaneous injection during 50 days.

Organ	Percentage of phosphatides renewed during the experiment	
	Lecithin	Cephalin
Liver	100	100
Marrow	100	100
Brain	75[1] (42[2])	81[1] (46[2])
Muscle	74[1] (65[2])	71[1] (62[2])

[1] Calculated on the assumption of formation inside the cells (with incorporation of plasma inorganic P).

[2] Calculated on the assumption of formation outside the cells (with incorporation of plasma inorganic P).

Discussion.

The fact illustrated by the results described above — that in experiments taking only a few hours, the cephalin shows a higher extent of renewal than the lecithin, while, in experiments taking one day or more, the opposite is the case — suggests that not all cephalin present in the organs is renewed at the same rate, some fractions showing a much faster turnover rate than others. These fractions could differ either in their chemical composition or in their location in the cells. Numerous chemically different cephalins and lecithins exist differing, for example, in the type of fatty acids they contain. It is, however, not probable that the difference in the chemical constitution is responsible for the remarkable difference in the turnover rate of the different cephalin fractions. The specific activity of successive fractions of cephalin crystallised repeatedly from alcoholic or other solutions does not vary appreciably (comp. p. 52). A much more probable explanation of the difference mentioned above is that in some parts of the cell a decidedly more pronounced enzymatic breakdown and building up of cephalin takes place than in others. In experiments of short duration, we mainly measure the rejuvenation taking place in these favoured districts. The behaviour of lecithin is different in that we do not encounter such a pronounced variation in the rate of turnover of different fractions. The average lecithin molecule is, however, renewed at a similar rate as the average cephalin molecule. This explanation is suggested by the fact that, while in experiments of short duration the cephalin P extracted from the liver, for example, is found to be more active than the lecithin P, in experiments of long duration both fractions are found to

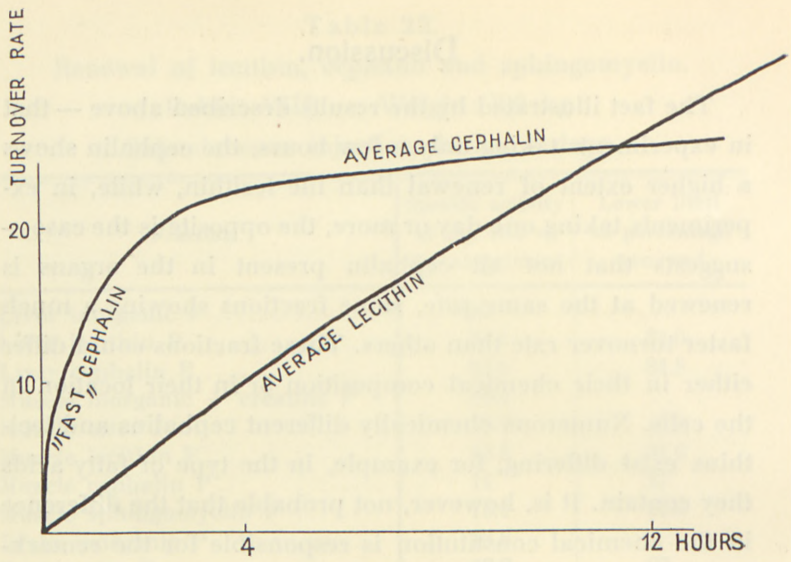

Fig. 5. Turnover of lecithin and cephalin in the liver.

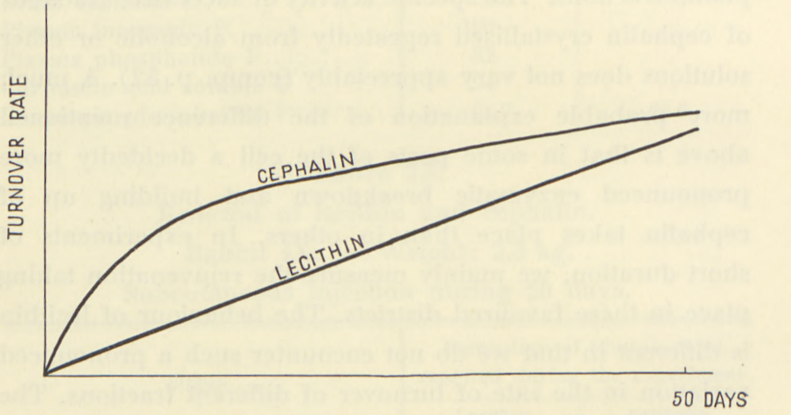

Fig. 6. Turnover of lecithin and cephalin in the brain.

have about the same activity. Not only cephalin and lecithin extracted from the organs of the rabbit show this behaviour, but also the phosphatide fractions secured from the organs of rats, frogs, hens, and of isolated cat liver.

That, in the case of the muscle and brain tissue, cephalin is found in experiments of long duration as well to have a faster turnover rate than lecithin is in no way in contradiction to the conclusion arrived at when investigating the liver fractions. All phosphatide fractions present in the muscle, and especially those in the brain, are renewed at a comparatively slow rate. This remark applies also to the "fast" cephalin fraction present which, though "fast" relative to the average cephalin or lecithin, is in fact "slow". This slowness has the effect that seven days do not suffice to reach the point where the amount of newly formed lecithin is larger than that of the newly formed cephalin. The considerations made above are illustrated by Figs. 5 and 6.

Brain phosphatide.

While, in the case of other tissues, the penetration of phosphate from the plasma into the interspaces can be considered as an almost momentary process and, accordingly, the specific activity of the plasma inorganic P can be taken to be equal to that of the extracellular inorganic P, it cannot in brain tissue. We find that after 4 hours only 1/3 of the amount of labelled phosphorus (incl. organic P) is present in the brain tissue, which we should expect to be present in the extracellular space alone in case of a prompt distribution of the labelled phosphate between the plasma and the interspaces. The extracellular space of the brain tissue was calculated from the chlorine or sodium distribution to amount to 30 per cent of the weight of the tissue[1].

In view of the foregoing statements, we cannot give

[1] J. F. Manery and B. Hastings, J. Biol. Chem. **127**, 657 (1939).

Table 24.

Formation of labelled phosphatides in the brain of the rabbit.

Duration of the experiment	Spec. activity of brain inorganic P / Spec. activity of plasma inorganic P	Spec. activity of brain phosphatide P / Spec. activity of plasma inorganic P	Spec. activity of brain phosphatide P / Spec. activity of brain inorganic P	Spec. activity of brain phosphatide P / Spec. activity of liver phosphatide P
215 min.	0.013	0.0001	0.0093	0.0032
250 min.	0.015	0.0002	0.016	0.005
11.5 hours	0.030	0.0033	0.11	0.022
9 days	0.19	0.054	0.29	0.063
50 days	0.56	0.43	0.77	0.43

exact figures for the specific activity of the extracellular and cellular inorganic P of the brain. Since these figures enter the calculation of the turnover rate of the brain phosphatides the calculation cannot be carried out. A further complication arises from the fact that the decomposition of the brain creatinephosphoric acid prior to the extraction of the inorganic P could not be avoided in our experiments. The brain creatine P may be appreciably less active than the brain inorganic P. This fact would lead to a dilution of the labelled inorganic P by non-labelled inorganic P. We record, therefore, in Table 24, a) the specific activity of the brain phosphatide P relative to the plasma inorganic P, b) relative to the brain total inorganic P, and c) relative to the liver phosphatide P. While the brain phosphatides are found to be much less active than the liver phosphatides, high values are obtained for the ratio of the specific activity of the brain phosphatide P and the brain inorganic P. Even if we divide these values by 2, to account

Table 25.

Formation of labelled lecithin and cephalin in the brain of the rabbit.

Duration of the experiment	Relative specific activity		
	Plasma inorganic P	Brain lecithin P	Brain cephalin P
250 min	100	0.0092	—
11.5 hours	100	0.25	1.08
19 hours[1]	100	0.40	1.04
50 days	100	42	46

[1] In this case, the total activity was injected at the start of the experiment.

for the diluting effect of the creatine[2] P, the resulting figures will still be high.

CHANGUS, CHAIKOFF and RUBEN[2] observed a progressive increase in the content of radioactive phosphatides in the brain on rats for about 200 hours after the administration of labelled phosphorus and it is of interest to note that, in a recent investigation, CHAIKOFF and his colleagues[3] found that the specific activity of the phosphatide P is not uniform throughout the central nervous system.

Experiments with rats.

The specific activity of lecithin P and cephalin P extracted from the rat's liver is given in Tables 26 and 27.

While the ratio of the specific activity of cephalin and lecithin P was found, after 3 hours, to be 1.33, after 24

[2] G. W. CHANGUS, J. L. CHAIKOFF and S. RUBEN, J. Biol. Chem. **126**, 493 (1938).

[3] B. A. FRIES, G. W. CHANGUS and J. L. CHAIKOFF, J. Biol. Chem. **132**, 24 (1940).

Table 26.

Specific activity of lecithin and cephalin in the rat's liver.
Weight of the rat: 200 gm.
All labelled phosphate was injected subcutaneously
at the start of the experiment;
190 min. later, the rat was killed.

Fraction	Percent of activity injected, found in 1 mgm. phosphatide P
Liver lecithin	0.21
Liver cephalin	0.28

Table 27.

Specific activity of different P fractions in the rat's liver.
Weight of the rat: 222 gm.
All labelled phosphate was injected subcutaneously
at the start of the experiment;
24 hours later, the rat was killed.

Liver fraction	Relative specific activity[1]
Labile P	100
Lecithin P	117
Cephalin P	82
Non-labile acid-soluble P	103
Protein P	31

[1] The figures are given relative to the labile acid-soluble P, the value of which, after 24 hours, closely corresponds to that of the inorganic P.

hours we find the value 0.7. Similarly, Chargaff[2] found, in experiments taking 24 hours, greater turnover figures for lecithin than for cephalin. He found the above ratio to be 0.8. It is also of interest to note that an early paper

[2] E. Chargaff, J. Biol. Chem. **128**, 592 (1939).

of ARTOM[1] and his colleagues contains data on the relative activity of lecithin and cephalin extracted from the liver of rats to which olive oil and labelled sodium phosphate was administered 9 hours previously. They state the above ratio to be about 0.6.

Experiment with frog.

The turnover figures of cephalin and lecithin extracted from the frog's liver were found, as would be expected, to be lower than the corresponding figures found in experiments with mammalia. The specific activity of the cephalin P was found to be much higher than that of the lecithin P.

Table 28.

Specific activity of different P fractions in the liver of a frog.

Labelled phosphate was injected into the lymph-sack of a frog kept at 20° all through the experiment (4 hours).

Liver fraction	Relative specific activity
Inorganic P	100
Cephalin P	7.8
Lecithin P	1.3

Ratio of specific activity of cephalin and lecithin = 6.

Experiments with laying hens.

That, in a laying hen, the specific activity of cephalin P of the liver is found, after the lapse of 5 hours, to be slightly

[1] C. ARTOM, C. PERRIER, M. SANTANGELO, G. SARZANA and E. SEGRÈ, Archiv Intern. Physiol. **45**, 35 (1937).

Table 29.

Specific activity of P fractions in the organs of a hen weighing 900 gm.

Labelled phosphate was administered to a laying hen by subcutaneous injection at the start of the experiment; the hen was killed 5 hours later.

Fraction	Relative specific activity
Plasma lecithin P	1.00
Plasma cephalin P	0.98
Liver lecithin P	2.76
Liver cephalin P	2.93
Liver sphingomyelin P	1.38
Liver protein P	0.15
Kidney lecithin P	1.15
Kidney cephalin P	1.69
Intestinal mucosa lecithin P	0.90
Intestinal mucosa cephalin P	1.05
Intestinal mucosa sphingomyelin P	1.10

higher than that of lecithin P, while in experiments with rabbits a very great difference was found, is just the result which we have to expect in view of the arguments discussed on p. 43. In the course of 5 hours, the phosphatide molecules present in the liver of the hen are renewed to an extent which in the case of the rabbit is first reached after the lapse of many hours. It is, therefore, not surprising that the fractions obtained from the hen's liver are similar to those secured from the rabbit's liver in experiments of much longer duration. In the kidneys of the laying hen the phosphatide molecules are renewed at a slower rate than in the liver and, in this organ, as was to be expected, cephalin is found to be markedly more active than lecithin.

The liver sphingomyelin of the laying hen which does

not enter the yolk to any appreciable extent is renewed at a decidedly lower rate than the petrol-ether soluble phosphatides. It is also of interest to note that the rate of rejuvenation of the protein P in the liver of the laying hen is about 20 times slower than that of the phosphatide P.

In the intestinal mucosa, cephalin and sphingomyelin are formed at a somewhat higher rate than lecithin. In the kidneys cephalin was found more active than lecithin. That the rate of renewal of phosphatides in the liver of laying hens is decidedly higher than in the intestinal mucosa or other organs was also found in our earlier researches[1].

Experiment with perfused cat liver.

The experiment on cat liver which was carried out with the kind help of Professor LUNDSGAARD also indicates the faster cephalin turnover in experiments of short duration. The fasting cat used in this experiment weighed 3.3 kg. Blood circulated for 70 min. through the isolated liver.

Table 30.

Specific activity of P fractions in the liver of a cat.

Duration of experiment: 70 min.

Fraction	Relative specific activity
Plasma inorganic P	100
Plasma lecithin P	0.18
Liver lecithin P	2.43
Liver cephalin P	4.05

Ratio of the activity of cephalin P and lecithin P = 1.67.

[1] G. HEVESY and L. HAHN, D. Kgl. Danske Vidensk. Selskab, Biol. Medd. XIV, 2 (1938).

Besides labelled phosphate of negligible weight, 500 mgm. alcohol was added to the blood at the start of the experiment and 500 mgm. glycocoll after 30 min.

When fractionating the alcoholic solution of the liver cephalin, the less soluble fraction was found to show the higher specific activity amounting to 4.47. The low lecithin activity of the plasma is, in view of the short duration of the experiment, not surprising. The labelled phosphate requires some time to penetrate into the liver cells, the formation of labelled lecithin takes some time as well and, finally, the release of the phosphatides into the plasma is far from being a momentary process.

Survey of the results.

In the course of 4 hours, an appreciable part of the petrol-ether soluble phosphatides present in the intestinal mucosa and the liver were found to be renewed. This result is in conformity with that found by Artom and his colleagues[1], by Chaikoff and his collaborators[2], and in this laboratory[3]. In Tables 31 and 32, a summary of the data obtained on the renewal rate of lecithin, cephalin and sphingomyelin fractions is given. In Table 32, the very different behaviour of lecithin from cephalin is clearly seen. While, in the case of lecithin, the labelled percentage increases more or less linearly with time, this is far from being the case with cephalin. We find an almost linear increase with time in the amount of labelled lecithin formed in the liver and the

[1] C. Artom, C. A. Perrier, M. Santangelo, G. Sarzana and E. Segrè, Archiv Internat. Physiol. **45**, 32 (1937).

[2] B. A. Fries, S. Ruben, J. Perlman and J. L. Chaikoff, J. Biol. Chem. **123**, 587 (1938).

[3] L. Hahn and G. Hevesy, Nature **144**, 204 (1939).

Table 31.

Extent of renewal of the petrol-ether soluble phosphatide mixture extracted from the organs of the rabbit in the course of 4 hours.

The results are computed from the figures of Tables 2—7.

Organ	Percentage of phosphatides renewed during the experiment	
	A[1]	B[2]
Small intestine (mucosa)	19.6	3.7
Liver	16.7	3.1
Lungs	8.1	1.2
Stomach	7.7	0.9
Muscle	7.3	0.11
Kidney	6.2	4.3
Spleen	5.2	0.74
Corpuscles	5.2	0.33
Heart	4.0	0.50

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

muscles, assuming that the formation of this compound takes place inside the cells. This linearity does not hold if we assume the formation of phosphatides to take place with incorporation of extracellular P. The bulk of the labelled liver lecithin could not be formed in the last mentioned way, since in that case (see column 5 of Table 32) nine times as much labelled lecithin should have been formed in the course of 12 hours than was found after 4 hours. Similar considerations apply to the muscle lecithin where in the course of 12 hours seventeen times as much labelled lecithin should have been formed as after 4 hours. Such an increase with time is highly improbable.

Table 32.

Extent of renewal of lecithin, cephalin and sphingomyelin in the organs of the rabbit in the course of experiments lasting 4 hours and 12 hours, respectively.

The results are computed from the figures in Tables 17—21.

Organ	Percentage of phosphatides renewed					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingomyelin	Lecithin	Cephalin	Sphingomyelin
	after 4 hours					
Small intestine (mucosa)	16.3	37	—	3.1	7.1	—
Liver	9.3	28	6.7	1.7	5.6	1.1
Muscle	2.3	21	15.1	0.03	0.33	0.23
Kidney	3.7	13	—	2.1	7.7	—
	after 12 hours					
Liver	25	26	15	14.6	15.3	8.8
Muscle	5.6	21	17	0.5	1.8	1.5

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.

[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

If we consider the two possibilities of the formation of cephalin, i. e. incorporation either of cellular or of extracellular labelled inorganic P, we arrive at the following result. If the labelled cephalin is formed inside the liver cells, as much as 1/4 became labelled within 4 hours; thus, 1/4 of the total cephalin present undergoes a rapid renewal, the remaining 3/4 being comparatively inert. In the course of the following 8 hours, hardly any further increase of the amount of newly formed cephalin can be noticed. That

also the remaining part of the cephalin is renewed, though at a very slow rate, is, however, shown by the fact that, after 9 days, most of the cephalin present at the start of the experiment was found to be labelled. Muscle cephalin beheaves in an analogous way.

If we now consider the possibility that the labelled cephalin is formed with incorporation of extracellular P, we arrive at an entirely different interpretation of the results. The amount of labelled liver cephalin formed in the course of 12 hours then works out to be about three times that formed during 4 hours. This result is quite plausible. The result obtained in the case of the muscle cephalin, where as much as five times more labelled cephalin should have been formed in the course of 12 than in 4 hours, seems less plausible. While it is unprobable that the cephalin present in the liver should have been formed with incorporation of extracellular inorganic P, we must envisage the possibility that a part of the cephalin located in the cell membranes is renewed with incorporation of inorganic P located inside the membrane. We discussed above the two extreme cases, formation of phosphatides with incorporation of cellular and of extracellular P. While penetrating through the cell wall, the inorganic P may experience a more or less continuous drop in its activity and the renewal of phosphatide molecules located in the cell membranes could take place by incorporation of "intermediary" labelled phosphate radicals.

It is of interest to remark that in a laying hen, where the liver has to supply large amounts daily of both lecithin and cephalin, the "slow" cephalin fraction is also renewed at a remarkable rate and the rejuvenation of the average lecithin and cephalin in the course of 5 hours hardly differs.

Difference between "fast" and "slow" cephalin.

That the organs contain a small cephalin fraction which is renewed at a fast rate and a larger one which is slowly renewed may possibly be due to a difference in the chemical composition of these fractions. Since different cephalin fractions obtained by fractional crystallisation of the total cephalin extracted from the organ in question did not show large variations, it is not probable that the above result can be explained as due to different rates of newformation of cephalins of different chemical composition.

In fractional crystallisation of alcoholic cephalin solutions, only minor differences in the specific activity of the fractions were noticed. The least soluble fraction extracted from the liver showed, for example, a turnover rate of 4.47, while the value found for the average fraction was 4.05. When organs were extracted first with ether and then with hot alcohol, the lecithin prepared from the first extract was found to be somewhat more active than was the lecithin prepared from the alcohol extract (see Table 19).

Since the renewal of cephalin is an enzymatic process, its velocity should be determined by the effectivity of the enzymes present. It is probable that that part of the cephalin which is located in such a region of the cells, where the enzymatic action is very pronounced, is renewed at a very fast rate. It is also probable that this "fast" fraction has a different biological significance from the "slow" fraction. The fact that the phosphatides have a much larger turnover in some organs than in others induced Sinclair[1] to distinguish between metabolic and non-metabolic phosphatides. The former ones found in the liver, for example, should

[1] R. G. Sinclair, Physiol. Rev. **14**, 357 (1934).

be involved in fat metabolism; the latter ones, found for example, in the muscle, should play an important role in building up cell membranes. Our results suggest the interpretation that we have in all the organs investigated a "fast" and a "slow" cephalin fraction as well. The "fast" fraction is the smaller one. To what extent the "fast" cephalin and other phosphatide fractions are involved in fat metabolism is under investigation.

It is of interest to remark that, if we want to calculate the amount of fatty acid which passed through the phosphatide stage in the liver, for example, we are not sure to arrive at a correct result by calculating from the known phosphatide turnover in the liver the amount of fatty acid or that of inorganic phosphate which passed through the phosphatide stage during the experiment. A minor percentage of the phosphatides present may, as discussed above, have a very high turnover rate, i. e. a minor percentage of the phosphatides may have been decomposed and built up several times during the experiment. It is obvious that such a process would make it possible that much larger amounts of fatty acids or inorganic P pass through the phosphatide stage than those calculated from the known turnover rate of the average phosphatide molecules. The possibility must, therefore, be envisaged that, in an organ, an appreciable amount of fatty acids may pass through the phosphatide stages in spite of the fact that the rejuvenation of the total phosphatide content of the organ in question is a slow process.

Summary.

Labelled sodium phosphate was administered to rabbits, rats, frogs and laying hens. In order to keep the concentration of the labelled phosphate in the plasma constant, labelled phosphate was injected from time to time throughout the experiments.

The specific activity of the inorganic P extracted from the plasma and the organs was measured at intervals. From these data the average specific activity of the cellular inorganic P prevailing during the experiment was calculated.

The phosphatides present in various organs were extracted as well, and the specific activity of the phosphatide P and also of the lecithin, cephalin and sphingomyelin P determined.

The knowledge of the average specific activity of the cellular inorganic P during the experiment and that of the phosphatide P at the end of the experiment permits us to calculate the extent of newformation (turnover) of the phosphatides on the assumption that this process takes place inside the cells. In case the phosphatide molecules are renewed with incorporation of extracellular inorganic phosphate, the specific activity of the latter enters the calculation.

The specific activity of cephalin P extracted from different organs was found in experiments of short duration (4 hours) to be much higher (up to 10 times) than that of lecithin P. With increasing time of experiment this difference was found to diminish. In the fractions obtained from the rabbits liver, after the lapse of 12 hours, both fractions showed the same activity. In organs like muscle

and brain, in which a slow phosphatide turnover takes place, an equal activity of lecithin and cephalin is only reached after the lapse of several days.

Sphingomyelin is renewed in the liver at a slower rate than the ethersoluble phosphatides. In the muscles, in experiments taking not longer than 12 hours, sphingomyelin was found to be appreciably more active than lecithin, but less active than cephalin; after the lapse of 9 days, sphingomyelin was found to be the most active fraction.

Two alternative explanations are put forward to explain the difference in the behaviour of cephalin and lecithin: (a) A part (about 1/4) of the cephalin present inside the cells is renewed at an appreciably higher rate than the average cephalin present, while the bulk of the cephalin showed a similar turnover rate as the average lecithin; or (b) a part of the cephalin located in the cell walls is renewed in situ with incorporation of inorganic phosphate which has a higher specific activity than the inorganic P located inside the cells.

In the course of 50 days, all phosphatide molecules present in the liver and the skeleton were found to be renewed. However, only 74 per cent of the lecithin and 71 per cent of the cephalin extracted from the muscles were newly formed in the course of the experiment. In the brain tissue, 1/4 or more of the lecithin and 1/5 or more of the cephalin molecules remained unchanged.

The amount of active lecithin and cephalin present in the plasma and corpuscles was determined. The active plasma phosphatide molecules are not formed in the circulation but in the organs and are led into the blood. Most of the individual phosphatide molecules present in the corpuscles were incorporated during the formation of

the erythrocytes, but some turnover also took place inside the corpuscles.

We wish to express our hearty thanks to Professor Niels Bohr and to Professor J. N. Brønsted for kindly putting numerous facilities at our disposal, to Professor E. O. Lawrence for the gift of the active phosphorus and to Professor E. Lundsgaard for valuable suggestions in connection with this work.

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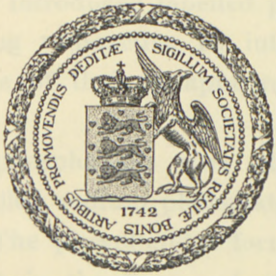
RATE OF PENETRATION OF PHOSPHATIDES THROUGH THE CAPILLARY WALL

BY

G. HEVESY AND L. HAHN



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Ions or molecules of crystalline substances present in the plasma can easily penetrate through the capillary wall. As soon as a few minutes after injecting labelled sodium ions ($^{24}Na^{+}$) into the jugularis, we find these ions proportionally distributed between the sodium ($^{23}Na^{+}$) ions of the plasma and those of the interspaces. On the other hand, colloidal particles like those formed by the proteins of the plasma do not under physiological conditions pass through the walls of the capillaries at any appreciable rate. The phosphatides present in the plasma can be expected to have an intermediary position as to their penetrability through the capillary wall between the crystalline constituents and the proteins present in the plasma. To determine the rate of penetration of the plasma phosphatides through the capillary wall, we introduced labelled phosphatides (phosphatides containing radioactive P) into the plasma and measured the rate of their disappearance from the circulation.

The labelled phosphatides were obtained in the following way. Labelled sodium phosphate was administered to a rabbit (A). The phosphatides formed, after the start of the experiment, in the liver and other organs of this rabbit become labelled; a part of these labelled phosphatides is liberated into the plasma. By injecting plasma of this rabbit (A) into the circulation of another rabbit (B),

we introduced labelled plasma phosphatides under strictly physiological conditions into the circulation. To avoid the increase of the plasma volume of rabbit B, we removed, previous to the injection of the labelled plasma, for example, 20 cc. blood of rabbit B. This blood was, after addition of heparin, gently centrifuged to separate the bulk of its plasma content which was then replaced by the labelled plasma of rabbit A. The blood thus obtained was injected into the jugularis of rabbit B. This rabbit, thus, gets its own corpuscles reincorporated, combined with the corresponding amount of labelled plasma of the other rabbit. An aliquot part of the plasma of rabbit A is kept to be analysed.

The labelled phosphatide molecules introduced into the circulation of rabbit B become distributed in the total plasma of the rabbit almost at once, the next step being the continuous escape of the labelled phosphatide molecules through the capillary wall and their replacement by other phosphatide molecules, originally located in the organs, which diffuse in the opposite direction, namely through the capillary wall, into the plasma. Since the phosphatide content of the plasma remains practically constant during the experiment, the exodus of a certain quantity of phosphatides must be followed by the influx of about the same amount. In view of the very minute turnover of phosphatides in the blood, the number of labelled phosphatide molecules which are decomposed in the plasma during the experiment can be neglected. The processes described above are going on under strictly physiological conditions. The replacement of ordinary phosphorus (^{31}P) by radioactive phosphorus (^{32}P) in some of the phosphatide molecules can certainly not be considered to entail the introduction

of a non physiological component into the circulation, as such a replacement cannot influence the chemical behaviour of the phosphatide molecules to any significant extent.

The rate at which the labelled phosphatides escape from the plasma of rabbits is seen in Tables 1 and 2, and also

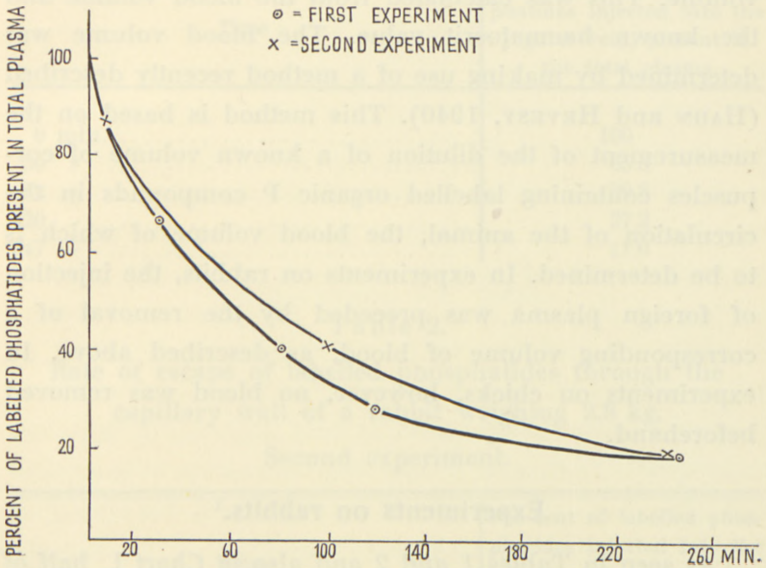

Chart I. Rate of disappearance of labelled phosphatide molecules from the plasma.

in Chart I. The figures of the tables were obtained by comparing the radioactivity of the phosphatides present in 1 cc. plasma samples of rabbit B, taken at different intervals, with that of the phosphatides of an equal plasma volume of rabbit A. The phosphatides were extracted by making use of BLOOR's method. After being converted into phosphate by wet ashing, an aliquot part of the solution obtained was used in the colorimetric measurement of the P content, another to secure an ammonium magnesium

phosphate precipitate, the activity of which was determined by a Geiger counter.

The calculation of the amount of labelled phosphatides present in the total plasma of the rabbit from that found in 1 cc. necessitates the knowledge of the total plasma volume. This was calculated from the blood volume and the known haematocrit value. The blood volume was determined by making use of a method recently described (Hahn and Hevesy, 1940). This method is based on the measurement of the dilution of a known volume of corpuscles containing labelled organic P compounds in the circulation of the animal, the blood volume of which is to be determined. In experiments on rabbits, the injection of foreign plasma was preceded by the removal of a corresponding volume of blood, as described above. In experiments on chicks, however, no blood was removed beforehand.

Experiments on rabbits.[1]

As seen in Tables 1 and 2 and also in Chart I, half of the labelled phosphatides introduced into the plasma leave the circulation by penetrating through the capillary wall in the course of about an hour. As the non-labelled phosphatides can be expected to show the same behaviour as the labelled ones, we can conclude that, from all phosphatide molecules present at the start of the experiment in the plasma, half will no longer be present after the lapse

[1] Some of the results obtained were previously published by us in a note to Nature (**144**, 204, 1939). — F. E. Haven and W. F. Bale (J. Biol. Chem. **129**, 23, 1939) injected emulsions containing labelled phosphatides prepared from the liver of the rat into the circulation of another rat and found the labelled phosphatides to accumulate mainly in the liver and the spleen.

Table 1.

Rate of escape of labelled phosphatides through the capillary wall of a rabbit weighing 2.4 kg.

First experiment.

Time	Per cent of labelled phosphatides injected into the jugular vein, present in the total plasma
0 min.	100
30 -	65.8
82 -	39.8
120 -	27.2
247 -	17.6

Table 2.

Rate of escape of labelled phosphatides through the capillary wall of a rabbit weighing 2.8 kg.

Second experiment.

Time	Per cent of labelled phosphatides injected into the jugular vein, present in the total plasma
0 min.	100
7 -	85.6
100 -	40.1
242 -	18.4

of about an hour, and will be replaced by others which were previously located in the organs.

Three objections may be raised against the conclusions drawn above: a) Labelled phosphatides can be decomposed in the plasma leading, for example, to the formation of labelled inorganic P; b) they can be incorporated into the

corpuscles; c) they can be synthesised in the body of rabbit B, into which labelled plasma was injected. In that case, besides a loss of the labelled phosphatides introduced into the circulation of rabbit B, a gain of such phosphatides due to a synthesis of labelled phosphatides in rabbit B would take place.

The objections mentioned above are, however, not justified, as

a) We recovered (see Table 6) more than 1/2 of the labelled phosphatides injected into the plasma of rabbit B 4 hours later in the organs investigated, in spite of the fact that the latter did not include the skin, the skeleton, and large parts of the digestive tract, which presumably took up an appreciable part of the labelled phosphatides. Furthermore, in the course of 4 hours, a non-negligible part of the phosphatides present in some of the organs and, thus, also that of the labelled phosphatides taken up by these organs, gets decomposed. In the liver, about 1/6 of the phosphatides present was found to be renewed in the course of 4 hours[1]. In view of the above considerations, the amount of phosphatides decomposed in the plasma in the course of a few minutes can certainly be disregarded.

b) That in the course of a few hours the replacement of corpuscle phosphatides by plasma phosphatides is a restricted one, is seen from the following figures. In two experiments, after the lapse of 4 hours, 2 resp. 1.3 per cent of the labelled phosphatides originally present in the plasma of rabbits were found to be located in the corpuscles.

As to objection c), the formation of labelled phosphatides does not take place in rabbit B to any significant extent

[1] G. Hevesy and L. Hahn, Det Kgl. Danske Vidensk. Selskab, Biol. Medd. XV, 5 (1940).

in view of the absence of a sufficient amount of labelled phosphate. This fact is seen from the following consideration: We administered to rabbit A 5×10^6 counts as phosphate and found the next day in the plasma of this rabbit 40.000 counts. We injected into rabbit B 20 cc. plasma containing 8.000 counts, of which 4.000 were due to phosphatide P and 4.000 to inorganic P. As from 5×10^6 inorganic P counts introduced 20.000 phosphatide counts were found after the lapse of a day in rabbit A, we can conclude that, within that time, less than 20 phosphatide counts were formed in rabbit B, thus an insignificant amount.

Experiments on chicks.

Labelled phosphate[1] was administered by subcutaneous injection to chicks (A_1, A_2, and A_3, respectively). After the lapse of a day, plasma samples of these chicks were taken. One part (1 cc.) of the sample was injected into the jugularis of the chicks B_1, C_1, D_1, E_1, B_2, C_2, D_2, and B_3, C_3, D_3, E_3, F_3, respectively; another part was analysed. After the lapse of 7 to 67 minutes, plasma samples of chicks B, C, D, E, and F, respectively, were taken and the activity of their phosphatide content determined; heparin was added to the blood before it was centrifuged. In Tables 3, 4, and 5, the results of these experiments are recorded. The time recorded in Tables 3 and 5 was reckoned from the middle of the time of injection, which took about one minute.

As seen in Table 3, after the lapse of 17.0 to 17.9 min., 1 cc. of the plasma of chicks B_2, C_2, and D_2, respectively, contains only about 7 per cent of the labelled phosphatide

[1] We are much indebted to Mrs. SVENDSEN for administering the labelled phosphate to the chicks.

Table 3.

Percentage of labelled phosphatides present in the plasma of chicks B_2, C_2, D_2, after injection of 1 cc. plasma of chick A_2 containing labelled phosphatides.

Chick	Weight of the chick	Total plasma volume	Time	Per cent of the labelled phosphatide injected	
				present in 1 cc. plasma	present in the total plasma
B_2	114 gm.	3.6 + 1 cc.	17.4 min.	8.02	36.9
C_2	127 -	4.1 + 1 -	17.9 -	7.34	37.4
D_2	134 -	4.3 + 1 -	17.0 -	7.12	37.7

present in 1 cc. of the plasma of chick A injected into chicks B_2, C_2, and D_2, respectively. This decrease is partly due to a dilution of the labelled phosphatides present in 1 cc. by the non-labelled phosphatides present in about 4 cc. plasma of chicks B_2, C_2, and D_2, and partly to an escape of the labelled phosphatides through the capillary wall into the organs and its replacement by non-labelled ones previously present in the organs. As seen in the last column of Table 3, from 100 labelled phosphatide molecules introduced into the circulation of the chicks, only about 37 were present in the plasma after the lapse of about 17 min.

Since the labelled phosphatides cannot be expected to show a different behaviour from the non-labelled ones, we can conclude that 63 per cent of all individual phosphatide molecules originally present are no longer in the plasma of the chick after the lapse of 17 min., being replaced by phosphatide molecules originally located outside the capillary wall.

In the first experiment which we carried out on chicks (see Table 4), we have chosen another procedure. We

Table 4.

Change in the specific activity of the plasma phosphatide of chicks B_1, C_1, D_1, E_1, after the injection of plasma of chick A_1 containing labelled phosphatides.

Chick	Weight of chick	Ratio of specific activity of the phosphatide P obtained after 7 and 67 min.
B_1	138 gm.	2.2
C_1	156 -	2.1
D_1	138[1] -	1.9
E_1	107[1] -	2.8

[1] These chicks have shown pronounced exsudates due to E-avitaminosis and were kindly put at our disposal by Dr. H. DAM. The injection was kindly carried out by Mrs. SVENDSEN. The above figures do not permit us to draw any conclusion as to a difference in the permeability of, for example, the muscle capillaries of normal chicks and chicks suffering from E-avitaminosis. To arrive at such a conclusion it would be necessary to compare the labelled phosphatide content of the muscle tissue of normal chicks and of chicks suffering from E-avitaminosis at the end of the experiment.

compared the activity of 1 mg. phosphatide P extracted from 1 cc. plasma of chick A with the activity of 1 mg. phosphatide P extracted from 1 cc. plasma of chick B, C, D, and E, respectively. Should the phosphatide concentration in the plasma of the different chicks used in this experiment be about the same, we could calculate from the data obtained the loss of labelled phosphatides through the capillary wall in the course of the first 7, and the consecutive 60 min. as well. When determining the phosphatide content of the plasma in our second experiment, we found, however, very pronounced differences between the plasma phosphatide contents of the chicks used. (Chick A_2 = 6.5 mg. %; B_2 = 7.5 mg. %; C_2 = 4.0 mg. %;

$D_2 = 4.8$ mg. %)[1]. From these variations in the phosphatide contents of the plasma we followed that, from the data obtained in the first experiment, we cannot calculate the loss of labelled phosphatides by the plasma in the course of the first 7 min., while we can state the loss sustained in the interval between 7 and 67 min. after the start of the experiment. It is this value which is recorded in Table 4.

Effect of histamin.

We also carried out experiments in which histamin was injected simultaneously with the plasma containing labelled phosphatides. The results of these experiments are seen in Table 5.

The administration of histamin did not much affect the appearance of chicks C_3 and D_3, while chicks E_3 and F_3 could not stand on their feet for the first 5—10 min. which elapsed after the injection of histamin. From the last mentioned two chicks, only small blood samples, about 0.4 cc., could be secured, while we collected several cc. from chicks which got no or only minor doses of histamin administered. The total plasma volume of the chick was calculated as described on p. 6. The average figure obtained for the labelled phosphatide content of the plasma of chicks C_3, D_3, E_3, and F_3, to which histamin was administered, 20 min. after the start of the experiment is about 7. The corresponding average figure for the labelled phosphatide content of the plasma of chicks B_3 and B_2, C_2, D_2 (see Table 3) is as well. No striking effect of the administration of

[1] Comp. also the great variations in the phosphatide content of the blood of chicks found by F. W. Lorenz, J. L. Chaikoff and C. Entenman, J. Biol. Chem. **123**, 577, 1938.

Table 5.

Percentage of labelled phosphatides present in the plasma of chicks B_3, C_3, D_3, E_3, and F_3 after the injection of 1 cc. plasma of chick A_3 containing labelled phosphatides.

Chick	Weight of the chick	Time	mg. histamin-dihydrochloride per gm. chick weight	Per cent of labelled phosphatides injected, present in	
				1 cc. plasma	total plasma per gm. body weight
B_3[1]	113 gm.	21 min.	0	8.1	37.8
C_3	104 gm.	23 min.	3×10^{-3}	5.5	23.8
D_3	95 -	23 -	5×10^{-3}	10.0	40.5
E_3	113 -	22 -	5×10^{-3}	4.0	18.5
F_3	124 -	19 -	1×10^{-2}	7	34.8

[1] Comp. also Table 3.

histamin on the permeability of the capillaries by phosphatides is, thus, found. In view of the large fluctuations shown by the values obtained in the experiments in which histamin was administered, the above result is, however, to be interpreted cautiously.

The permeability of different artificial membranes to phosphatides was investigated by Süllmann and Verzár (1934). They found that through such membranes which are permeable to water blue and Congo red the plasma phosphatides can penetrate as well.

Uptake of labelled phosphatides by the organs.

In the preceding chapters, we discussed the rate at which labelled phosphatide molecules located in the plasma penetrate through the capillary wall. We will now describe

experiments which were carried out in order to determine to what extent the various organs took up the labelled phosphatides which left the circulation. We arrive at these figures by extracting the phosphatides of the organs and by determining their activity.

In Table 6, the percentage of labelled phosphatides introduced into the circulation, present at the end of the experiment in several organs, is recorded in the third column. The fourth column of the table contains data on the labelled phosphatide content of the interspaces computed on the assumption that all the labelled phosphatides present are to be found in the extracellular volume. For the extracellular volume of the organs of the rabbit we utilised the figures arrived at by Manery and Hastings (1939). In the fifth column, the distribution of the labelled phosphatides between equal volumes of the plasma and the extracellular fluid of the organs in question is shown on the assumption that the labelled phosphatides are solely to be found in the interspaces of the organ in question. The conclusion to be drawn from the figures of this column are discussed on page 16. Correctly, we should not compare the distribution of labelled phosphatides between equal volumes of plasma and of extracellular tissue spaces, but the specific activities of phosphatides of the plasma and those of the extracellular phosphatides. In view, however, of the fact demonstrated that the phosphatide molecules penetrate easily through the capillary wall, the phosphatide content of the interspace presumably not much differs from that of the plasma[1].

[1] As to the phosphatide content of the lymph from the leg of the rabbit, Aakuma (1937) states an average value of 1.7 mg. per cent, while 2.1 mg. per cent were found by him in the plasma.

Table 6.

Labelled phosphatides found in the organs of rabbit B after the lapse of 4 hours.

Organ	Weight	Percentage of the labelled phosphatides injected into the vein, present in		Distribution coefficient[1] of labelled phosphatides between equal volumes of extracellular water and plasma water
		the blood-free organ	1 cc. extracellular fluid[1]	
Liver	62 gm.	28.9	2.17	9.8
Kidneys	9 -	0.88	0.19	0.85
Muscles	910 -	2.5	0.018	0.082
Heart	5 -	0.21	0.12	0.54
Spleen	1.2 -	0.06	0.16	0.72
Small intestine mucosa	46 -	1.1	0.065	0.29
Lungs	10 -	1.0	0.22	1.0
Brain	6 -	0.05	0.022	0.10
Plasma	79 gm.	17.6	0.22	..

[1] Calculated on the assumption that no penetration of labelled phosphatides into the cells took place.

In another experiment, only the labelled phosphatide content of liver and muscles were determined. The liver, weighing 85 gm., contained 38 per cent of the labelled phosphatides injected after the lapse of 4 hours, while in the blood-free muscles, weighing 1060 gm., 2.7 per cent of the labelled phosphatides administered were present.

The figures given above relate to the labelled phosphatide content of organs of rabbits killed by bleeding. While such organs have only a comparatively small blood content, this cannot be entirely disregarded. Some of the labelled phosphatides present in the organs will be due to their

blood content. In the muscles of the rabbit we found, by making use of the method of Eichelberger and Hastings (1937), that the blood content amounted to 0.5 per cent of the organs' weight. In the experiment described above, in which the weight of the muscles was 1060 gm. and the total plasma of the rabbit amounted to 97 cc., the blood present in the muscles contained 1.0 per cent of the labelled phosphatides injected. In the case of the liver, the corresponding figure works out to be less than 1 per cent; in the case of the other organs the correction is insignificant.

As seen in Table 6, the labelled phosphatide content of all the organs but that of the liver can be interpreted as being present in the interspaces, though this must not actually be the case. The liver contained, after the lapse of 4 hours, about ten times more phosphatides as can be explained by an uptake of the liver interspaces. This result suggests the explanation that not only the capillary wall but also the membrane of the liver cells is very easily permeable to phosphatides. The capillary wall of the lungs, kidneys, spleen, heart, small intestine, brain, and muscles is but fairly permeable, its permeability decreasing in the above sequence. The uptake of labelled phosphatides by 1 gm. muscle makes out only about 1/170 part of the labelled phosphatides taken up by 1 gm. liver. The corresponding figure for the small intestine mucosa is about 1/20.

Formation and exchange of phosphatides in the liver.

It is of interest to compare the amount of phosphatides synthesized in the liver with the amount which reaches the liver through an exchange process from the plasma. In the first case, we investigate the formation of labelled phos-

phatide molecules, in the second case no new labelled molecules were formed but all the labelled phosphatide molecules present were taken up by the liver from the plasma. This uptake is presumably followed by the release of a similar amount of phosphatide molecules previously present in the liver. An alternative explanation would be that the uptake of phosphatide molecules from the plasma by the liver is followed by a destruction of these molecules in the liver, the phosphatides lost by the plasma being replaced by phosphatides synthesised in other organs and liberated into the circulation.

As found by us, in the course of 4 hours 150 mg. liver phosphatides were newly formed, while during the same time 52 mg. phosphatides are carried from the plasma into the liver; if this amount is not replaced, at least to a large extent, by an equal amount of phosphatides migrating in the opposite direction, then it must be supplied by another source to the plasma. The organ responsible for such a supply must be one in which phosphatide molecules are formed at an appreciable rate. This is primarily the case — besides the liver — in the small intestine. We have, therefore, to ask if the amount of phosphatides supplied during 4 hours by the intestine into the circulation suffices to compensate the uptake of phosphatide molecules by the liver from the plasma. SÜLLMANN and WILBRANDT (1934) determined the amount of phosphatides carried into the circulation by the intestinal lymph of the rabbit. They found that up to 1/2 mg. phosphatide P can be carried by the lymph stream in the course of 4 hours, thus appreciably less than given off by the plasma to the liver during the same time. As the amount of phosphatides brought into the circulation from the intestine does not suffice to com-

pensate the loss of phosphatides by the plasma due to the uptake of phosphatide molecules by the liver, we can hardly expect the amount released by other organs to compensate the loss of the phosphatides. We have, thus, to conclude that in the liver not only a very marked turnover of phosphatides takes place, but that phosphatide molecules exchange also with great ease between the liver cells and the plasma.

Calculation of the amount of phosphatides given off by the plasma to the liver.

We saw that, after the lapse of 4 hours, 29 to 38 per cent of the labelled phosphatide molecules originally present in the plasma were found in the liver of rabbits. We wish to calculate from the average of these figures the total amount of phosphatides which, originating from the plasma, reached the liver in the course of 4 hours. When calculating this amount, we must envisage that large amounts of labelled phosphatides were taken up by the liver and, to some extent, by other organs as well and were replaced by non-labelled ones. These processes clearly lead to an increase of the sensitivity of the radioactive indicator in the course of the experiment. While, at the start of the experiment, 1 count indicates, for example, 1 μ mg. phosphatide P, at the end of the experiment it will indicate the presence of 5 μ mg.

Let us denote by L_0 the concentration of the labelled phosphatide molecules of the plasma at the start of the experiment, and by L_t that found after the lapse of t hours. The amount of phosphorus corresponding to L_0 (average of the values obtained in two experiments) was found to

be 2.4 mg. The decrease of the labelled phosphatide content of the plasma is assumed to take place according to the equation

$$L_t = L_0 e^{-\lambda t},$$

where λ is the constant of disappearance (analogous to the decay constant of radioactive bodies). If the liver alone would take up phosphatide molecules from the plasma, the amount of labelled phosphatides which, coming from the plasma, were located in the liver, would be equal to $L_0 - L_t$. As this is not the case, we must determine experimentally the percentage of the labelled plasma phosphatides present in the liver at the end of the experiment, which we denote by E. To arrive at the figure giving the percentage of the total amount of plasma phosphatide molecules (X) which were found in the liver after the lapse of t hours, we must multiply E by

$$\frac{\lambda t}{1 - e^{-\lambda t}} = Y.$$

From $\lambda = 0.69$ hour^{-1}, and $t = 4$ hours, it follows[1] that

$$Y = 3.$$

The value obtained for Y is too high, as the decrease of the labelled phosphatide content of the plasma takes place in the later stages of the experiment at a slower rate than according to the equation mentioned above. By taking into account this deviation we arrive at the values

$$Y = 2.6 \text{ and } X = 87.$$

[1] We are much indebted to Mr. ARLEY for kindly carrying out this calculation.

From the fact that the phosphatide content of the plasma of the rabbit amounted to 60 mg. it follows that, from the phosphatide molecules present in the liver after the lapse of 4 hours, 52 mg. were such as migrated from the plasma into the liver during the experiment.

Rate of penetration of phosphatides from the organs into the plasma.

We can draw conclusions as to the rate of penetration of labelled phosphatides from the organs into the plasma from an entirely different type of experiments as from those discussed in this paper. We administer labelled inorganic phosphate to rabbits and follow the rate at which labelled phosphatides accumulate in the plasma of such rabbits. Since the amount of labelled phosphatides formed in the plasma can practically be disregarded, we can follow that the labelled phosphatides present in the plasma were brought there from the organs, primarily from the liver. If, in first approximation, we disregard the labelled phosphatides liberated from other organs into the plasma, we find that, in the course of 4 hours, 1.1 per cent of the labelled phosphatides formed during that time in the liver were brought into the circulation, which amount corresponds to about 1/6 of the plasma's phospatide content. We arrive then at the result that about 16 per cent of the plasma phosphatides were replaced, in the course of 4 hours, by labelled phosphatides formed in the liver in the course of the experiment. As, in the early stages of the experiment, the labelled phosphatide content of the liver was much lower than in the later stages, we can expect the amount of total phosphatides liberated from the liver

into the plasma in the course of 4 hours to be larger than 16 per cent of the phosphatide content of the latter.

In the course of 12 hours, we found the labelled plasma phosphatide content to amount to 1/18 of that of the liver. In this experiment, an almost proportional partition of the labelled phosphatide molecules between plasma phosphatides and liver phosphatides was obtained, as the distribution coefficient of the labelled phosphatides between liver phosphatides and plasma phosphatides works out to be 0.76.

Summary.

Plasma of rabbits containing labelled phosphatides was injected to other rabbits. Plasma samples of the last mentioned rabbits were taken at intervals and their labelled phosphatide content determined. The labelled phosphatide content of the organs was determined as well. The labelled phosphatides were found to disappear at a fairly rapid rate from the circulation. Half of those originally present left the circulation in the course of about 1 hour.

The labelled phosphatide molecules penetrate at a fast rate into the interspaces of the liver, at a much slower rate into that of other organs; the sequence of the decreasing rate of penetration being lungs, kidneys, spleen, heart, small intestine, brain, and muscles.

The accumulation of labelled phosphatides in the liver in the course of 4 hours was ten times larger than expected in the case that the interspaces alone contained these phosphatides. From this fact follows a very great permeability of the cell walls of the liver to phosphatides. This is not the case for the other organs investigated. In view of the small amounts of phosphatides which penetrate, in the

course of 4 hours, from the plasma into the muscles and the brain, we can conclude that the exchange of phosphatides between the cells of these organs and the circulation is almost negligible.

The total amount of phosphatides taken up from the plasma by the liver in the course of 4 hours was found to be 52 mg. This uptake is accompanied by a migration of a similar amount in the opposite direction. Not only is the rate of turnover of phosphatides in the liver very high, the exchange of phosphatide molecules between the liver cells and the plasma takes place at a much higher rate than the corresponding process between other organs and the circulation.

In the course of 17 min., about 65 per cent of the labelled phosphatides originally present in the plasma of the chicks left the circulation.

Administration of large doses of histamin had no very striking effect on the rate of penetration of phosphatides through the capillaries of the chick.

We wish to express our hearty thanks to Professor Niels Bohr for numerous facilities kindly put at our disposal, to Professor A. Krogh for many valuable suggestions, and to Dr. H. Dam for his effective help in carrying out the experiments on chicks.

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RATE OF RENEWAL OF THE ACID SOLUBLE ORGANIC PHOSPHORUS COMPOUNDS IN THE ORGANS AND THE BLOOD OF THE RABBIT

WITH A NOTE

ON THE DURATION OF LIFE OF THE RED BLOOD CORPUSCLES

BY

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In a paper published recently in these Proceedings[1], the rate of renewal of the phosphatide molecules present in various organs of the rabbit and other animals was discussed. In the present publication, data on the rate of new-formation of acid soluble phosphorus compounds are communicated. The acid soluble organic P compounds represent a great variety of chemically very different bodies: esters as, for example, hexosephosphate, nucleotide compounds as adenosintriphosphate, phosphagen, and other compounds. These compounds[2] are renewed at a comparatively fast rate in the organs in contradistinction to the phosphatides and nucleoproteins[3]. Furthermore, while the rate of new-formation of the phosphatides in the circulation is almost negligible, the acid soluble phosphorus compounds are renewed at a remarkable rate in the corpuscles. These facts justify the consideration of the acid soluble phosphorus compounds from our view-point as a definite group of the phosphorus compounds present in the body.

[1] G. Hevesy and L. Hahn, Det Kgl. Danske Vidensk. Selskab, Biol. Medd. XV, 5 (1940).

[2] With the exception of adenylic acid [T. Korzybski and J. K. Parnas, Z. physiol. Chem. **255**, 195 (1938)] and, possibly, of other not yet known minor components of the acid soluble P mixture.

[3] L. Hahn and G. Hevesy, Nature, April 6, 1940.

Experimental method.

Labelled P as sodium phosphate was administered by intravenous or subcutaneous injection to rabbits all through the experiment in order to keep the activity of the plasma inorganic P at a constant level. After the lapse of some hours or days, the animal was killed by bleeding. The fresh organs were placed in liquid air and were extracted immediately with cold 10 per cent trichloroacetic acid. The inorganic phosphate present in the extract was precipitated as ammonium magnesium phosphate. The filtrate obtained was then hydrolysed with 1 n H_2SO_4 for 7 min. at 100° to split off the labile P which was then precipitated as ammonium magnesium salt. The filtrate obtained after the last mentioned operation was hydrolysed 100 min. to split off the phosphate radical of the hexosephosphate present. In order to avoid several consecutive precipitations of ammonium magnesium phosphate which lead to an accumulation of very appreciable amounts of ammonium salt in the soluble fraction, we usually divided the filtrate obtained after precipitation of the inorganic phosphate present as such in the tissue into aliquot parts. One aliquot part was hydrolysed for 7 min., the phosphate split off was precipitated, and the filtrate obtained was hydrolysed for 100 min. Another aliquot part was hydrolysed for 7 min., the filtrate obtained was hydrolysed for 12 hours, and so on. The phosphate of the creatinephosphoric acid was split off by heating the solution for 30 min. to 40°. In some cases, the total acid soluble organic P was converted into phosphate and was investigated in toto. The ammonium magnesium phosphate precipitates obtained were dissolved in diluted hydrochloric acid and an aliquot part was used for a colori-

metric P determination. To another aliquot part about 80 mgm. non-active sodium phosphate was added; the total P present in the solution was then precipitated as ammonium magnesium salt. The radioactivity of these precipitates was determined by the aid of a GEIGER counter.

Though the separation of the different acid soluble P compounds described above is far from being quantitative, it sufficed in most cases for our purpose.

In the experiments with blood, as anti-coagulent, ammonium oxalate was used. The corpuscles were centrifuged off and washed twice with a physiological sodium chloride solution. In experiments in vitro, the blood was kept in a $CO_2 - O_2$ atmosphere and was shaken, after addition of labelled sodium phosphate of negligible weight, for 30—190 min. in a thermostat at 37°.

Rate of new-formation.

As labelled phosphorus atoms can only be incorporated into organic molecules in the course of a synthetic process, the radioactivity of the organic phosphorus compounds isolated from an organ is a measure of the rate of its total or partial resynthesis. It is, however, not permitted to compare the specific activity (activity per mgm. P) of the hexosemonophosphate extracted from the kidney and the muscle, for example, and to conclude from the fact that the hexosemonophosphate extracted from the kidney is much more active than that secured from the muscle, that the rate of new-formation[1] of hexosemonophosphate is

[1] The significance of the notion of the rate of new-formation is discussed in the paper by G. HEVESY and L. HAHN, Det Kgl. Danske Vidensk. Selskab, Biol. Medd. XV, 5 (1940).

correspondingly larger in the kidney. The incorporation of labelled P atoms into the hexosemonophosphate molecules must be preceded by a diffusion of the labelled inorganic P into the cells of the organ. If this diffusion process is slow, the rate of formation of labelled hexosemonophosphate molecules is bound to be comparatively slow, in spite of a possibly very fast rate of new-formation of the hexosemonophosphate molecules inside the cells of the organ in question. In fact, the labelled inorganic phosphate molecules penetrate very much faster into the kidney cells than into the muscle cells. To get proper information on the rate of renewal of an organic compound in an organ, we have to compare the specific activity of the P isolated from the organic compound in question at the end of the experiment with the average value of the specific activity of the cellular inorganic P prevailing during the experiment. The considerations mentioned above are discussed in detail in the publication cited above on the turnover rate of phosphatides. In this paper is described the method which permits us to calculate from the specific activity of the tissue inorganic P, the specific activity of the plasma inorganic P, the extracellular space of the organ, and the inorganic P content of the organ and plasma the average specific activity of the cellular inorganic P during the experiment. From the latter magnitude and the specific activity of the P of the organic phosphorus fraction at the end of the experiment, we can calculate what percentage of the organic compound in question is newly formed during the experiment, if only the extent of new-formation is restricted.

If a large fraction of the hexosemonophosphate molecules, for example, is newly formed during the experiment, we can no longer disregard the number of hexosemono-

phosphate molecules which were decomposed and resynthesised more than once during the experiment. If such a repeated new-formation takes place, it will have the effect that the active hexosemonophosphate molecules present at the end of the experiment cannot be longer considered as having been formed with participation of inorganic P which had an activity corresponding to the average activity prevailing during the experiment. The inorganic P atoms, which had an activity corresponding to a late stage of the experiment, will clearly be found to a larger extent incorporated in hexosemonophosphate molecules than those P atoms the activity of which corresponds to an early stage of the experiment.

Let us consider active hexosemonophosphate molecules which were formed during the first stage of the experiment and which were again newly formed during the last minute of the experiment. If the second process were not forthcoming, we should find molecules of small activity; if the opposite were the case, we should find the molecules to be strongly active. When calculating the fraction of the hexosemonophosphate molecules which were newly formed (once or several times) during the experiment from the ratio

$$R = \frac{\text{specific activity of hexosemonophosphate P at the end of the experiment}}{\text{average specific activity of inorganic P during the experiment}}$$

we overestimate the percentage of hexosemonophosphate which was renewed during the experiment. This will be especially the case if the ratio

$$\frac{\text{rate of renewal}}{\text{rate of interpenetration}}$$

is large, as, for example, in the case of the corpuscles.

When calculating from the ratio

$$\frac{\text{specific activity of organic P at the end of the experiment}}{\text{average specific activity of inorganic P during the experiment}}$$

the extent of renewal of the acid soluble P mixture in the corpuscles, we arrive at a value of 199 per cent (see Table I). Such a calculation, for reasons stated above, supplies the upper limit of the extent of renewal. The lower limit is given by the ratio

$$\frac{\text{specific activity of organic P at the end of the experiment}}{\text{specific activity of inorganic P at the end of the experiment.}}$$

The actual value clearly lies very much nearer to the lower than to the upper limit.

Table I.

Extent of renewal of the total organic acid soluble P in the organs of the rabbit.

Rabbit II. Weight 2.6 kg.

Intravenous injection during 215 min.

Organ	A Specific activity of the tissue inorganic P at the end of the experiment	B Specific activity of the cellular inorganic P at the end of the experiment	C Average specific activity of the cellular P during the experiment	D Specific activity of the organic P at the end of the experiment	$\frac{D}{C} \times 100$ Upper limit of the percentage renewed	$\frac{D}{B} \times 100$ Lower limit of the percentage renewed
Plasma	100	—	—	—	—	—
Corpuscles	12.7	12.7	6.4	12.7	199	100
Kidney	87.4	87.1	77.8	33.6	43.2	38.6
Small intestine, mucosa	47.4	45.2	22.6	24.0	106	53.1
Liver	44.0	40.6	20.4	14.3	70.2	35.2
Lungs	36.5	26.9	13.4	9.5	71.0	35.3
Spleen	30.8	28.5	14.3	—	—	—
Stomach	25.9	23.6	11.8	6.9	58.5	29.2
Heart	25.5[1]	21.4	10.8	8.6	79.6	40.2
Brain	1.32	—	—	0.56	—	—

[1] The inorganic P extracted from the heart contains partly such inorganic P atoms which were formed through decomposition of creatine-phosphoric acid prior to the extraction. As the specific activity of the creatine P is, after the lapse of 4 hours, lower than that of the inorganic P (comp. the muscle values in Table III!), the specific activity of the cellular inorganic P of the heart is in fact higher than that stated above and, correspondingly, the values of the rate of renewal of the organic acid soluble P compounds in the heart are smaller than those stated in the last and the last but one column of Table I.

Table II.

Extent of renewal of different fractions of the organic acid soluble P.

Rabbits II, III, and IV (average).

Intravenous injection during 4 hours.

Organ	Time of hydrolysis in 1 n H_2SO_4 at 100°	Spec. activity of the organic P at the end of the experiment: Average specific activity of the cellular inorganic P during the experiment. (Upper limit of the percentage renewed)	Spec. activity of the organic P at the end of the experiment: Specific activity of the cellular inorganic P at the end of the experiment. (Lower limit of the percentage renewed)
Liver	0—7 min.	152	76
Liver	non-hydrolysed	66	33
Kidney, cortex...	0—100 min.	64	57
Kidney, cortex...	100 min.—12 hs.	47	42
Kidney, cortex...	non-hydrolysed	29	26
Kidney, cortex..	hydrolysed in 1 n NaOH at 80°	48	43

Table III.

Specific activity of acid soluble P fractions extracted from the organs of the rabbit.

Rabbit VII. Weight 2.4 kg.

Subcutaneous injection during 11.5 hours.

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle inorganic P	25
Corpuscle P hydrolysed 15 hours in 1 n H_2SO_4 at 100°	25
Corpuscle P hydrolysed 15—120 hours in 1 n H_2SO_4 at 100°	25
Corpuscle non-hydrolysed residue	13.0
Muscle inorganic P	15.5
Muscle creatine P	8.5
Marrow inorganic P[1]	13.1
Marrow organic P	36.8
Brain inorganic P	3.0
Brain organic P	2.3

[1] The low value is presumably due to the presence of traces of slightly active bone P in the marrow sample.

Table IV.

Specific activity of acid soluble P fractions extracted from the organs of the rabbit.

Rabbit VIII. Weight 2.0 kg.

Subcutaneous injection during 9 days.

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle total acid soluble P	94
Muscle inorganic + creatine P	40
Muscle ester P	18.7
Brain inorganic + creatine P	18.8
Brain ester P	17.3

Table V.

Specific activity of acid soluble P fractions extracted from the organs of the rabbit.

Rabbit IX. Weight 2.5 kg.

Subcutaneous injection during 50 days.

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle total acid soluble P	100
Muscle inorganic + creatine P	88
Muscle ester P	77
Brain inorganic + creatine P	56
Brain ester P	68

Table VI.

Specific activity of acid soluble P fractions extracted from the corpuscles of the rabbit.

Rabbits II, III, and IV (average).

Intravenous injection during 4 hours.

Time of hydrolysis in 1 n H_2SO_4 at 100°	Specific activity at the end of the experiment
Inorganic P (present as such in the corpuscles)...	100
0—7 min.	100
7—100 min.	100
7 min.—12 hours	100
Non-hydrolysed in the course of 12 hours (residue)	87
Non-hydrolysed in the course of 24 hours (residue)	77

Table VII.

Experiments in vitro with rabbits blood.

Corpuscle fraction	Duration of the experiment	Specific activity at the end of the experiment
Inorganic P	30 min.	100
Hydrolysed 7 min.	30 -	77
Hydrolysed 7 min.—12 hours	30 -	16
Non-hydrolysed	30 -	13
Inorganic P	60 -	100
Hydrolysed 7 min.	60 -	90
Hydrolysed 7 min.—12 hours	60 -	41
Non-hydrolysed	60 -	28
Inorganic P	90 -	100
Hydrolysed 7 min.	90 -	82
Hydrolysed 7 min.—12 hours	90 -	57
Non-hydrolysed	90 -	46
Inorganic P	190 -	100
Organic acid soluble P	190 -	57

Table VIIIa.

Effect of temperature on the distribution of ^{32}P between plasma and corpuscles.

Rabbits blood after addition of labelled phosphate of negligible weight is shaken for 90 min.

Fraction	Temperature	
	37°	5°
Plasma inorganic P	78	96.7
Corpuscle inorganic P	3.8	0.62
Corpuscle organic P	18.2	2.64

Table VIIIb.

Effect of temperature on the distribution of ^{32}P between plasma and corpuscles.

Relative specific activity of the P fractions of the blood.

Fraction	Temperature	
	37°	5°
Plasma inorganic P	100	100
Corpuscle inorganic P	13.8	0.37
Corpuscle pyrophosphate P	11.3	0.36
Corpuscle non-hydrolysed P	6 9	0.087

Table VIIIc.

Effect of temperature on the ^{32}P fractions of the corpuscles.

Relative specific activity of the P fractions of the corpuscles.

Fraction	Temperature	
	37°	5°
Corpuscle inorganic P	100	100
Corpuscle pyrophosphate P	82	84
Corpuscle non-hydrolysed P	50	23.5

Discussion.

A. Renewal of the acid soluble P compounds in the organs.

B. Renewal of the acid soluble P compounds in the corpuscles.

A. Renewal of the acid soluble P compounds present in the organs.

As seen in Table I, in the course of 4 hours a very appreciable part of the average acid soluble P compounds present in many of the organs was renewed. A very active turnover takes place in the mucosa of the small intestine. One half or more of the molecules of the organic acid soluble P compounds present in this organ became renewed in the course of 215 min. This very marked rate of new-formation of the organic acid soluble P compounds is of interest in connection with the view put forward by VERZÁR and others on the role of intermediary phosphorylation processes in the resorption of sugar from the intestine[1]. The highest value for the specific activity of the acid soluble organic P was found in the kidneys. The labelled inorganic P diffuses faster into the cells of the kidneys than into those of any other organ. The high value of the specific activity of the acid soluble kidney P is, to some extent, due to the fact that the cellular inorganic P within 215 min. acquires a higher value in the kidneys than in other organs. If due regard is taken to this phenomenon we find that, in spite of the fact that the specific activity of the intestinal acid soluble P is lower than that of the corresponding fraction extracted from the kidneys,

[1] F. VERZÁR and E. J. MCDOUGALL, Absorption from the intestine. London 1936. Comp. also E. LUNDSGAARD, Z. physiol. Chem. **261**, 19 (1939).

the rate of renewal in the intestinal mucosa is greater than in the kidneys.

The rate of renewal of the organic acid soluble P molecules in the liver and in the lungs (see Table I) is also quite appreciable. The comparatively high value found for the ratio of the specific activities of the organic P and inorganic P in the case of the brain tissue is, at least to some extent, due to an extremely low activity of the average inorganic P of the brain. It is a puzzling result that the total activity found in the brain tissue, due to the presence of active inorganic and organic P, is smaller than that we should expect to find in the interspaces of the brain alone when assuming a proportional distribution of the active inorganic P between the plasma and the extracellular space of the brain tissue. In this calculation, the extracellular space is taken to be 30 per cent of the weight of the brain, as found from the distribution figures of chlorine and sodium[1] between the plasma and the brain tissue. Our results suggest the assumption that the labelled phosphate ions penetrate at a very slow rate through the capillaries of the brain or, alternatively, that the figures obtained by determining the distribution of chlorine or sodium between the plasma and the brain do not represent the proper extracellular space of the brain. It is for these reasons that we did not state in Table I any figures for the rate of renewal of the acid soluble P compounds present in the brain.

Table II contains data on the activity of different organic P fractions extracted from the kidneys and the liver. The phosphate obtained after 7 min. hydrolysis contains, as well known, besides P split off from creatinephosphoric acid, the labile P of the adenosintriphosphate molecules.

[1] J. F. Manery and B. Hastings, J. Biol. Chem. **127**, 657 (1939).

That the adenosintriphosphate molecules present in the muscles are reorganised at a fast rate was found in our previous experiments[1]. MEYERHOF and his collaborators[2] studied the rate of reorganisation of the adenosintriphosphate molecule with incorporation of active inorganic P in experiments in vitro and found this process to take place at a very fast rate. Data on the activity of the phosphorus obtained by hydrolysing the organic acid soluble phosphorus extracted from perfused cat liver for 7 min. are given by LUNDSGAARD[3].

Our experiments lead to the result that at least 76 per cent of the 7 min. product extracted from the liver of the rabbit became renewed in the course of 215 min. In LUNDSGAARD's perfusion experiment, the specific activity of the 7 min. fraction was found, after 90 min., to amount to 60 per cent of that of the inorganic P extracted from the plasma at the end of the experiment.

As seen in Tables II-VII the more readily hydrolysable compound is renewed at a faster rate than the less readily hydrolysable one. That even those compounds which resist treatment with 1 n H_2SO_4 at 100° for 12 hours or more are renewed, however, at a very appreciable rate is seen in Tables II and III. More than 1/4 of the non-hydrolysable residue of the organic acid soluble P fraction secured from the kidneys was, for example, found to be renewed in the course of 215 min. (see Table II.)

After the lapse of so long a time as 9 and 50 days (see Tables IV and V), the muscle inorganic + creatine P has

[1] G. HEVESY and O. REBBE, Nature **141**, 1097 (1938). G. HEVESY, Enzymologia **5**, 138 (1938).

[2] O. MEYERHOF, P. OHLMEYER, W. GENTNER and H. MAIER-LEIBNITZ, Biochem. Z. **298**, 398 (1938).

[3] E. LUNDSGAARD, Skand. Arch. f. Physiol. **80**, 291 (1938).

only reached 40 and 88 per cent, respectively, of the specific activity of the plasma inorganic P. After the lapse of 50 days, the specific activity of the ester P of the muscles was found to be 77 per cent of that of the plasma inorganic P. A detailed investigation of the rate of renewal of the acid soluble P compounds present in the muscles of the frog will be published shortly.

B. Renewal of the acid soluble P compounds present in the corpuscles.

1. Phosphorylation processes going on inside the corpuscles.

In our early investigations[1] on the circulation of phosphorus, using radioactive P as an indicator, we found that the organic acid soluble P compounds of the red blood corpuscles are normally in a state of flux, being continuously decomposed and resynthesised. Labelled phosphate ions were found to penetrate into the corpuscles at a fairly slow rate and to take part in very rapid phosphorylation processes inside the corpuscles. Labelled hexosemonophosphate introduced into the plasma was found not to penetrate at any significant rate into the corpuscles. However, the labelled phosphate present in such hexosemonophosphate molecules after being split off diffuses as inorganic phosphate into the corpuscles and is incorporated inside the erythrocytes partly into hexosemonophosphate molecules. Presumably, the P atoms of the plasma diffuse exclusively or almost exclusively as phosphate ions into the corpuscles.

That phosphorus compounds, as hexosephosphoric acid,

[1] L. Hahn and G. Hevesy, C. R. Lab. Carlsberg **22**, 188 (1938). G. Hevesy and A. H. W. Aten, Det Kgl. Danske Vidensk. Selskab, Biol. Medd. XIV, 5 (1939).

triosephosphoric acid, phosphopyruvic acid, phosphoglyceric acid, and so on, take an important part in glycolytic processes going on in the corpuscles was emphasised by v. EULER and BRANDT[1], and others. According to the views of MEYERHOF, PARNAS, and others, in the course of the glycolytic cycle, hexosediphosphate, for example, is found to be formed through the interaction of dextrose with adenosintriphosphate. Hexosediphosphate is maintained in enzymatic equilibrium with two molecules of triosephosphate. The last mentioned compound reacting with pyruvic acid forms phosphoglyceric acid which is converted into phosphopyruvic acid and this, in turn, reacts with adenylic acid in the resynthesis of adenosintriphosphate. The last mentioned compound is also formed by direct phosphorylation of adenylic acid from inorganic phosphate or by transfer of the phosphate radical of glycerophosphate to adenylic acid. The synthesis of adenosintriphosphate is a very rapid process and the active inorganic phosphate ions which penetrate into the corpuscles will soon be found to be incorporated in adenosintriphosphate molecules. The participation of the active adenosintriphosphate molecules in the synthesis of various organic P compounds will lead to the formation of active hexosephosphate, active phosphoglyceric acid, and so on, in the corpuscles. In this connection, the result obtained by DISCHE[2] is of interest: he found that the total phosphate transferred

[1] H. v. EULER and K. M. BRANDT, Z. physiol. Chem. **240**, 215 (1936). Comp. also H. LAWACZECK, Biochem. Z. **145**, 351 (1924); NEGELEIN, Biochem. Z. **158**, 121 (1925); M. MARTLAND, Biochem. J. **19**, 117 (1925); P. RONA and K. IWASAKI, Biochem. Z. **184**, 318 (1917); H. K. BARRENSCHEEN and B. VASARHELYI, Biochem. Z. **230**, 330 (1931); H. K. BARRENSCHEEN and K. BRAUN, Biochem. Z. **231**, 144 (1931).

[2] Z. DISCHE, Die Naturwiss. **24**, 462 (1936).

to glucose added to human erythrocytes originates from adenosintriphosphate.

Important evidence that the organic acid soluble phosphorus compounds and, primarily, diphosphoglycerate of the red blood corpuscles constitute a labile phosphorus reserve of considerable consequence serving various functions was presented in recent years by Guest and his colleagues[1]. Some of their findings are described in what follows.

The development of ricketts in rats is associated with decreases in all fractions of the acid soluble phosphorus. During the first five days, the concentration of inorganic phosphorus and adenosintriphosphate phosphorus drops abruptly to a low level and then remains constant for 25 days and longer. The decrease in the organic acid soluble phosphorus is accounted for almost entirely, after the first few days, in the diphosphoglycerate fraction. Guest and Rappaport state that diphosphoglycerate makes out about half of the acid soluble phosphorus present in the corpuscles.

In experiments carried out on dogs after nephrectomy, it was found that, due to the failure of excretion of the vaste endogenous P, a large increase in the inorganic P content of the blood takes place, which is followed by a corresponding increase in the acid soluble organic P content of the corpuscles. The increase is mainly due to the rise of the diphosphoglycerate content of the corpuscles, the increase in organic acid soluble P and in diphosphoglycerate P being 47 and 43 mgm., respectively, per hundred cc.

They found, furthermore, that the increase of phos-

[1] A summary of many of their results is to be found in the paper by G. M. Guest and S. Rappaport, American J. of Diseases of Children 58, 1072 (1939).

phorus excretion in the urine during acidosis comes partly from mobilised diphosphoglycerate of the corpuscles. As an effect of pyloric obstruction, an increase of the acid soluble P content amounting to 37 mgm. Eq per kg. corpuscle water of the dog was found to take place. From this increase, 32 mgm. Eq were due to the rise in the glycerophosphate content.

These and numerous other findings clearly show that the acid soluble phosphorus compounds of the red corpuscles are readily synthesised and decomposed in the blood through reactions of the glycolytic cycle. That these processes take place in the corpuscles at a remarkable speed was shown by us when making use of radioactive phosphorus as an indicator. We have, thus, two independent lines of evidence as to the remarkably high rate of turnover of phosphoglycerate and some other phosphorus compounds present in the corpuscles.

By comparing the specific activity of the inorganic P of the corpuscles with that of the P extracted from various organic compounds present in the corpuscles we get information on the rate of resynthesis of these compounds. The comparison of the specific activity of the inorganic P present in the corpuscles with that of the inorganic P present in the plasma informs us, on the other hand, on the rate of penetration of phosphate ions from the plasma into the corpuscles.

2. *Rate of new-formation of the acid soluble P compounds present in the corpuscles.*

As seen in Table VII which gives the result of experiments in vitro, the product of 7 min. hydrolysis has, after the lapse of 30 min., a specific activity amounting to 77 per

cent of that of the corpuscle inorganic P. The product hydrolysed between 7 min. and 12 hours, which contains besides hexosephosphate P and other fractions appreciable amounts of diphosphoglycerate P as well, is markedly less active than the readily hydrolysed fraction, while the specific activity of the P of the non-hydrolysed residue is only 1/8 of that of the corpuscle inorganic P. This fraction[1] consists mainly of 2, 3-diphosphoglyceric acid P though it contains also P of the adenylic acid which amounts, in the corpuscles of the rabbit, to about 5—10 mgm. per cent, thus to about 1/10—1/20 of the total acid soluble P of the corpuscles.

In experiments in vivo taking about four hours, all but the non-hydrolysed fraction were found to be entirely renewed; only about 1/5 of the last mentioned fraction, presumably mainly its adenylic acid content[2], was found to be unchanged. Diphosphoglyceric acid is, thus, renewed at a high rate as well.

Rate of penetration of plasma inorganic P into the corpuscles.

To obtain information on the rate of penetration of the inorganic phosphate of the plasma into the corpuscles, we have to compare the specific activity of the plasma inorganic

[1] E. Greenwald, J. Biol. Chem. **63**, 339 (1925); H. Jost, Z. physiol. Chem. **116**, 171 (1927); S. E. Kerr and A. Autaki, J. Biol. Chem. **121**, 531 (1927); E. Warweg and G. Stearns, J. Biol. Chem. **115**, 567 (1936); S. Rappaport and G. M. Guest, J. Biol. Chem. **129**, 781 (1939); A. Lennerstrand and M. Lennerstrand, Arkiv f. Kemi, Miner. og Geol. 13 B, No. 15 (1939).

[2] S. E. Kerr and L. Daoud, J. Biol. Chem. **109**, 304 (1937) state that, out of 88 mgm. per cent organic acid soluble P found in the corpuscles of the rabbit, 16 mgm. per cent are pyrophosphate P and 8 mgm. per cent adenylic acid P.

P with that of the corpuscle inorganic P. After the lapse of 11.5 hours (see Table III), this ratio is found to be 4, showing that the rate of penetration of the phosphate ions from the plasma into the corpuscles and vice versa is slow, a much slower process than the reorganisation of most of the acid soluble organic P compounds present in the corpuscles. After the lapse of nine days, the ratio of the specific activity of the plasma inorganic P and the corpuscle average acid soluble P is only slightly larger than 1 (1.06) (after so long a time, the activity of the average corpuscle acid soluble P acquired almost the same value as shown by the inorganic P of the corpuscles); and after the lapse of fifty days, a completely proportional distribution of the labelled P atoms between the plasma P and the P of the acid soluble P compounds present in the corpuscles is attained. While, after the lapse of 11.5 hours, the chance of a normal distribution of a P atom which diffused into the corpuscles between organic and inorganic P is almost = 1, the corresponding figure for the distribution of an inorganic P atom between plasma and corpuscles is only of the order of magnitude of 1/4.

The interesting phenomenon that an individual phosphate ion, while penetrating fairly slowly into the corpuscles, is incorporated at a remarkably fast rate into organic molecules present in the corpuscles finds many analoga in the processes going on in various organs. It is especially conspicuously shown in the study of the penetration of labelled phosphate into the muscle cells and of that of the rate of renewal of the acid soluble P compounds present in these cells; the former process being slow, the latter process being, in the case of some of the compounds, very fast.

There can hardly be any doubt that the large majority of the P atoms present in the molecules of most of the acid soluble organic P compounds of the corpuscles were incorporated into these molecules inside the corpuscles and reached the erythrocytes as inorganic phosphate ions which passed from the plasma into the corpuscles. The possibility that hand in hand with the process mentioned above a slow exchange of, for example, organic phosphoglycerate between plasma and corpuscles takes place cannot be disregarded. In view of the very low content of organic acid soluble P compounds of the plasma, if a migration of such compounds between corpuscles and plasma would take place, it should be mainly directed from the corpuscles into the plasma. In view of the fast rate of renewal going on in the corpuscles and the fast turnover of the acid soluble P compounds in the plasma, the investigation of a migration of organic acid soluble P molecules from the corpuscles into the plasma or vice versa encounters great difficulties.

In the above connection it is of interest to remark that Solomon, Hald and Peters[1] found, in a recent investigation, that phosphate esters present in the corpuscles are restrained from escaping by some force in addition to the membrane of the corpuscles. The restraining force is presumably a chemical aggregation or combination with substances of large molecular size. They arrived at the result mentioned above by the following observation. When filtering blood which was hemolysed by freezing, the ultrafiltrate obtained at 7° did not contain any appreciable amount of organic P, while the opposite was the case when

[1] R. Z. Solomon, P. M. Hald and J. P. Peters, J. Biol. Chem. **132**, 721 (1940).

saponin was used to obtain hemolysis. Frozen blood acts much as does intact blood so far as phosphates are concerned. The organic esters remain intact as long as the blood is kept cold and their combination with substances of high molecular size remains unpaired. This is not the case when saponin is added. Under the action of this agency the binding forces break down, and the organic phosphate esters can enter the ultrafiltrate. At 37° the phosphate esters can be ultrafiltrated even if the blood was hemolysed by freezing. In experiments in vitro with intact blood at 37°, during 18 hours no appreciable amount of organic phosphate ester was found to escape from the corpuscles into the plasma. These results support the view that the P atoms present in the phosphate ester molecules of the corpuscles reach the plasma, and vice versa, after being converted into constituents of inorganic phosphate ions.

As seen in Tables VIII a, b and c, with decreasing temperature the rate of penetration of ^{32}P from the plasma into the corpuscles and also the rate of its incorporation into organic P compounds strongly decreases. While at 37°, in the course of 90 min., 22 per cent of the ^{32}P originally present in the plasma diffused into the corpuscles, at 5° only 3.3 per cent of the ^{32}P originally present in the plasma found their way into the corpuscles. The comparison of the specific activity of the inorganic P present in the corpuscles at 37° and 5°, respectively, leads to the result that this activity is 37 times larger at 37° than at 5°. A similar comparison of the specific activity of the organic P of the corpuscles (exclusive the labile P of adenosintriphosphate) leads to a ratio of 80. It is of interest to note that a decrease of the temperature hardly affects the very fast rate of new-formation of adenosintriphosphate molecules, since more

than 80 per cent of the labile P of the adenosintriphosphate present in the corpuscles became renewed during the experiment both at high and at low temperatures.

Summary.

Labelled phosphate was administered to rabbits all through the experiments in order to keep the activity of the inorganic phosphate of the plasma at a constant level. The experiments took 215 min. to 50 days. The comparison of the specific activities of the organic P and the cellular inorganic P extracted from the organs leads to the result that in the course of 215 min. more than one half of the acid soluble P compounds present in the mucosa of the small intestine became renewed. Next the intestinal mucosa, the fastest rate of turnover was found to take place in the kidneys, liver and lungs.

From the various organic acid soluble phosphorus compounds the most readily hydrolysable ones were found to be renewed at the fastest rate. Fractions containing mainly phosphoglycerate were found to be renewed at an appreciable rate as well.

While the rate of formation of labelled acid soluble organic P compounds inside the corpuscles is rapid, the diffusion of labelled phosphate ions from the plasma into the corpuscles is a slow process. In the course of 12 hours, only about 1/4 of the P atoms of the acid soluble phosphorus compounds of the corpuscles entered into exchange equilibrium with the P atoms of the plasma phosphate, while most of the molecules of the compounds mentioned above were renewed during this time inside the erythrocytes. The contrast between the rate of interpenetration of labelled

phosphate and that of its incorporation into several of the acid soluble phosphorus compounds inside the cells is also found in the case of the muscles.

Labelled phosphate was found to penetrate into the brain tissue at an exceedingly low rate.

We wish to express our most hearty thanks to Professor Niels Bohr for numerous facilities most kindly put at our disposal.

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ON THE DURATION OF LIFE OF THE RED BLOOD CORPUSCLES

Several methods have been applied to determine the lifetime of the red blood corpuscles[1].

1. The number of the corpuscles is artificially increased and the time observed after the lapse of which the normal level is re-established.

2. The number of the corpuscles is artificially diminished and the time necessary to obtain the normal level is determined.

3. Blood of another species or another type of blood is introduced into the circulation and the rate of disappearance of the "foreign" corpuscles is observed by using microscopic or serologic methods.

4. Determination of the time of excretion of the products of destruction of the blood corpuscles.

5. Observation of the rate of increase of the reticulocyte figure which follows the recovery after haemorrhage.

In what follows, we shall outline a method which much differs from those enumerated above. This method makes use of the application of isotopic indicators.

a) We administer to a rabbit, for example, a compound of an element which is normally to be found in the cor-

[1] A detailed survey of these methods was recently given by E. SCHIØDT, Acta Med. Scand. XCV, fasc. I (1938).

puscles. This element contains an isotopic indicator. We can then expect that the corpuscles formed after the start of the experiment will contain some of the labelled element administered. After the administration af radioactive iron, for example, we will find the haemoglobin of the newly formed corpuscles of the rabbit to contain labelled iron. We introduce some of these labelled corpuscles into the circulation of another rabbit. Hand in hand with the destruction of these corpuscles in the circulation of the second rabbit, there takes place a decrease of the activity of the corpuscles of this rabbit which decrease is a measure of the duration of life of the erythrocytes introduced.

b) We introduce labelled iron, for example, into the circulation, keep the specific activity (activity per mgm. Fe) of the plasma at a constant level, and follow the change of the ratio

$$\frac{\text{activity of haemoglobin Fe}}{\text{activity of plasma Fe}}$$

with time. The rate of increase of this ratio will inform us on the rate of formation of new corpuscles.

In the preliminary experiments to be described in this note we used labelled phosphorus as an indicator and followed both a) the rate of decay of the radioactive organic P compounds extracted from the corpuscles and b) the rate of activation of organic phosphorus molecules found in the corpuscles. We are describing our preliminary experiments in this note, since we have no more hope in a near future to come into possession of the strong ^{32}P preparations which would enable us to continue our experiments.

a I. Rate of disappearance of labelled phosphatides incorporated into the corpuscles.

The method denoted above as a) can be applied only if the labelled P is unable to leave the intact corpuscles. If this is not the case, the decrease in the activity of the corpuscles is no longer a measure of the rate of destruction of the erythrocytes.

Table 1.

Change in the activity of the corpuscle phosphatides with time.

The corpuscles were formed in rabbit A and introduced into the circulation of rabbit B.

Time	Activity
7 min.	100
5 hours	91
3 days	43
7 —	27
13 —	19.8
25 —	11.4
39 —	5.27

In a former investigation, we found that a significant part of the labelled phosphatides present in the corpuscles can exchange with the phosphatide molecules of the plasma[1]. Presumably, the phosphatides in the surface layer of the stroma can take part in such an exchange process. Furthermore, some replacement of the labelled phosphatide P by non-labelled inorganic P takes place inside the corpuscles giving the phosphatide P atoms a possibility to escape as labelled inorganic P from the intact corpuscles into the

[1] L. Hahn and G. Hevesy, Nature **144**, 72 (1939).

plasma. A part of the labelled phosphatides present in the corpuscles can, thus, be lost without the decay of the erythrocytes in which they are located. The fall in the activity of the corpuscle phosphatides in the earlier stages of the experiment (see Table 1) will, thus, to a large extent be due to other effects than the disintegration of the corpuscles containing these molecules.

These facts explain the rapid decrease of the activity of the phosphatide fraction in the early stages of the experiment. With increasing time, the rate of decay of the corpuscle phosphatide activity becomes slower and slower. In the last stage of the experiment, between the 25th and the 39th day, 54 per cent of the activity present the 25th day was found to be lost. Should this loss be solely due to the decay of "old" corpuscles containing labelled phosphatides, then the figure mentioned above would indicate the percentage of about 29 days old corpuscles[1] destroyed in the circulation in the course of 14 days to be 54. The figure given above represents thus the upper limit of the percentage of the 29 days old corpuscles destroyed in the course of 14 days.

a II. Rate of disappearance of labelled organic acid soluble compounds incorporated into the corpuscles.

The rate of renewal of the acid soluble P compounds present in the corpuscles is, with the exception of that of adenylic acid and possibly of some minor constituents, a fast one. Correspondingly, the active acid soluble P molecules present in the corpuscles which are suspended in an inactive plasma will soon lose most of their activity. These

[1] The active corpuscles were formed in rabbit A in the course of 7 days.

acid soluble P compounds can, thus, not be used as indicators in the determination of the lifetime of the corpuscles. Adenylic acid can, however, be presumably applied to that purpose. We found that, after the lapse of 39 days, the activity of the acid soluble P of the corpuscles amounted to half of its value measured after the lapse of 25 days. This activity was presumably due to that of adenylic acid, since the other acid soluble P compounds lost their activity in the early stages of the experiment. By considering the activity of the acid soluble residual fraction we arrive thus at about the same value for the decay rate of corpuscles as we found by using labelled phosphatides. As the next step, we intended to isolate the adenylic acid P by making use of the method of Fiske[1] and to follow the rate of its decay in the corpuscles. For reasons stated above (p. 29), these experiments could not be carried out.

It is of interest to remark that the method outlined in this chapter shows some resemblance to that method of determining of the duration of life of the corpuscles in which blood of another species or another type of blood is introduced into the circulation and the rate of disappearance of the "foreign" corpuscles observed. While, however, in the last mentioned method corpuscles of another species are used, which may decay with a different speed as do the corpuscles under physiological conditions, the indicator method makes use of corpuscles of the same species. The replacement of a minute percentage of the ^{31}P atoms present in the P compounds of the corpuscles by ^{32}P atoms can hardly have any influence on the biochemical behaviour of these corpuscles. The same applies to the weak β-radiation emitted by the active P compound

[1] C. H. Fiske, Proc. Nat. Acad. Sc. **20**, 25 (1934).

of the corpuscles. The remark made on p. 29—that in such experiments phosphorus preparations of very appreciable activities have to be applied— does not contradict the above statement. The corpuscles injected into rabbit B will, at the start of the experiment, contain less than 1/1000 of the activity administered to rabbit A and, in the later phases of the experiment, still less.

b. Rate of increase in the labelled phosphatide content of the corpuscles.

The corpuscles formed in a medium containing active phosphatide molecules will necessarily contain such molecules. If only the active phosphatide content of the organs producing corpuscles is kept at a constant level, the rate at which the activity of the phosphatides present in the corpuscles reaches this level is a measure of the rate of formation of the corpuscles. As in the case discussed under a I, in the earlier phases of the experiment, some of the phosphatide molecules of the corpuscles are replaced by phosphatide molecules previously located in the plasma. Therefore, and also for other reasons mentioned on p. 30, only the figures obtained in the late stage of the experiment permit us to draw conclusions on the rate of formation of corpuscles. The increase in the activity of the corpuscle phosphatides is seen in Table 2. In these experiments, the level of the activity of the plasma inorganic phosphate was kept constant.

We found the marrow phosphatides to have reached the activity level of the inorganic P of the plasma and, thus, the maximum value obtainable after the lapse of nine days.

Table 2.

Ratio of the specific activity of the corpuscle phosphatide P and the marrow phosphatide P.

(The specific activity of the marrow phosphatide P reached its maximum value after the lapse of 9 days).

Rabbit	Time in days	Percentage of marrow activity reached
B	9	66
D	25	77
D	31	99
D	50	97

We have, therefore, to compare the activity values of the corpuscle phosphatides obtained after that date. As seen in Table 2, after the lapse of nine days, the specific activity of the corpuscle phosphatides reached 66 per cent of the maximum activity. If half of the corpuscles present after the lapse of nine days is replaced by newly formed corpuscles in the course of the following 14 days, $66 + 1/2\ (100 - 66) = 83$ per cent of the maximum activity should have been obtained. After the lapse of 16 days, 77 per cent were obtained.

As the corpuscle activity approaches the maximum value the method becomes less and less sensitive. It is, therefore, practically only applicable in a fairly narrow range. The investigation of the rate of activation of the corpuscle phosphatides or the corpuscle adenylic acid is, therefore, a less suitable method of determination of the lifetime of corpuscles than is the measurement of the decay rate of transfused active corpuscles. The former me-

thod has, however, the advantage that the transfusion of corpuscles from one rabbit to another one can be avoided and, consequently, the possible errors inherent in the introduction of corpuscles of a rabbit into the circulation of another rabbit can be eliminated.

We mentioned on p. 29 the determination of the change in the activity of the haemoglobin iron as an example for the application of isotopic indicators in the determination of the time of life of the corpuscles. HAHN, BALE, LAWRENCE and WHIPPLE[1] have administered labelled iron to dogs and found that some of the active iron rapidly entered the corpuscles. This observation by no means excludes the possibility of applying labelled iron in the determination of the lifetime of the corpuscles. The penetration of similar amounts of labelled Fe into the corpuscles as are present in the plasma, as found by them, cannot be interpreted as a sign of a swift renewal of haemoglobin molecules with incorporation of active Fe inside the corpuscles. This would only be the case if the specific activity of the haemoglobin Fe of the corpuscles reached a value similar to that shown by the plasma iron. We can infer from the figures published by the authors just mentioned that this was not the case. It is, therefore, quite possible that labelled iron or any other labelled component of the haemoglobin could be used as an indicator in the determination of the lifetime of the corpuscles. Should the renewal of haemoglobin molecules with incorporation of other iron atoms take place inside the corpuscles at a rate which is faster than the rate of production of the corpuscles, this

[1] P. F. HAHN, W. F. BALE, E. O. LAWRENCE and G. H. WHIPPLE, J. Am. Med. Assoc. **111**, 2285 (1938); J. Exptl. Med. **69**, 739 (1939).

might frustrate the application of the method. In this case, just as when applying labelled P as an indicator, the observation of the rate of decay of the activity of the haemoglobin iron in another animal is more likely to be successful than the observation of the formation of corpuscles containing labelled haemoglobin which, from a physiological point of view, might be preferable.

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BLÜTENBIOLOGISCHE STUDIEN. I.

DIMORPHIE UND MONOMORPHIE BEI *ARMERIA*

VON

JOHS. IVERSEN

WITH A SUMMARY IN ENGLISH

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VORWORT

Ausgangspunkt der vorliegenden Arbeit war die Beobachtung der Blütendimorphie bei *Armeria*, die ich im Sommer 1938 während eines Aufenthalts auf Skallingen machte. Die ersten Stadien der Untersuchung wurden im Skallinglaboratorium ausgeführt, wo ich die besten Arbeitsbedingungen fand. Später habe ich die Untersuchung noch anderorts fortgesetzt.

Bei meiner Arbeit mit *Armeria* bin ich von verschiedenen Personen und Institutionen aufs freundlichste unterstützt worden. Allen, die mir geholfen haben, danke ich herzlich. Meinen besonderen Dank möchte ich richten an Museumsinspektor Dr. O. Hagerup, København, Professor Dr. Rolf Nordhagen, Bergen, und Direktør M. P. Porsild, Den danske arktiske Station Disko, durch deren Hilfeleistungen meine Arbeit sehr wesentlich gefördert worden ist.

Die Zeichnungen wurden von Frk. Ingeborg Frederiksen mit grosser Sorgfalt ausgeführt.

I. Pollen- und Griffeldimorphie bei *Armeria*.

Im Sommer 1938 machte ich die Beobachtung, dass *Armeria vulgaris* zwei scharf getrennte Pollentypen aufweist, die stets an verschiedenen Pflanzen gefunden werden. Der naheliegende Gedanke, dass die beiden Pollentypen verschiedenen Rassen dieser polymorphen Art angehören, erwies sich bald als irrig. Gleichviel ob man Populationen in nassen, salzigen Marschwiesen oder im trockenen, salzfreien Dünensande untersuchte, war das Ergebnis dasselbe: überall wurde gefunden, dass etwa 50 % der Pflanzen den einen, und etwa 50 % den anderen Pollentypus besassen. Offenbar handelte es sich um eine blütenbiologische Erscheinung. Eine genauere Untersuchung ergab denn auch, dass die Pollendimorphie mit einer Griffeldimorphie verbunden war.

Besser als viele Worte erhellen die Figuren 2 und 3 die beiden Typen bei *Armeria*, die ich im folgenden als Linie A und Linie B bezeichnen will.

Die Pollenkörner der Linie A besitzen eine sehr grobe, bienenwabenartige Netzstruktur. Die Wände, welche die »Kammern« trennen, sind durch Verschmelzung hoher, nach aussen verdickter Papillen entstanden. Da diese Verschmelzung nicht vollkommen ist, sind die Wände von langgestreckten Löchern durchbrochen; an der Aussenseite sind sie mit Dörnchen gewimpert. Die Griffel zeichnen sich durch sehr schwach gewölbte Narbenpapillen aus; die Narben besitzen dadurch eine fast glatte Oberfläche.

Bei Linie B ist die Struktur der Pollenexine sehr viel

feiner: bei oberflächlicher Betrachtung bemerkt man meist nur zahlreiche kleine Wärzchen; bei genauerer Beobachtung sieht man aber, dass sich die Wärzchen unten vereinigen und zusammen eine netzförmige Struktur ähnlicher Art wie bei Linie A bilden, nur sind die Maschen sehr viel feiner, kleiner und niedriger. Dörnchen und Löcher sind kaum ohne Immersionsobjektiv zu erkennen. Die Griffel unterscheiden sich durch stark vorspringende Narbenpapillen; die Narben haben daher eine rauhe (papillöse) Oberfläche. Ausserdem sind sie schlanker als bei Linie A (vgl. Fig. 2 und 3).

In der Länge der Griffel und Staubgefässe finden sich dagegen keine deutlichen Unterschiede zwischen den Linien A und B.

Der Sinn dieser eigentümlichen Differenzierung der Pflanzen in zwei Typen tritt ausserordentlich schön hervor, wenn man die Gynoecien junger Früchte herauspräpariert und im Mikroskop untersucht.

Fig. 4 und 5 stellen zwei solche Gynoecien dar. Wie man sieht, sind die Griffel fast bis zum Grunde frei. Im untersten Drittel der Griffel findet sich ein dichter Bestand von Haaren; dieses Merkmal ist charakteristisch für die Gattung *Armeria* zum Unterschied von der nahe verwandten *Statice*. Die obere Hälfte der Griffel wird von den Narben eingenommen.

Im Gewirr von Haaren zu unterst auf den Griffeln findet man beinah immer zahlreiche Pollenkörner, die dort hängen geblieben sind. Sie gehören fast ausschliesslich zu derselben Linie wie der Fruchtknoten, es handelt sich also offenbar um Pollenkörner derselben Blüte. Damit dies auf den Bildern (Fig. 4 und 5), die sonst nicht schematisiert sondern direkt nach der Natur gezeichnet sind, deutlich

hervortreten konnte, sind die A-Pollenkörner weiss, die B-Pollenkörner schwarz dargestellt.

Abgesehen von dem Haargürtel zu unterst auf den Griffeln, wo gestrandete Pollenkörner leicht im dichten Gewirr zurückgehalten werden, haften auf den Griffeln junger Früchte im allgemeinen nur solche Pollenkörner, die auf den Narben gekeimt, und deshalb durch die hinunterwachsenden Pollenschläuche in den Griffeln fest verankert sind. Zu meiner Freude konnte ich nun feststellen, dass fast alle auf den Narben haftenden Pollenkörner zu der entgegengesetzten Linie des Fruchtknotens gehören, d. h. auf A-Narben findet man nur B-Pollenkörner, auf B-Narben nur A-Pollenkörner (vgl. Fig. 2 u. 3). Die Figuren 4 und 5 stellen willkürlich gewählte Beispiele eines A- und B-Fruchtknotens dar. Auf letzterem sieht man neben zahlreichen A-Pollenkörnern auch ein einzelnes, welches zur eigenen Linie gehört. Das kann vorkommen; ich habe 60 Gynoecien, die sich gleichmässig auf die beiden Linien verteilen, bezüglich der anhaftenden Pollenkörner mit folgendem Ergebnis untersucht[1]: Auf den A-Narben wurden 14 A-, aber 310 B-Pollenkörner gefunden, auf den B-Narben 19 B-, aber 343 A-Pollenkörner. Mit andern Worten: 96 % der Pollenkörner auf den A-Griffeln gehörten zur B-Linie, und gleicherweise 95 % der Pollenkörner auf den B-Griffeln zur A-Linie. Die vereinzelten Pollenkörner auf den Narben, die zur eigenen Linie gehören, sind wohl z. T. blos zufällig hängen geblieben[2], ebenso wie die Pollenkörner von

[1] Die jungen Früchte wurden vorher kurz mit heisser verdünnter Kalilauge gespült.

[2] Ausnahmsweise habe ich aber doch ein vereinzeltes gekeimtes B-Pollenkorn auf der B-Narbe nachweisen können; der Wuchs des Pollenschlauchs war in dem einen Fall, den ich näher untersuchte, abnorm: der Schlauch wuchs aufwärts gegen die Griffelspitze.

Gramineen, Plantago, Aster u. a. Pflanzen, die man ab und zu auf den Narben junger *Armeria*-Früchte antrifft. Ursprünglich haben dort sicher viele solcher Staubkörner gehaftet, mit der Zeit fallen sie aber ab, da sie unter normalen Verhältnissen nicht durch Keimschläuche verankert werden. Daraus muss man folgenden Schluss ziehen: A-Pollenkörner vermögen nur in B-Narben, B-Pollenkörner nur in A-Narben hineinzuwachsen. Demnach sind diese beiden Typen also auch physiologisch verschieden: sie sind intersterile Linien, es muss eine ständige Kreuzung zwischen ihnen stattfinden, sonst geschieht keine geschlechtliche Vermehrung.

Um diese Auffassung experimentell prüfen zu können, habe ich einige Kulturversuche angestellt. Aus einem dichten *Armeria*-Bestand auf Skallingen habe ich drei grosse Blöcke ausgegraben und mit Wurzeln und Erde in grossen Kästen nach Als versandt, wo ich sie in drei Gärten eingepflanzt habe, die mehrere Kilometer voneinander und noch weiter entfernt von natürlichen *Armeria*-Wuchsorten gelegen waren. Der eine ausgepflanzte Bestand enthielt sowohl A- als auch B-Pflanzen. Aus den andern beiden Beständen hatte ich vorher alle A- bezw. B-Pflanzen entfernt, so dass in beiden nur die eine Linie vorhanden war. Diese »reinen« Bestände wuchsen in ganz isolierten Höfen, aus deren Gärten ich vorsichtshalber die angepflanzten Gartenformen von *Armeria* sorgfältig entfernt hatte; die Möglichkeit, dass meine Kulturen durch fremde *Armeria*-Pollen bestäubt würden, war also soweit möglich ausgeschlossen.

Die Auspflanzung wurde 1938 vorgenommen, und schon im folgenden Jahr konnte festgestellt werden, dass weder die A- noch die B-Anpflanzung irgend eine Frucht gebildet hatte;

die Fruchtstände beider Bewachsungen waren taub. Dagegen war die Fruktifikation normal und reichlich in dem dritten, gemischten Bestand. Dieser Versuch hat also bestätigt, dass beide Linien vollständig selbststeril sind.

Die Bestäubungsbiologie von *Armeria* ist bereits von mehreren Verfassern untersucht worden (z. B. Knuth 1891, Warming 1906, S. 309), doch ist die Dimorphie bei *Armeria* offenbar bisher übersehen worden. Die Blüten von *Armeria* sind protandrisch. Anfangs sind die mit Pollen bedeckten Antheren nach innen gerichtet, während die Griffel nach aussen abstehen. Später krümmen sich die Griffel nach innen, und Knuth hält es für wahrscheinlich, dass nunmehr Selbstbestäubung eintreten kann, falls eine Insektenbestäubung ausgeblieben sein sollte. Warming meint dagegen, dass eine Selbstbestäubung nur durch den Wind herbeigeführt werden könnte, da die Narben und Antheren sich auch in diesem Zustande nicht berühren.

Nun ist es ja leicht zu verstehen, dass diese verschiedenen Bewegungen der Antheren und Griffel wie auch die Protandrie nicht dazu dienen die Selbstbestäubung anfangs zu hindern, später eventuell zu fördern, da eine Selbstbefruchtung ausgeschlossen ist.

Damit ein Fruchtansatz entstehen kann, muss Kreuzbestäubung eintreten, und die Blüten von *Armeria* sind denn auch vorzüglich auf Insektenbestäubung eingerichtet (Farbe, Duft, Nektar). Knuth hat über 30 verschiedene Insekten als Besucher beobachtet, hauptsächlich Hummeln, Bienen, Fliegen und Schmetterlinge. Man findet auch fast immer reichliche Fruktifikation bei unserer heimischen *Armeria*, und die grosse Anzahl von Staubkörnern auf den Narben der jungen Früchte zeugt ja ebenfalls von dem Fleiss der Insekten.

II. Monomorphe *Armeria*.

Armeria vulgaris setzt, wie wir gesehen haben, nur dann Frucht an, wenn die Narben mit Pollen der entgegengesetzten Linie bestäubt werden, was infolge der Grösse und Klebrigkeit der Pollenkörner nur durch Vermittlung von Insekten geschehen kann.

Wie verhält es sich nun aber mit den hocharktischen *Armeria*-Formen, besonders der *Armeria labradorica*, die noch an der Nordküste Grönlands gedeiht? Hier müssen die Bedingungen für Insektenbestäubung zum mindesten äusserst ungünstig sein.

Ich untersuchte deshalb aus Grönland mitgebrachtes Material. Es stellte sich dabei heraus, dass hier die Dimorphie fehlte. Wie aus Fig. 6 erhellt, stehen sowohl die Pollenkörner als auch die Narben morphologisch so ziemlich in der Mitte zwischen denen der Linien A und B der *Armeria vulgaris*. Die Skulptur der Pollenexine ist bedeutend gröber als bei der B-Linie; manche Pollenkörner stehen denen der A-Linie sehr nahe, immerhin ist die Skulptur bei den meisten deutlich zarter und niedriger und die Maschen sind kleiner und zahlreicher (Fig. 6). Bemerkenswert ist eine starke Variabilität selbst innerhalb desselben Staubbeutels; bei *Armeria vulgaris* war sie bei beiden Linien nur sehr gering. Die Griffel besitzen fast ebenso stark hervorspringende Narbenpapillen wie bei der B-Linie der dimorphen Form, in der Dicke der Narben entsprechen sie dagegen eher der A-Linie.

Die Narben waren dicht besetzt mit gekeimten, durch Pollenschläuche fest verankerten Staubkörnern (Fig. 6), die völlig denjenigen entsprachen, die noch in den Staubbeuteln derselben Blüte vorhanden waren.

Eine Prüfung der reichhaltigen arktischen Sammlungen des Botanischen Museums, København, ergab, dass *Armeria* in der ganzen Arktis monomorph ist (siehe Fig. 10).

Die Monomorphie wird von Selbstfertilität begleitet. Diese prinzipiell so wichtige Erscheinung geht deutlich aus folgenden Tatsachen hervor:

1. Selbst in jungen Blüten findet man gewöhnlich zahlreiche gekeimte Staubkörner auf den Narben. Infolge der Protandrie kann Selbstbestäubung nämlich schon eintreten, ehe die Blüten sich öffnen. Trotzdem die Griffel zu diesem frühen Zeitpunkt ihre normale Gestalt noch nicht erreicht haben, wachsen die Pollenschläuche in die Narben hinein. Wie später (S. 31) näher dargelegt wird, ist die merkwürdig unentwickelte, plumpe Gestalt des Gynoeciums der monomorphen Armerien (vgl. Fig. 4 und 5 mit Fig. 7) als direkte Folge einer solchen sehr früh erfolgten Selbstbefruchtung anzusehen. Nach der Häufigkeit dieser Erscheinung zu urteilen, muss Selbstbefruchtung die Regel, Fremdbefruchtung dagegen eine seltene Ausnahme sein.

2. Die Fruktifikation der monomorphen *Armeria* ist offenbar immer gut. Auf meine Anfrage schreibt mir Herr Direktor M. P. Porsild, Den danske arktiske Station Disko, wie folgt: »Ich erinnere mich nicht, jemals Insektenbesuch gesehen zu haben, welcher Bestäubung herbeiführen könnte, und doch ist die Fruchtbarkeit sehr gross.«

Es ist klar, dass diese Selbstfertilität von ausserordentlich grossem Vorteil für eine hocharktische Pflanze ist, die nicht durch den Wind bestäubt wird: sie wird unabhängig von den äusserst spärlich vorkommenden Insekten. Auch ist die frühe Befruchtung — ehe noch die Griffel voll entwickelt sind — insofern ein Vorteil, als damit die Ent-

wicklung der Früchte beschleunigt wird, was bei dem kurzen Sommer der Arktis von besonderer Bedeutung sein kann.

III. Systematische Probleme.

a. Über die Einteilung von *Armeria*.

Armeria labradorica und verwandte Formen werden von einigen Forschern als selbständige Arten aufgefasst, während andere sie nur als schwache Formen innerhalb der sehr polymorphen Art *A. vulgaris* bewerten. Es war denn auch bisher nicht möglich ein Merkmal zu finden, das nicht auch bei irgend einer Vulgaris-Form auftritt. Das neue Merkmal aber, die Monomorphie, trennt die arktischen Formen scharf von allen europäischen Arten. Um über das gesamte Areal der monomorphen Armerien Klarheit zu erhalten, habe ich Herbarienmaterial aus den botanischen Museen in København, Bergen, Stockholm, Upsala und Göteborg besichtigt, das mir von den erwähnten Museen freundlichst zur Verfügung gestellt worden war.

Als Ergebnis der Untersuchung konnte folgendes festgestellt werden (siehe Fig. 10 und 11): Während *Armeria* in Nordasien und Amerika ausschliesslich monomorph ist, finden sich in dem europäisch-mediterranen Verbreitungsgebiet nur dimorphe Arten, — wenn wir von dem Vorkommen von *Armeria labradorica* im nordöstlichsten Zipfel von Europa absehen. Die Areale der monomorphen bezw. dimorphen Armerien sind scharf getrennt, nur auf Grönland und in Lappland berühren sie sich (vgl. S. 22—23).

Hiermit dürfte der systematische Wert dieses Merkmals

erwiesen sein. In Wirklichkeit lässt sich die sehr gleichförmige Gattung *Armeria* in zwei wohlbegrenzte Sektionen — *Dimorphae* und *Monomorphae* — einteilen. Die geographische Verbreitung dieser Sektionen geht ohne weiteres aus Fig. 11 hervor.

Die übliche auf Wallroth (1844) und Boissier (1848) zurückgehende Einteilung beruht auf Merkmalen der Frucht. Je nach dem, ob die Fruchtkelche nur auf den Rippen oder auch auf den Zwischenfeldern behaart sind, werden die Arten den Sektionen *Barbatae* Wallr. (*Pleurotrichae* Boiss.) oder *Mastrucatae* Wallr. (*Holotrichae* Boiss.) zugeteilt. Spätere Forscher wie Druce (1903) und Blake (1917) wollen ebenfalls diesem Merkmal besonderen Wert beilegen. Dieser Auffassung kann ich nicht beipflichten.

Zweifellos sind die Behaarungsmerkmale der Frucht erblich fixiert: ein pleurotriches Individuum verbleibt pleurotrich unabhängig von den Umweltbedingungen; soweit hat Druce recht in seiner Erörterung (1903, S. 69—70). Dadurch unterscheidet sich dieses Merkmal aber keineswegs von andern Merkmalen wie etwa der Behaarung des Schaftes, der Behaarung der Blätter oder der Form der Hüllblätter; auch sie sind bei demselben Individuum durchaus konstant. Ferner ist die Grenze zwischen pleurotrichen und holotrichen Pflanzen nicht so scharf wie verschiedene Autoren ausführen. Es gibt alle Übergänge zwischen ganz kahlen und völlig behaarten »Zwischenfeldern«; irgendwo muss die Grenze willkürlich gelegt werden. Viel wesentlicher ist aber ein anderer Einwand: Eine Reihe von Arten enthalten sowohl pleurotriche als auch holotriche Formen. Es gibt an der dänischen und norwegischen Küste wohl kaum eine *Armeria*-Population, bei

der nicht holotriche und pleurotriche Individuen Seite an Seite stehen (vgl. auch Turesson 1922 und Knud Jessen 1938, S. 229). Hier zwei oder mehr Arten zu unterscheiden, ist einfach unmöglich. Nicht besser geht es bei anderen natürlichen systematischen Einheiten; Wallroth's und Boissier's Einteilungsprinzip führt zu einer allzu grossen Zersplitterung der Gattung in Arten, das geht schon aus der Unmenge von Arten in De Candolles Prodromus hervor. Die meisten dieser »Arten« sind nur als Varietäten oder Ökotypen aufzufassen.

Dagegen ist die Einteilung von *Armeria* in *Dimorphae* und *Monomorphae* sehr leicht durchzuführen; Übergangsformen scheinen ganz zu fehlen.

b. Sektion *Monomorphae*.

Der monomorphe Formenkreis umfasst folgende der in Boissier's Monographie in D. C. Prodromus angeführten Arten: *Holotrichæ: A. labradorica, A. sibirica, A. androsacea. Pleurotrichæ: A. arctica, A. scabra, A. chilensis, A. brachyphylla, A. andina, A. macloviana, A. sanguinolenta.* Da der systematische Wert dieser Arten sehr umstritten und eine Übersicht nunmehr, nach Ausscheidung der dimorphen Arten, leichter zu erreichen ist als früher, soll hier der Versuch gemacht werden, eine neue Bearbeitung des monomorphen Formenkreises — d. h. der amerikanischen und nordasiatischen Arten — zu geben.

Die verschiedenen arktischen Formen (*A. labradorica, A. sibirica, A. arctica, A. scabra* und *A. sanguinolenta*) lassen sich zu einer einzigen Art vereinigen, die als *A. scabra* Willd. zu bezeichnen ist. Dieser Name ist anscheinend in Vergessenheit geraten, er ist aber bedeutend älter als die

andern (Willd. herb. Roem. et Sch. 1817—20). Ich habe das Typ-Exemplar von *A. scabra* nicht gesehen, es kann sich aber nur um eine monomorphe Form handeln. Als Fundort wird »Asia borealis« angegeben; ich habe monomorphe Pflanzen aus Nordasien gesehen, die sehr gut mit Wallroth's und Boissier's Beschreibungen übereinstimmen.

Innerhalb von *A. scabra* — im weiteren Sinne — finden sich, wie angeführt, sowohl holotriche als auch pleurotriche Sippen, und es scheint, dass der Behaarung des Fruchtkelches in diesem Falle wirklich systematische Bedeutung zukommt (vgl. Blake 1917, S. 3—4). In Nordamerika stellte Blake fest, dass das gesamte untersuchte Material aus den östlichen Teilen (incl. Grönland) zur holotrichen *A. labradorica* gehörte, während er im Westen nur die pleurotriche *A. arctica* vorfand. Diese Feststellung kann ich auf Grund des von mir untersuchten Herbariummaterials bestätigen (siehe Fig. 10), doch findet sich die holotriche Sippe auch in den North West Territories, und im nördlichen Alaska finden sich Übergangsformen. Auf der anderen Seite der Bering Strasse setzt sich das Areal der holotrichen Sippe ohne Unterbrechung fort im Areal der *A. sibirica*, welche von der *A. labradorica* kaum verschieden sein dürfte. Malte (1934) schreibt allerdings, dass *A. sibirica* »according to the original description in D. C. Prodr., p. 678, has obtuse leaves and the bracts slightly shorter than the fruiting calyx. All the Canadian Arctic *Armeria* which the writer has seen has the leaves from sharply acute to acutish, and the bracts are as long or longer than the fruiting calyx.« Dieses Urteil ist aber unhaltbar, wenn man grösseres Material untersucht. Die Form der Blattspitze wechselt beträchtlich, ich kann aber nicht sehen, dass sich die sibirische *Armeria*

in dieser Hinsicht anders verhält als die nordamerikanische. Ich habe Pflanzen aus Grönland und aus Kanada mit extrem stumpfen Blättern gesehen; anderseits besitzen die meisten sibirischen Herbarienexemplare, die ich besichtigt habe, zugespitzte Blätter. Viel besser steht es auch nicht um das andere Merkmal. Die Brakten sind an sibirischen wie an amerikanischen Exemplaren ungefähr von der Länge der Fruchtkelche, durchschnittlich dürften sie an sibirischen etwas kürzer, an amerikanischen etwas länger sein, doch gibt es sowohl hier wie dort Ausnahmen von der Regel. Wenn Boissier als Unterschied angibt, dass *A. sibirica* im Gegensatz zu *A. labradorica* kahle Schäfte besitzt, so ist das vollends irreführend, da auch *A. labradorica* am häufigsten kahle Schäfte besitzt. Zweifellos sind also *A. sibirica* und *A. labradorica* in der gleichen Unterart zu vereinigen, die dann als *A. scabra* subsp. *labradorica* zu bezeichnen ist; »*A. labradorica* Wallr. 1844« ist nämlich älter als »*A. sibirica* Turcz.« ex. Boissier 1848. Boissier (1848) schreibt: (Turcz. in pl. Dahur. exs.), nach freundlicher Mitteilung von Dr. A. Bescherer, Genève, findet sich aber im Herbarium DC. keine Diagnose, und der Name *sibirica* ist nicht mit Turczaninoffs sondern mit Boissiers Handschrift geschrieben.

Die nordamerikanischen pleurotrichen Armerien sind neuerdings von Blake (l. z.) behandelt. Blake vereinigt sie (*A. arctica, A. sanguinolenta* und *A. andina β californica*) zur *Statice arctica* (Cham.) Blake, die er der holotrichen *Statice labradorica* (Wallr.) Hubbard & Blake entgegenstellt. Meiner Ansicht nach stehen aber die arktisch-borealen pleurotrichen Formen (*A. arctica*) der *A.*labradorica* näher als der pazifischen *californica*, welche von Boissier als Varietät von *A. andina* aufgefasst wurde. Die nordasiatischen

pleurotrichen Armerien schliessen sich ohne Lücke an Alaskas und so vereinigen wir sie in der subsp. *arctica*, die sich von der gewöhnlich kahlen *A.*labradorica* noch durch behaarte Blätter unterscheidet. Das Verbreitungszentrum dieser Unterart liegt am Bering Meer, im Norden geht sein Areal in das der *A.*labradorica* über. Auch ausserhalb des zusammenhängenden Areals der subsp. *arctica* werden noch im arktischen Sibirien gelegentlich pleurotriche Fruchtkelche angetroffen, und an der Grenze der beiden Unterarten finden sich Übergangsformen.

Die kalifornische *Armeria* ähnelt im Behaarungsmerkmal des Fruchtkelchs zweifellos der pleurotrichen Form von *A. scabra*. Schon habituell weicht sie aber bedeutend ab; alle Teile der Pflanze sind grösser und gröber, die Blätter sind breiter und verschmälern sich nicht gegen die Spitze, so wie es bei *A. scabra* fast immer der Fall ist; die Blattspitze ist sehr stumpf oder häufig ganz abgerundet. Das beste Unterscheidungsmerkmal liefern wohl die Blüten. Während die Kronblätter bei *A. scabra* klein sind und die Spitzen der Kelchblätter kaum überragen, sind sie bei der kalifornischen Form bedeutend grösser und ragen etwa ein paar Millimeter aus dem Blütenkopf hervor. Dazu kommen die sehr abweichenden klimatischen Anforderungen: *Armeria californica* wächst in einem gemässigten Regenklima, *A. arctica* ist arktisch-boreal. Will man nicht alle monomorphen Formen zu einer einzigen Art vereinigen, so muss *A. californica* von den arktischen Formen getrennt werden.

Die Systematik der südamerikanischen *Armeria* ist schwierig. Auf den Falklandsinseln findet sich eine anscheinend recht distinkte Form, die der kalifornischen *Armeria* nahe steht und habituell kaum von ihr zu unterscheiden ist; doch sind die Blüten kleiner. Auch die *Ar-*

meria der hohen Anden steht zufolge Boissier der kalifornischen Form sehr nahe. Diese drei Formen sind wohl am besten als geographische Varietäten oder Unterarten derselben Art aufzufassen, welche dann den Namen *A. macloviana* bekommt; dieser Name ist nämlich älter (1831) als *A. andina*.

Die zweite südamerikanische Art ist die sehr polymorphe *A. chilensis*. Von *A. macloviana* unterscheidet sie sich durch schmälere, mehr oder weniger zugespitzte Blätter und durch ihren abweichenden, weniger groben Habitus. Die Art tritt in verschiedenen Varietäten oder Ökotypen auf. Es gibt eine dürre Steppenform (*A. chilensis* s. str.) mit sehr schmalen, fast borstenartigen, sklerомorphen, steif aufrechten oder etwas gekrümmten Blättern, die getrocknet eine hellgraue Farbe annehmen. An den feuchten Gestaden der Magellan Strasse wächst die an *Armeria maritima* erinnernde var. *magellanica* mit schlafferen, getrocknet dunkleren Blättern und behaarten Blättern und Schäften. Breitblättrige Übergangsformen zu *A. macloviana* treten auf.

Boissier's *A. androsacea* soll sich durch holotriche Früchte von den andern südamerikanischen Arten unterscheiden. Das Merkmal versagt aber, die Behaarung des Fruchtkelches variiert weitgehend, es kommen holotriche Formen vor, die in andern Merkmalen *A. chilensis* mit ihren Varietäten oder *A. macloviana* mit ihren Varietäten vollständig entsprechen.

Wir erhalten demnach folgende Einteilung der Sektion *Monomorphæ*:

A. Die ganze Pflanze ist kräftig und grob. Blätter breit und derb, oben nicht verschmälert, Spitze abgerundet oder mindestens sehr stumpf. Kahl.
A. macloviana (Cham.) comb. nov.

Der Typus auf den Falklandsinseln. Krone klein.

subsp. *andina* (Poeppig), comb. nov. Hochgebirge der Anden. Polsterpflanze mit stark verlängerter Sprossachse.

subsp. *californica* (Boiss.), comb. nov. Pazifisches Nordamerika. Krone ziemlich gross, etwa 2 mm länger als der Kelch.

B. Blätter schmächtiger, oben verschmälert und meist mehr oder weniger zugespitzt.

I. Nördlich zirkumpolar. Krone klein, etwa von der Länge des Kelchs oder wenig länger.

A. scabra (Willd.) comb. nov.

subsp. *arctica*. (Cham.) nov. comb. (syn. *A. sanguinolenta* Wallr.). Fruchtkelch pleurotrich. Blätter typisch behaart. Arktisch-boreal. Alaska—Ostsibirien mit Kamtschatka.

subsp. *labradorica* (Wallr.) nov. comb. (syn. *A. sibirica* Turcz.). Fruchtkelch holotrich, Blätter selten behaart. Hocharktisch-zirkumpolar.

II. Südamerika. Krone klein, etwas länger als der Kelch. Meeresstrand und Hügelland im südlichen Chile und Argentinien.

A. chilensis Boiss.

Polymorph. Wichtig ist var. *magellanica*: Blätter und Schäfte behaart. Magellan-Länder.

c. *Armeria vulgaris* Willd.

Wie viele Arten etwa man innerhalb der Sektion *Dimorphae* unterscheiden kann, ist vorläufig noch ganz unklar,

zweifellos lässt sich aber nur ein Bruchteil von Boissiers Arten aufrechterhalten; eine neue monographische Bearbeitung wäre sehr erwünscht.

Im nördlichen Europa unterscheidet Boissier 5 Arten. *Holotrichae: A. maritima, A. pupigera. Pleurotrichae: A. pubescens, A. elongata* und *A. Halleri.* Diese und andere Formen dürfen als Varietäten oder Ökotypen aufgefasst werden, jedenfalls gehen sie völlig ineinander über und gehören deshalb zur gleichen Art (siehe Gams 1927, Turesson 1922, Willy Christiansen 1932).

Den besten Überblick über den grossen Formenkreis der *Armeria vulgaris* erhält man, wenn man ihn vom ökologischen Gesichtspunkt aus betrachtet. Gemeinsam für alle Arten der Gattung *Armeria* ist ein Bedürfnis: sie müssen infolge ihres ganzen Aufbaus an lichtoffenen Orten wachsen, sie vertragen keine Beschattung. Daher findet sich *Armeria* nur dort, wo Wald oder anderer dichter Pflanzenwuchs fehlt, — ob das nun durch ungünstiges Klima, hohen Salzgehalt im Boden, periodische Überschwemmungen oder menschliche Kultureingriffe bedingt ist.

Der Hochgebirgstypus wächst oberhalb der Waldgrenze und ist eine Polsterpflanze mit kurzen, breiten und derben Blättern. Hierher gehört *A. alpina* aus der alpinen Region Mittel- und Südeuropas. Sie ist der geographisch am besten isolierte Typus und wird daher gern als gute Art angesehen. Ein breiter Waldgürtel trennt sie von andern Wuchsorten der *Armeria.* Nur am Fuss der Pyrenäen, an der atlantischen Küste, besteht die Möglichkeit der Mischung mit Strandformen, und gerade hier findet man denn auch Übergangsformen zur *A. vulgaris* var. *maritima.*

Eine habituell sehr ähnliche, breitblättrige, alpine Form

findet sich im Hochgebirge Islands. Sie wird gewöhnlich zur *A. maritima* gerechnet, weil Schaft und Blätter behaart sind; doch handelt es sich um einen ausgeprägten Hochgebirgsökotypus. Geographisch ist sie von der Strandform weniger gut isoliert als *A. alpina*, es fehlt der trennende Waldgürtel; Zwischenformen sind daher häufiger. Diese Rasse verdient grössere Aufmerksamkeit, weil sie ein Bindeglied zwischen *A. vulgaris* und *A. alpina* darstellt. In den schottischen Hochgebirgen wächst offenbar dieselbe alpine Rasse (Herbariumexemplar, etikettiert: Ben Lawers at 3000 ft. Perth: Scotland. 7. 88. C. A. Bennet). Es scheint, dass *Armeria vulgaris* var. *planifolia* Syme sich auf diese Form bezieht (vgl. Druce 1903), weshalb auch die isländische alpine Pflanze als var. *planifolia* zu bezeichnen ist. Auch auf den Färöer Inseln findet sich diese Varietät; und es ist interessant, dass, nach freundlicher Mitteilung von Dr. Tyge Böcher, diese alpine Form früher blüht als die Strandform auf den Färöern. Druce gibt nämlich ebenfalls an, dass *A. planifolia* 3 Wochen früher blüht als der »Typus«. Der Unterschied zwischen *A. alpina* und *A. vulgaris* var. *planifolia* besteht namentlich darin, dass erstere typisch kahl ist, während letztere jedenfalls auf den Blättern Haare trägt. Das Merkmal ist aber nicht immer stichhaltig. Von 22 untersuchten *A. alpina*-Exemplaren aus den Pyrenäen besassen 4 behaarte Blätter. Auch ist der Schaft bei *A. alpina* nicht immer kahl und bei var. *planifolia* nicht immer zottig. Der Fruchtkelch ist bei der schottisch-isländischen Hochgebirgsrasse bald pleurotrich, bald holotrich. *A. alpina* ist gewöhnlich pleurotrich, doch hebt Gams (1927) hervor, dass auch holotriche Individuen vorkommen. Es finden sich demnach keine durchgehenden Unterschiede zwischen *A. alpina* und *A. vulgaris* var. *planifolia*, und es ist deshalb

vielleicht richtiger, *alpina* als Unterart in den Formenkreis von *A. vulgaris* einzubeziehen.

Var. maritima, der Typus des Meeresstrandes, hat niedrige, behaarte Schäfte und kurze, schmale, graue und etwas sukkulente Blätter (Blattanatomie siehe Turesson 1922). Das Wurzelsystem ist ziemlich schwach. Die Hüllblätter sind stumpf oder mit kurzer aufgesetzter Spitze (Abbildungen bei Christiansen 1932). Diese Rasse ist ausgeprägt halophil und gleichzeitig die am meisten atlantische von allen *A. vulgaris*-Formen (vgl. Christiansen 1932).

Var. *elongata* wächst auf trockenem Sandboden. Sie hat tiefgehende Wurzeln, hohe, schlanke und kahle Schäfte, dünne, etwas verbreiterte Blätter und ist die kontinentalste Rasse der sonst überwiegend atlantischen *A. vulgaris* (vgl. Christiansen l. z.). Die Verbreitung dieser Form ist durch die menschliche Waldrodung stark begünstigt worden. Sie ist zweifellos eine sehr junge Rasse, die entweder aus der var. *maritima* (Ruhland 1915) oder aus der var. *alpina* (Gams 1927) hervorgegangen ist. Im ersten Falle sind die binnenländischen Vorkommnisse nach der menschlichen Waldrodung vom Meeresstrande aus, im anderen Falle von den Alpen aus besiedelt worden. Beide Möglichkeiten haben viel für sich.

In Dänemark finden sich hauptsächlich Übergangsformen zwischen den Varietäten (Ökotypen) *maritima* und *elongata* (Iversen 1936), man hat sie als var. *intermedia* zusammengefasst. Falls *A. elongata* — wie Gams (l. z.) annimmt — aus der *A. alpina* hervorgegangen ist, so ist var. *intermedia* als Kreuzungsprodukt zwischen der var. *maritima* und der var. *elongata* anzusehen. Besteht Ruhland's Auffassung zurecht (siehe oben), so ist *A. elongata*

dagegen aus dem Formenkreis der *A. intermedia* hervorgegangen.

Turesson (1927) hat eine Reihe von *Armeria*-Populationen statistisch untersucht, woraus sehr instruktiv hervorgeht, wie sich der Formeninhalt von der schwedischen Westküste zur Ostseeküste und zum Binnenland nach und nach ändert.

Äusserst spezialisiert sind die Ökotypen var. *Halleri* und var. *purpurea* (*A. alpina* var. *purpurea*). Die erstere steht der var. *maritima* nahe und findet sich an Stellen, wo jahrhundertlanger Bergbau einen an Schwermetallen reichen Boden geschaffen hat und wo der Wald nicht gedeihen mag (siehe Gams 1927 und Christiansen 1932). Die var. *purpurea* endlich hat sich den speziellen Verhältnissen am Bodensee angepasst. Sie wächst dort in der im Sommer wasserbedeckten, im Frühjahr und Herbst aber sehr trockenen Strandzone, wo nur einige speziell angepasste Formen zu wachsen vermögen (Baumann 1914).

Alle diese Formen gehen, wie gesagt, ineinander über. Dagegen fällt *Armeria *labradorica* im nördlichen Norwegen und in Grönland vollständig ausserhalb des Formenkreises von *Armeria vulgaris*. Es finden sich keinerlei Übergangsformen, im Gegenteil, hier wo *A. vulgaris* mit *A. *labradorica* zusammenstösst, ist der Unterschied zwischen ihnen besonders auffällig. *A. vulgaris* ist in Nordnorwegen und Grönland eine halophile Küstenpflanze, während *A. *labradorica* in der öden Felsenheide (»Fjeldmark«) zu Hause ist. *A. vulgaris* tritt im nördlichen Norwegen und an der grönländischen Nordgrenze (Dunholmene) in einer sehr niedrigen, stark behaarten Form auf, während *A. *labradorica* daselbst hochwüchsig und fast kahl ist. Die Blütenblätter sind bei der *A. vulgaris* auch an der Nordgrenze

viel grösser als der Kelch, während die Krone von *A. *labradorica* klein und unansehlich ist. Trotzdem das beste Unterscheidungsmerkmal — die Dimorphie bei *A. vulgaris* — bisher unbekannt war, haben die norwegischen Botaniker die »*A. sibirica*« stets richtig bestimmt. Auch Kolderup Rosenvinge, der die grönländische Vegetation aus eigener Anschauung kannte, hatte eine richtige Auffassung von der Grenze zwischen *A. vulgaris* und »*A. sibirica*«, was seine stichhaltigen Bestimmungen beweisen. Spätere »Revisionen« des grönländischen Materials haben dagegen Verwirrung hervorgebracht, die scharfe Grenze wurde verwischt. Unglücklicherweise wurden auch *A. vulgaris*-Formen auf den Färöern und anderorts zu *A. »sibirica«* gestellt, und so ist die falsche Auffassung entstanden, dass diese Arten an ihren Grenzen ineinander übergehen.

d. Verzeichnis des untersuchten *Armeria*-Materials.

Blüten monomorph:

1. Armeria scabra subsp. **labradorica.**

Arktisches Europa.

Norwegen: *Troms Fylke:* Maalselven: Langfjeldene (J. N.V.); Dödesfjeldet (Holmboe 1914); Storefjeldet (Holmboe 1914); Mauken (Holmboe 1915); Rostafjeld (E. Taylor 1907). Lyngen: Birtavarre (Haglund & Källström 1899, Notö 1897); Paratind (Holmboe 1914); Vestre Rappovarre (Holmboe 1914); Østre Rappovarre (Holmboe 1914); Nuorttavagge (Sam. Mårtenson 1914); Lulle (Holmboe 1911); Favresvarre (Holmboe 1914); Storfjord (Notö 1909); Agjeg (Holmboe 1905); Nordreisen: (A. Blytt 1891; A. Notö 1896); Venevarre (A. E. Fridtz 1898, Notö 1904); Gatbovarre (A. Sandmark 1917); Javroaivpe (Peter & Selander 1906). *Finmarken:* Vardöe, Svartnæs (Th. M. Fries 1864; Sommerfelt 1858). Rörselv Porsangriæ (J. M. Normann); Silbatjokka v. Skoganvarre (R. Nordhagen 1934).

Kola: *Lapponia murmanica:* Semostrow (Brotherus 1887). *Lapponia varsugae:* Tshavanga (Kihlman 1869).

Waigatsj: South Shore W. (N. W. I. 1897); Cap Grebenij (Kjellman & Lundström 1875).

Arktisches Nordasien.

Uralia polaris: distr. superiore Sob, Jeletzky road (Gorodkov 1924).

Jennisseisk: Galtschicha 71° N. (A. Tolmatchew 1926); Insula Nasonowski ostrow 71° N. (Enander 1914); Dudinka (H. W. Arnell 1876); fl. Boganida 71°10′4″ (Exp. Sibir. Acad. 1843); Taimyr 73°30′4″ (Exp. Sibir. Acad. 1843).

Jakutsk: Ad fl. Olenek et Lenam infer. (Czekanowski 1875); Bulkur pr. 72° (N. H. Nilsson 1898).

Bering Strasse: Pitlekaj 67°0′5″ N., 173°24′ W.[1].

Zentralasien.

N-Mongolei und Changai: Mongoi-dabosch (Ikonnikov-Galitzky 1926).

Arktisches Nordamerika.

Alaska: Tigara near Point Hope[2] (Knud Rasmussen 1924).

North West Territories: Orpigtujaq isl. in Baker Lake (Birket Smith 1922, Bangsted 1924), Bernard Harbour 68°47′ N. 114°46′ W. (Frits Johansen 1915); Chesterfield (P. Freuchen 1923); Chesterfield Inlet 63°40′ 93°0′ W. (M. O. Malte 1928); Repulse Bay (Parry 1821).

Baffin Land: Cape Dorset 64°10′ N. 76°30′ W. (M. O. Malte 1928); Eclipse Sound east of Mitimatalik (P. Freuchen 1924); Mitimatalik, Ponds Inlet (Th. Mathiassen 1923). Lake Harbour 62°49′ N. 69°55′ W. (M. O. Malte 1927).

King William Land: Malerualik (Knud Rasmussen 1923); Gjøa Harbour 68°38′ N. 96°26′ W. (Godfred Hansen 1904).

Ellesmereland: 76°23′ N. 81°30′ W. (H. G. Simmons 1899).

North Devon Island: Dundas Harbour 74°33′ N. 87°17′ W. (M. O. Malte 1927).

Halbinsel Labrador: Port Burwell, Hudson Strait (J. M. Macoun 1910; M. O. Malte 1927; Frits Johansen 1927). Head of Nachwak Bay, Torngate Region (Woodworth 1926). Wakeham Bay, Hudson Strait (Frits Johansen 1927, M. O. Malte 1928); Erik Cave. Cape Wolstenholm (Frits Johansen 1927). Labradoria rev. Heldenberg (Dr. Barth 1892). Labrador 1840.

[1] Übergang zur var. *arctica*: pleurotriche und holotriche Individuen gemischt. Blätter spärlich behaart — kahl.

[2] Übergang zur var. *arctica*. Holotrich, aber Blätter behaart.

Quebec: Gaspé County. Mt. Albert 900—1050 m. (J. F. Collins and M. L. Fernald 1905).

Newfoundland: Region Bay of Islands, Blomidon Mountains (M. L. Fernald and K. M. Wiegand 1910). Straits of Belle Isle, Savage Point (M. L. Fernald u. a. 1925).

Nord-Grönland: *Adam Bierings Land* (P. Freuchen 1912). *Thule Distr.* Inglefield Bay (G. Olsen 1917); Murchison Sound, Mac Connich Bay 77°40′ (J. Noe Nygaard 1921); Thule (Lauge Koch 1916).

West-Grönland: *Upernivik Distr.* Toppen af Tassiusak 73°27′ (Ryders Eksp. 1887); Ingnerit Fjord 72°3′ (M. P. Porsild 1911). *Umanak Distr.* Umanatsiak (1843). *Godhavn Distr.* Ekigtok (M. P. Porsild 1898). *Godhavn* (M. P. Porsild 1939). *Ritenbek Distr.* Disko. Vajgatskysten, Iquagnak (M. P. Porsild 1902) Atanikerdluk (N. Hartz 1890). *Jakobshavn Distr.* Pakitsok 1833, *Christianshaab Distr.* Colon. Christianshaab. Kunguak. 1835. Kunguak in Tasiursak 69°3′ (S. Hansen 1888). *Egedesminde Distr.* Egedesminde (R. Brown 1867); *Holsteinborg Distr.* Tiggak i Ndr. Strømfjord (P. H. Sørensen 1894); Holsteinborg (Deichmann 1909); Sarfanguak (Warming-Holm 1884); Itivnek i Ikertok Fjord (Warming-Holm 1884); Ungoriarfik, N. Strømfjord (Kornerup 1879). *Godthaab Distr.* Zwischen Kapisilik und Kangersunek 64°30′ N. (Johs. Iversen 1938).

Ost-Grönland: *Scoresby Sund*: Moskusoksefjorden 73°44′; NW-Küste von Loch Fyne 73°55′; Westküste von Ymer Ø 73°20′; NW.-Küste von Clavering Ø 74°25′ (Seidenfaden 1929); Sabine Ø 74°50′ (C. Kruuse 1900); Danmarks Havn (Lundager 1908); Hvalrosodde (Lundager 1908); Ella Ø (Th. Sørensen 1932). *Angmagsalik Distr.* Tasiusak nahe Angmagsalik (C. Kruuse 1902; Bøgvad 1920; M. Jørgensen 1933).

2. **Armeria scabra** subsp. **arctica.**

Nordasien.

Tschuktschen-Halbinsel: St. Lawrence Bay, 65°30′ N., 171° W. (Kjellman 1879); *Kamtschatka*: Klutschenskaja volcano (R. Malaise 1927); Lopatka[1] (Rieder).

Nordamerika.

Alaska: Sin. Schischmaren (Cham. 1816); Port Clarence 65°5′ N. 166° W. (Kjellman 1879); Priblof islands: St. Paul (E. Hultén 1932); Kadiak Island, Vicinity of Karluk (Cloudsley Rutter 1903).

[1] Fruchtkelche zwischen den Rippen schwach behaart.

3. Armeria macloviana subsp. **californica.**

British Columbia: British Columbia (Wm. Scott 1893); Vancouver Island, Distr. of Renfrew (Rosendal, J. Brand 1901); Vancouver Island, vic. of Victoria (J. Macoun 1893).

Washington: Marysville (J. M. Grant 1928).

Oregon: By the sea, Chetko (Th. Howell 1884).

California: Santa Cruz (Johs. Clausen 1928); Marin Co. Point Reyes (H. P. Bracelin 1930); Pacific Grove (A. D. E. Elmer 1903; Skjøt Petersen 1928).

4. Armeria macloviana subsp. **andina.**

Prov. Nuble. Bänas de Chillan, Aguas Calientes. alt. ca. 2200 m. (E. Werdermann 1927); Lago Nahuel Huapi, Sierra Lopez nordsluttning (E. Ljungner 1933); Patag. andina, Zeballos-Gio-Passet (C. Skottsberg 1908).

5. Armeria macloviana s. str.

Falklands-Inseln: West Falkland. Roy Cove (C. Skottsberg 1907);

I. de los Estados: Puerto Cook (Castellanos 1934); Puerto St. Juan (Castellanos 1934).

6. Armeria chilensis.

Distr. Chubut: Valle Koslowsky (C. Skottsberg 1908).

Distr. Santa Cruz: In campo suffruticosa ad prædium Richmond (Durén 1905). Tchuelchen, 250 m (A. Donat 1928); Tør Mark. Lat. S. 49°4′ Long. W. 72°12′. 205 m (J. Högberg 1901).

Distr. Magallanes: Inter Eberhardt et R. Tres Pasos (O. Borge 1899); Prope Sandy Point (W. R. F. Hohenacker);

Tierra del Fuego: Canal Beagle, allm. Klippstrand (M. Gusinde 1922).

Blüten dimorph.

Folgende Arten oder Varietäten sind untersucht worden: *A. allioides, A. alpina* mit var. *purpurea, A. alpinifolia, A. berlengensis, A. cæspitosa, A. cariensis, A. choulettiana, A. duriæi, A. fasciculata, A. gaditana, A. juncea, A. maderensis, A. majellensis, A. mauritanica, A. nebrodensis, A. plantaginea, A. rumelica, A. splendens, A. undulata, A. vulgaris* mit ihren Varietäten var. *elongata*, var. *halleri*, var. *maritima*, var. *planifolia.*

Folgende grönländische Fundorte gehören zur *A. vulgaris:*

Süd-Grönland: Tumia 59°55′ (Eberlin 1885); Kematuisalik, Kitsigsut Øerne ca. 60° (A. Jessen 1894); Øen Akia ved Julianehaab (G. Meldorf 1899); Na-ah. Torsukatak (1829); Ilua (Lundholm 1889); Ataneritsok, Igaliko (Rosenvinge 1888); Sermilik, Itivdlersuak Bugt (Bøgvad 1932); Nunarsuak 60° (Lindholm 1890); Eqaluit, Igaliko Fj. 60°47′ (A. E. Porsild & M. P. Porsild 1925); Cap Farvel (Sylow 1881); Sisardlugtok i Igaliko Fj. (C. Petersen 1880); Narsak (Rosenvinge 1888); Ivigtut (Lindhard 1899).

Ost-Grönland: Dunholmen, 69°55′ (O. Hagerup 1924).

IV. Über die biologische Bedeutung von Blütendimorphie und Parasterilität.

Die Blütendimorphie bei *Armeria* ist ja eine Erscheinung die sehr an die Dimorphie der heterostylen Pflanzen erinnert; nur die traditionelle Definition des Heterostylie-Begriffs hindert uns daran, *Armeria* als heterostyl zu bezeichnen. Seit Darwin (1862) ist die Frage nach der Bedeutung (dem »Zweck«) der Heterostylie dahin beantwortet worden, dass durch sie eine Selbstbestäubung verhindert, die Fremdbestäubung dagegen stark gefördert wird; in neuester Zeit wurde jedoch die Richtigkeit dieser Deutung bezweifelt (Ubisch 1925).

Auf die Blütendimorphie von *Armeria* ist diese Erklärung nicht anzuwenden. Selbstbefruchtung ist infolge der vorhandenen Selbststerilität ausgeschlossen, und es ist nicht einzusehen, wie eine Förderung der Fremdbestäubung durch die Dimorphie stattfinden sollte. Vielmehr ist die Dimorphie als eine an und für sich ganz bedeutungslose Begleiterscheinung der physiologischen Differenzierung anzusehen. Es verhält sich offenbar hier so wie oft in der Natur, wo bedeutungslose morphologische Merkmale (Zähnelung der Blätter, Behaarung u. s. w.) mit ent-

scheidenden physiologischen Unterschieden gekoppelt sind.

Falls dies richtig ist, bedeutet die Dimorphie nur, dass sich das Vorhandensein zweier physiologisch verschiedener Linien bemerkbar macht. Danach ist es gerechtfertigt, diese physiologische Seite des Problems in den Vordergrund des Interesses zu rücken.

Die Bedeutung der physiologischen Differenzierung ist bei *Armeria* natürlich dieselbe wie bei anderen selbststerilen Pflanzen. Bei mehr oder minder ausgeprägter Selbststerilität muss man immer das Vorkommen mehrerer konstitutionell verschiedener intersteriler Linien annehmen (Correns 1912), die sich allerdings in der Regel morphologisch nicht unterscheiden lassen.

Der Sinn dieser merkwürdigen, im ganzen Pflanzenreich so häufigen Einrichtungen ist offenbar folgender: Die aufgezwungene oder jedenfalls ungemein geförderte Kreuzung bewirkt, dass die vielen Gene, welche die Art besitzt, beständig umgruppiert werden. Unter der grossen Zahl von Samen, die hervorgebracht werden, sind kaum zwei vollkommen gleich hinsichtlich ihrer Erbanlagen. Die Art ist deshalb sozusagen geschmeidig, sie vermag sich den Veränderungen im Wuchsort, Klima u. s. w. anzupassen, immer verfügt sie über neue Kombinationen von Erbanlagen, unter denen die natürliche Zuchtwahl die jeweils besten auswählt.

Der Formenkreis von *Armeria vulgaris* ist ein typisches Beispiel hierfür. Auf Skallingen z. B. wachsen Individuen mit kahlen und behaarten Schäften, von hohem und niedrigem Wuchs, mit pleurotrichen und holotrichen Fruchtkelchen u. s. w., Seite an Seite und spotten wegen ihrer freien Merkmalkombinationen allen Klassifikationen. Die ökologische Amplitude des Formenkreises ist ausser-

ordentlich gross: *Armeria* wächst in der nassen, stark salzigen Marsch so gut wie im trockenen, ausgelaugten Dünensande. Nun ist es höchst unwahrscheinlich, dass dieselbe Pflanze unter so extrem verschiedenen Bedingungen gedeihen kann, und man muss deshalb eine physiologische Variabilität annehmen, die der morphologischen entspricht, die aber trotz ihrer Unanschaulichkeit für die Pflanzenart und damit auch für das Verständnis unseres Problems viel wichtiger ist. Auf Skallingen bilden alle diese morphologischen und physiologischen Varianten eine einzige grosse Familie; in der Marsch wie auf der Düne findet man dieselben morphologischen Merkmalkombinationen, wenn sich auch die statistische Häufigkeit der Merkmale allmählich von der Marsch zu Düne hin ändert. Zweifellos sind alle Formen, auch die physiologischen Extreme — etwa die Marschform — stark heterozygotisch und spalten deshalb morphologisch und physiologisch sehr verschiedenartige Typen ab.

Eine »homotypische«, selbstfertile Art tendiert dagegen zur Homozygotenbildung. Dadurch werden viele Rassen gebildet, die jede für sich bestimmten Umweltbedingungen angepasst ist. Da die Diasporen einer solchen Rasse genau die gleichen speziellen Typen hervorbringen wie die Eltern es waren, ist eine Population aus solchen Pflanzen nicht anpassungsfähig, wenn die Umweltbedingungen sich ändern.

Ein Formenkreis von Selbstbestäubern erstarrt sozusagen in isolierten Sippen, während ein kreuzender sich ständig erneuert. Das muss sich auch in dem Formeninhalt der Populationen eines Selbstbestäubers ausdrücken.

Thorvald Sørensen (1938) hat in einer sehr interessanten Arbeit über Variation und Vererbungsverhältnisse bei *Ranunculus auricomus* darauf aufmerksam gemacht,

dass die Variabilität bei den Ranunkelarten von verschiedenem Charakter ist. Während z. B. *Ranunculus acer* die gewöhnliche binomiale Variabilität hinsichtlich verschiedener Merkmale aufweist, ist die Variation bei der sich hauptsächlich vegetativ vermehrenden *Ranunculus repens* und bei der pseudogamen *Ranunculus auricomus* sprungweise, d. h. die Individuen innerhalb eines Bestandes gehören entweder zu derselben Form oder sie können in mehrere verschiedene, scharf trennbare Formen unterschieden werden. Ungefähr ebenso — wenn vielleicht auch kaum so ausgeprägt — muss sich auch die Variabilität eines Selbstbestäubers verglichen mit der eines Fremdbestäubers verhalten. Leider verfüge ich nicht über das notwendige Beobachtungsmaterial, um die Variabilität der monomorphen und dimorphen Armerien an ihren Standorten vergleichen zu können.

V. Ist Monomorphie oder Dimorphie das Ursprüngliche bei *Armeria*?

Wie schon erwähnt, fasse ich Monomorphie und Selbstfertilität bei *Armeria* als eine sekundäre Erscheinung auf; ich tue es auf Grund folgender Beobachtungen und Erwägungen:

1. Die dimorphen Arten sind selbststerile Insektenbestäuber, die monomorphen selbstfertile Selbstbestäuber. Da der ganze Blütenbau bei den *Plumbaginaceen* auf Insektenbestäubung eingerichtet ist, müssen die Selbstbestäuber aus Insektenbestäubern abgeleitet werden.

2. Die Blüten der monomorphen Arten scheinen — verglichen mit dimorphen — in gewisser Hinsicht reduziert zu sein. Im Verhältnis zu den schlanken und eleganten Griffeln bei *Armeria vulgaris* verbleiben die Griffel bei den

monomorphen Armerien gewöhnlich merkwürdig embryonisch und plump, mit schwach entwickeltem Haargürtel (vgl. Fig. 3 und 4 mit Fig. 7). Wie erwähnt ist eine sehr früh erfolgte Selbstbefruchtung als Ursache hierfür anzusehen; infolge der Protandrie und Selbstfertilität kann Selbstbefruchtung schon vor der normalen Entfaltung der Griffel eintreten. Nach erfolgter Befruchtung wird die weitere Entwicklung der Griffel abgebrochen, die Blütenblätter kräuseln sich und ziehen sich ein. Eine südamerikanische Pflanze bildete eine überraschende Ausnahme. Sie zeichnete sich durch schlanke, wie bei *A. vulgaris* aus den Blüten weit herausragende Griffel aus. Ich vermutete endlich eine dimorphe amerikanische *Armeria* gefunden zu haben. Eine nähere Untersuchung ergab aber, dass die Staubbeutel steril waren. Aus diesem Grunde hatte keine Selbstbestäubung stattfinden können, die Griffel hatten ihre normale — bei den monomorphen Armerien sonst unterdrückte — Gestalt angenommen. Das deutet darauf hin, dass die Selbstfertilität — die mit der Monomorphie zusammenhängt — sekundären Ursprungs ist.

3. Nicht nur *Armeria*, sondern auch die nächstverwandten Gattungen (*Statice* und *Limoniastrum*) besitzen genau dieselbe Pollen- und Griffeldimorphie (vgl. Fig. 8 und 9), und auch die Gattung *Acantholimon*, die im Griffelbau bedeutend abweicht, hat genau dieselben beiden Pollentypen. Offenbar ist diese Dimorphie ein gemeinsames Merkmal der ganzen Unterfamilie *Staticoideae*, nur innerhalb *Armeria* habe ich Monomorphie beobachtet. Daraus darf man wohl schliessen, dass schon die Stammform der erwähnten Gattungen dimorph gewesen ist.

4. Das Verbreitungszentrum von *Armeria* ist das mediterran-lusitanische Gebiet. Hier findet sich die Mehrzahl

der Arten, und von hier aus hat sich die Gattung ausgebreitet (vgl. Maury 1886). Alle untersuchten Arten dieses Gebiets sind aber dimorph (siehe S. 26). Monomorph sind nur einige einander nahestehende Arten, die fern ihrer ursprünglichen Heimat neue Gebiete besiedelt haben. Falls Monomorphie das primäre wäre, müsste man annehmen, dass sich monomorphe Relikte in der ursprünglichen Heimat — etwa in isolierten Hochgebirgen — erhalten hätten.

5. Die Entstehung der selbstfertilen monomorphen *Armeria* lässt sich als Anpassung an den Insektenmangel in der Arktis zwanglos erklären. Wie in einer folgenden Arbeit eingehend dargelegt werden soll, ist Alaska als Ausgangspunkt der monomorphen *Armeria* anzusehen. Hier ist sie offenbar mutativ entstanden und hat — dank ihrer Selbstfertilität — in dem Refugium bei Alaska als einzige *Armeria* die Eiszeit überdauert, worauf sie sich nach Süden, Osten und Westen ausgebreitet und neue Arten gebildet hat, die aber selbst in insektenreichen Gebieten die einmal eingebüsste Parasterilität nicht wieder zurückbekommen.

In einer jüngst erschienenen Abhandlung über dimorphe (heterostyle) und monomorphe (homostyle) *Primula*-Arten der Sektion *Candelabra* diskutiert Ernst (1938) eingehend die phylogenetischen Probleme. Frühere Untersuchungen von Ernst hatten zu der Hypothese geführt, dass für den Formenkreis der Gattung *Primula* eine selbstfertile, monomorphe Stammform anzunehmen ist, aus der dann durch mutative Abänderungen die heterostylen Formen entstanden seien. In seiner neuen Arbeit weist Ernst nun innerhalb der Sektion *Candelabra* eine Reihe solcher monomorphen Arten nach, und fasst sie entsprechend seiner Theorie als relativ ursprüngliche »phylogenetische Relikte« auf.

Dass die dimorphe Blütenplastik letzten Endes aus einer

monomorphen abzuleiten ist, sehe ich auch als höchst wahrscheinlich an. Die Monomorphie der jetzigen monomorphen Armerien ist aber, wie ich zu beweisen versucht habe, keine primäre sondern eine abgeleitete Erscheinung. Die Entstehung der Dimorphie geht bei *Plumbaginaceen* sicher weit zurück. Eine andere Frage ist es natürlich, ob dasselbe auch bei *Primula* der Fall ist.

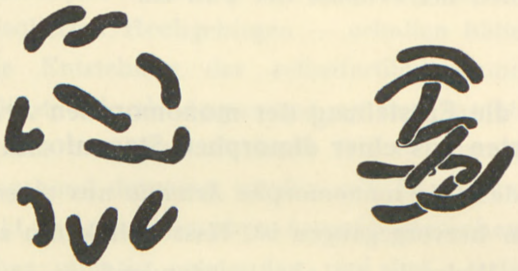
VI. Über die Entstehung der monomorphen *Armeria*-Arten aus einer dimorphen Stammform.

Daran dass die monomorphe *Armeria* aus einer dimorphen Form hervorgegangen ist, lässt sich kaum zweifeln; auf welche Weise ist sie aber entstanden?

Der Gedanke lag nahe, dass eine Chromosomenverdopplung zu Grunde lag. Dann würde die monomorphe *Armeria* eine Parallele zu *Empetrum hermaphroditum* darstellen, die ja nach Hagerups (1927) Untersuchungen tetraploid ist. *Empetrum hermaphroditum* ist zwitterig geworden anstatt zweihäusig, d. h. monomorph anstatt dimorph.

Um die Frage entscheiden zu können, habe ich Herrn Professor Rolf Nordhagen, Bergen, und Herrn Direktor M. P. Porsild, Disko, gebeten, mir lebendes bezw. fixiertes Material von *Armeria *labradorica* aus Grönland bezw. Lapland zuzusenden. Beide Herren waren so liebenswürdig meinen Wunsch zu erfüllen. Vergleichsweise bekam ich fixiertes Material der beiden Linien von *Armeria vulgaris* von meinen beiden gepflanzten Beständen auf Als. Dr. Olaf Hagerup übernahm freundlichst die zytologische Untersuchung und stellte fest, dass die arktische *Armeria* dieselbe Chromosomenzahl besitzt wie die heimische (siehe Fig. 1).

Aus Hagerups Untersuchung geht also hervor, dass die Monomorphie bei *Armeria labradorica* nicht durch Chromosomenverdopplung entstanden ist. Dies steht in Einklang mit den Ausführungen von Ernst (1938) betreffs der monomorphen *Primula*-Arten: »es kann«, sagt Ernst (S. 217), »von einer sekundären Entstehung des Blüten-



a. b.

Fig. 1 a. *Armeria vulgaris*, B Linie. $^{20}/_{5}$ 39. n = 9. I. Teilung in der Mikrospore. × 1920. b. *A.*labradorica*. Disko 1939. I. Teilung in der Mikrospore. × 1920 (O. Hagerup).

monomorphismus als Folge einer Chromosomenverdopplung in der *Candelabra*-Sektion keine Rede sein.«

Die monomorphe *Armeria* muss, da keine Chromosomenverdopplung vorliegt, offenbar durch mutative Änderung einer A- oder B-Linie entstanden sein. Man könnte nun erwarten, dass experimentelle Untersuchungen diese Frage beleuchten würden. Das scheint in der Tat der Fall zu sein. Bei Kreuzungsversuchen zwischen monomorphen (*Armeria *labradorica* aus Finmarken) und dimorphen Pflanzen (*A. vulgaris* var. *maritima*, Dänemark) konnte festgestellt werden, dass die Pollenkörner der monomorphen Pflanzen wohl in die eigenen Narben und in die Narben der B-Pflanzen, nicht aber in die der A-Pflanzen hineinwachsen konnten. Der Pollen der monomorphen Pflanzen entspricht also physiologisch —

wie auch morphologisch — dem Pollen der A-Linie der dimorphen *Armeria*. Anderseits konnte ausser dem eigenen Pollen sowohl der B-Pollen als auch der A-Pollen im Griffel der monomorphen Pflanze wachsen. Diese Beobachtung lässt sich am einfachsten folgendermassen erklären: Im Fruchtknoten der A-Pflanzen wird ein Hemmstoff gebildet, der die Keimung der A-Pollen hemmt, während ein anderer Hemmstoff in den B-Griffeln das Keimen der B-Pollenkörner hemmt[1]. Bei der selbstfertilen monomorphen *Armeria *labradorica* fehlt sowohl der eine wie der andere Hemmstoff, und man kann daher annehmen, dass die monomorphe *Armeria* ursprünglich aus einer A-Pflanze entstanden ist, indem die Bildung des Hemmstoffes im Griffel unterdrückt worden ist.

VII. Schluss.

In der vorliegenden Arbeit ist nur ein Teil der Probleme, die mit Dimorphie und Monomorphie bei *Armeria* zusammenhängen, eingehender behandelt worden. Die phylogenetischen Probleme sind im vorigen Abschnitt aufgeworfen worden, eine gründliche Bearbeitung auf geologischer und pflanzengeographischer Grundlage bleibt aber einer folgenden Arbeit vorbehalten. Die physiologisch-genetische Seite der Problemstellung konnte diesmal auch nur kurz berührt werden. Die notwendigen experimentellen Untersuchungen sind im Gange, können aber, da mehrere Generationen von Pflanzen erforderlich sind, erst nach einigen Jahren fertig werden.

[1] Das Vorhandensein eines solchen Hemmstoffes wurde von Yasudo (1932) im Griffel der selbststerilen *Petunia violacea* nachgewiesen.

Summary.

1. We find a marked pollen- and style-dimorphism in *Armeria vulgaris* and the closely related genera, *Statice* and *Limoniastrum* (Figs. 2—3 and 8—9).

2. Both types (line A and line B) are completely parasterile, since the A-pollengrain can only grow in the B-stamen while the B-pollengrain grows but in the A-stamen (Figs. 2—5). This observation has been confirmed in culture experiments.

3. The arctic *Armeria*, however, is monomorphic and self-fertile. The style stands nearest to line B, the pollengrains approach mostly line A, but both of them are somewhat intermediary (Figs. 6—7).

4. According to Dr. O. Hagerup's analysis, the dimorphic *Armeria vulgaris* has the same number of chromosomes as the monomorphic *Armeria *labradorica* (Fig. 1).

5. According to the typical features of dimorphism and monomorphism, respectively, the very homogeneous *Armeria* can be separated into two distinctly different groups, *Dimorphae* and *Monomorphae*.

6. The species growing in the European-mediterranean area are dimorphic; in South America, North America and North Asia, however, only monomorphic species are to be found (Figs. 10—11).

7. An attempt has been made to give a new classification of the species within the monomorphic group (see p. 13—18). The different forms of *Armeria vulgaris* Willd. are discussed.

8. Parasterility is important because of its promoting influence on the mixing of genes; the variability of *Armeria vulgaris* is in agreement with this interpretation. Dimorphism in itself, however, is presumably without any importance.

9. Dimorphism and parasterility are primary characteristics of *Armeria;* monomorphism and self-fertility, however, are secondary.

10. In the styles of the monomorphic *A. *labradorica* grow both A-pollens and B-pollens of *A. vulgaris.* Furthermore, it is to be noted that the pollens of *A. *labradorica* could grow in the B-styles but not in the A-styles of *A. vulgaris.*

11. We must, therefore, assume that the monomorphic *A. *labradorica* primarily originates from an A-line by recession of an inhibitor in the style.

12. The physiological and phylogenetic problems involved in dimorphism and monomorphism of *Armeria* will be discussed in following papers.

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INHALTSANGABE

Indleveret til Selskabet den 13. Juni 1940.
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TAFELN

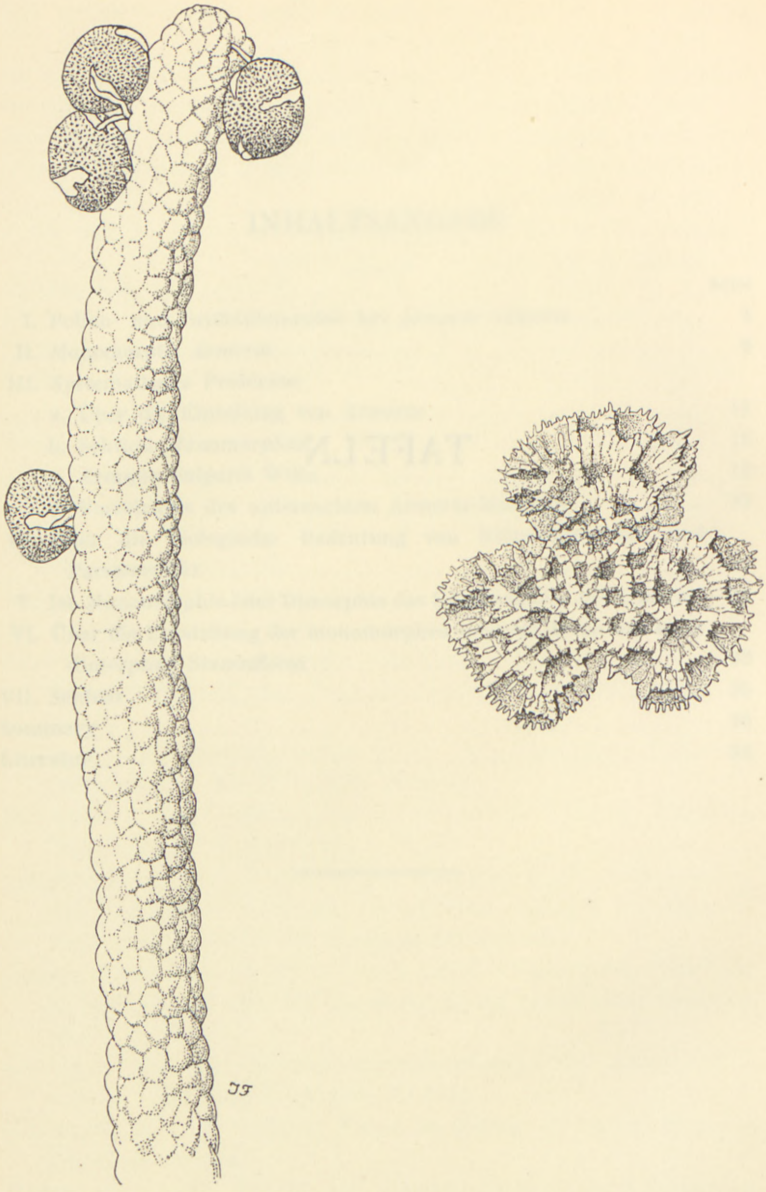


Fig. 2. *Armeria vulgaris.* Griffelspitze (× 250) und Pollenkorn (× 500) der A-Linie. Auf der Narbe 4 gekeimte B-Pollenkörner.

Fig. 3. *Armeria vulgaris*. Griffelspitze (× 250) und Pollenkorn (× 500) der B-Linie. Auf der Narbe 4 gekeimte A-Pollenkörner.

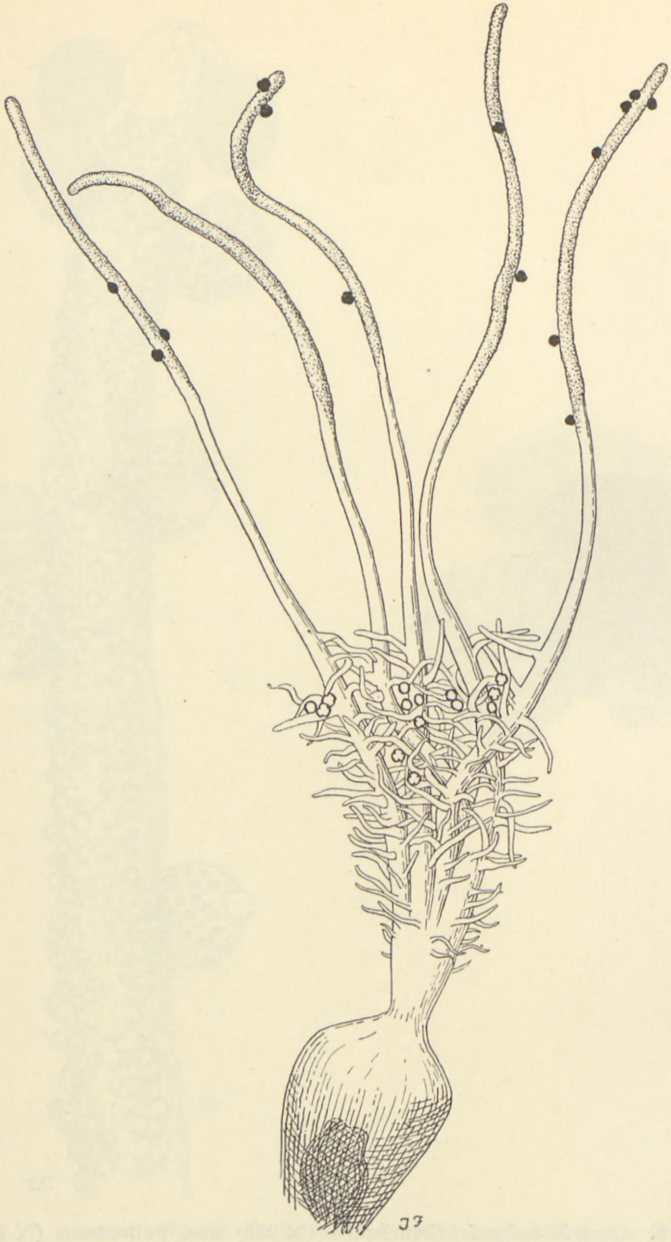


Fig. 4. Befruchtetes *Gynoecium* der Linie A. Pollenkörner der A-Linie weiss, der B-Linie schwarz gezeichnet. × 25.

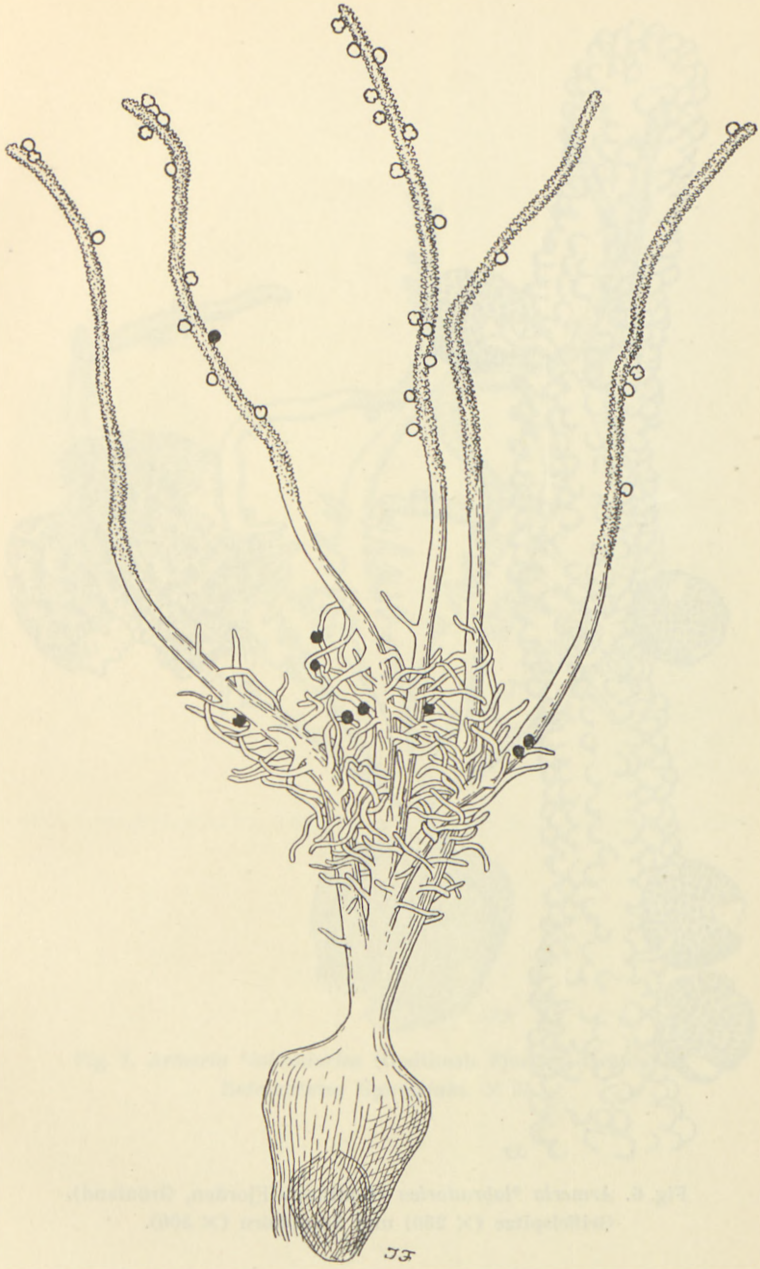


Fig. 5. Befruchtetes *Gynoecium* der B-Linie (vgl. Fig. 3). × 25.

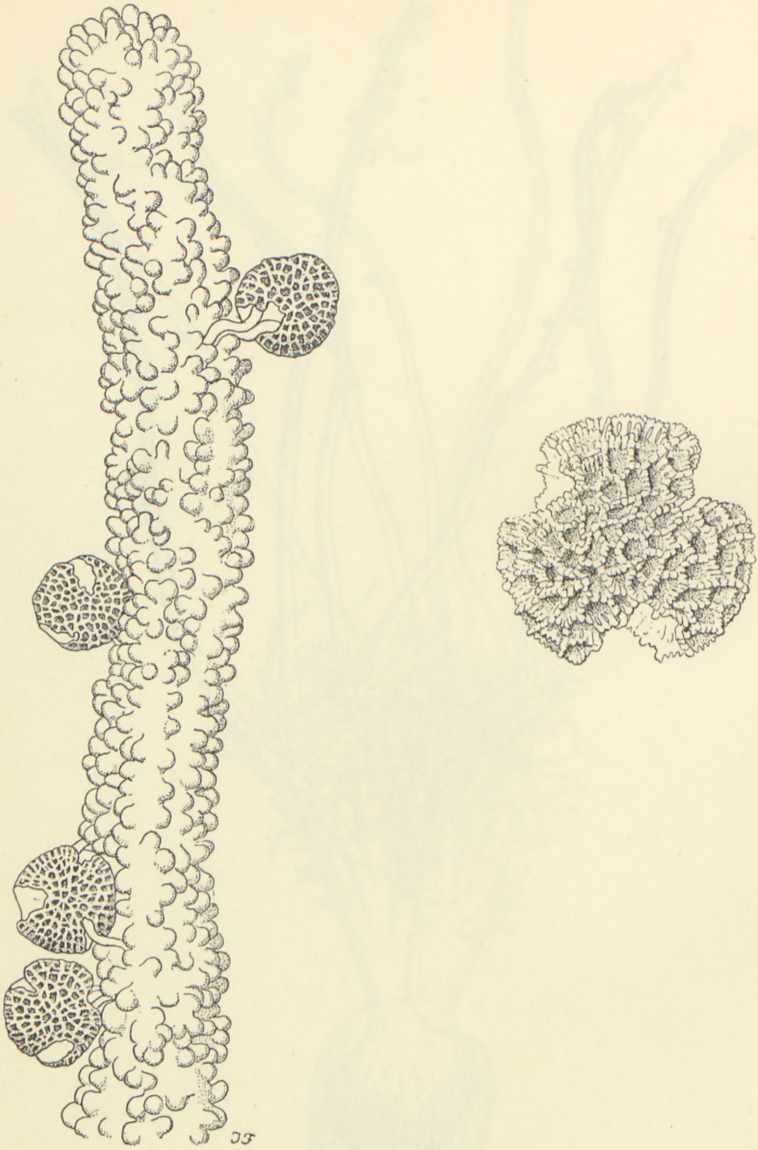


Fig. 6. *Armeria *labradorica* (Godthaab Fjorden, Grönland). Griffelspitze (× 250) und Staubkorn (× 500).

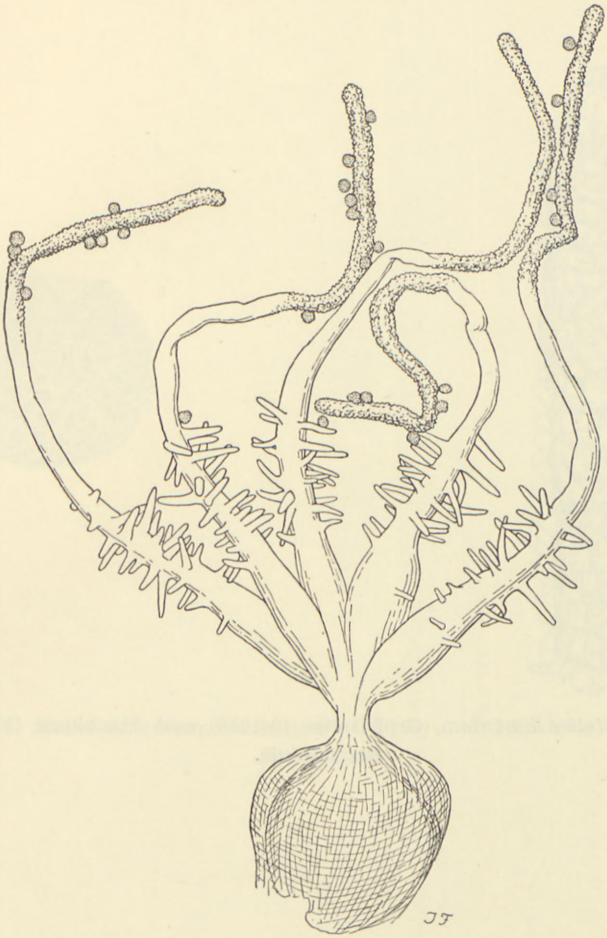


Fig. 7. *Armeria *labradorica* (Godthaab Fjorden, Grönland). Befruchtetes *Gynoecium*. × 25.

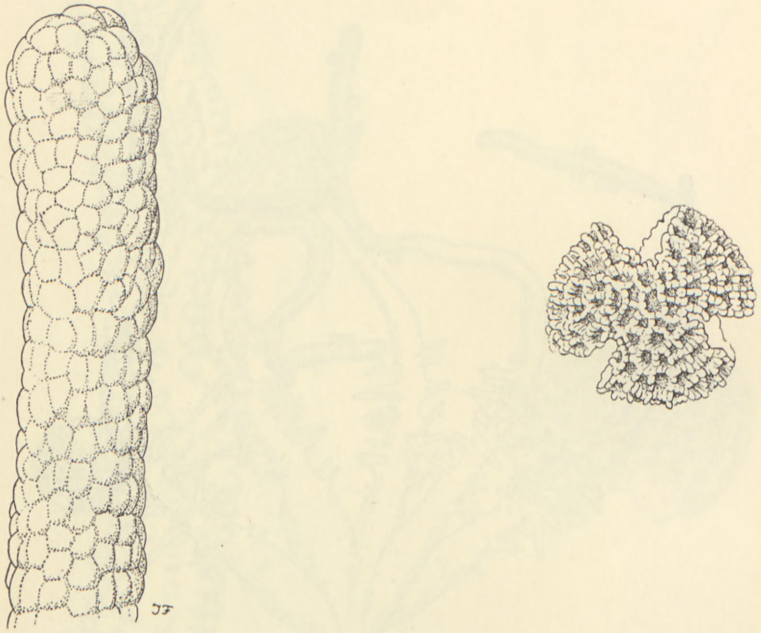
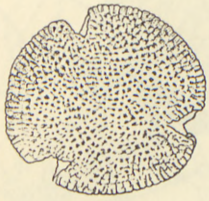


Fig. 8. *Statice limonium*, Griffelspitze (× 250) und Staubkorn (× 500) der A-Linie.



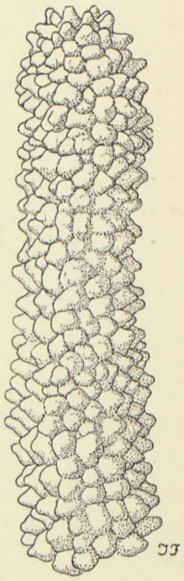
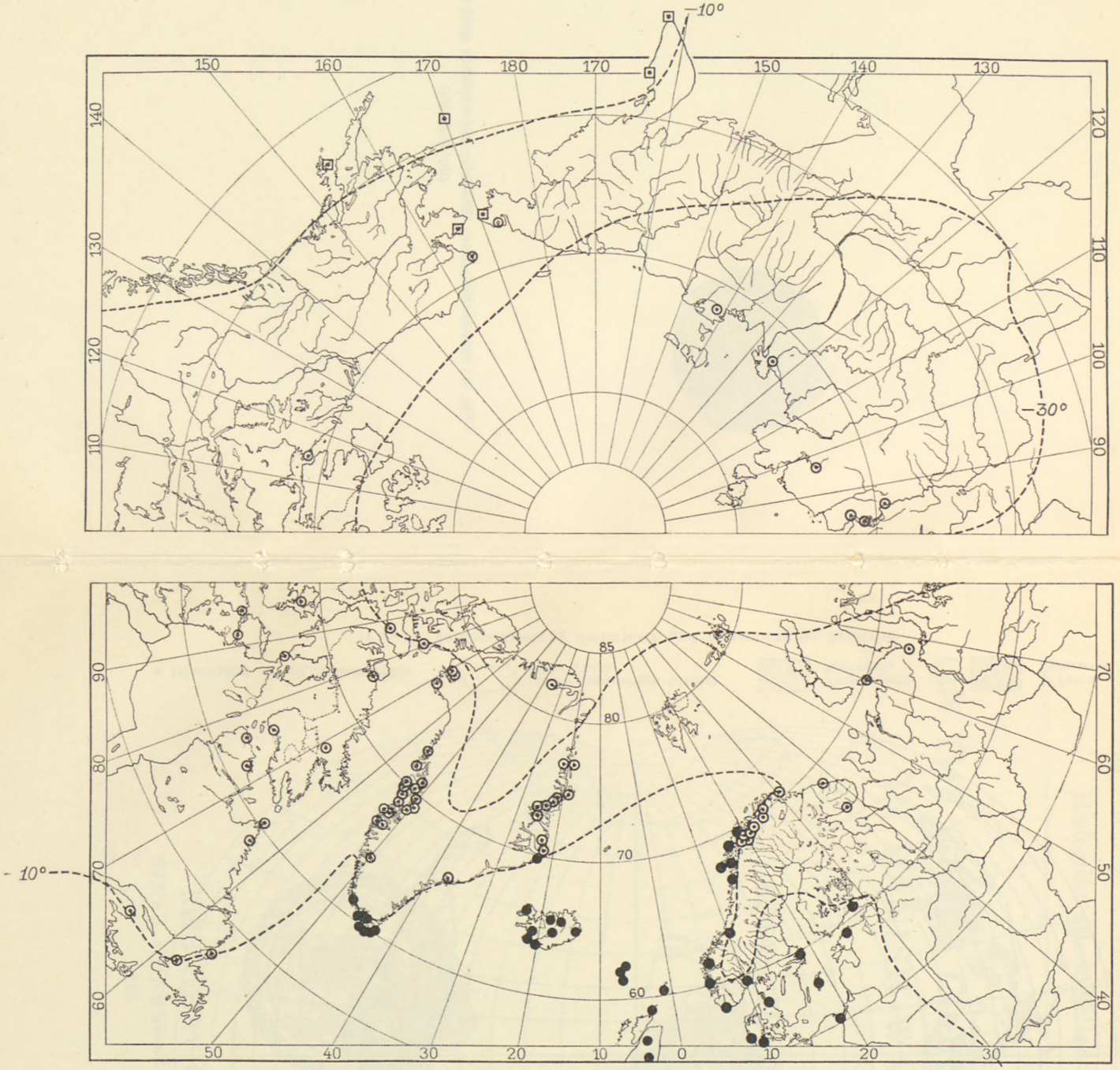


Fig. 9. *Statice limonium*, Griffelspitze (× 250) und Staubkorn (× 500) der B-Linie.



● *A. vulgaris*
Dimorphe *Armeria*

⊙ *A. *labradorica*
⊡ *A. *arctica*
Monomorphe *Armeria*

Januar Isothermen (÷ 10° und ÷ 30°).

Fig. 10. Verbreitung dimorpher und monomorpher Armerien im Norden.

Fig. 11. Totalausbreitung dimorpher und monomorpher Armerien.

Det Kgl. Danske Videnskabernes Selskab.
Biologiske Meddelelser. **XV**, 9.

ON THE GENUS *CODIUM* STACKH. IN DANISH WATERS

BY

SØREN LUND

KØBENHAVN
EJNAR MUNKSGAARD
1940

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Of the genus *Codium* two species inhabit the Danish waters: *C. dichotomum* (Huds.) Setchell and *C. fragile* (Sur.) Hariot, both belonging to the group *Tomentosa* De Toni. They are characterised by having an erect, cylindrical, ramiferous thallus expanding into a holdfast-cushion at the base. Several erect shoots generally grow from the basal portion. The characteristic feature of the anatomical structure of the erect frond is that it consists of a central part of long hypha-like threads, running lengthwise, branching and interwoven, and a peripheric part of large, serried, cylindrical or club-shaped vesicles (utricles), resembling palisades. The utricles projecting at right angles from the central part are formed at the apices of the pith-filaments (or sometimes laterally?). The latter change the direction of their growth, bending towards the periphery of the frond swelling when reaching the former. The bottom part of the utricles usually develop one or more lateral branches, which generally continue the growth of the pith-filaments, themselves likewise producing utricles at their tips after the course of a short or a long time. In several cases the lateral branches, however, grow directly into new utricles.

The utricles as a rule do not branch off; they contain a number of nuclei and numerous small chromatophores chiefly found at the upper end. Often they bear at the upper end one or more hairs (or the scars from them).

The hairs contain a rather small number of chromatophores.

The reproduction is by motile gametes, which develop in gametangia. The position of these is lateral, on the utricles. In addition, in *C. dichotomum* there is a vegetative propagation by means of vegetative buds.

Transverse walls do not exist. On the other hand, local pad-shaped thickenings of the external walls occur, the so-called plugs. Such bodies are seen between the utricles and the pith-filaments, at the bases of the gametangia and the hairs and in the pith-filaments. In *C. dichotomum* between the utricles and the vegetative buds, too.

Regarding the general structure reference may be made to Oltmanns (1922, p. 386) and Schmidt's monography (1923), regarding the taxonomy to the latter treatise.

1. Codium dichotomum (Huds.) Setchell.

Setchell 1931, p. 357; Suneson 1939, p. 57, fig. 1 A.

Codium tomentosum (Huds.) Stackh.; Gibson and Auld 1900, pl. 1—3, figs. 1—21 (—fig. 13 a, b, e); K. Rosenvinge 1920, p. 129; Schmidt 1923, p. 39, figs. 20—21; van Goor 1923, fig. 1 d.

f. *proliferum* Kütz.; Vouk 1936, p. 15, Taf. IV, fig. 2, Taf. V, fig. 2.

f. (?) *dichotomum* Vouk 1936, p. 15, Taf. I, fig. 1.

In the attached state this species is noticed in the northern Kattegat only, near Frederikshavn, in which place it grows on wood, stones, shells of *Balanus* and *Modiola*, tubes of worms and the like. It often occurs in the upper sublittoral region, from a little below the surface of the water down to the depth of a few metres. This is the case, for instance, in the harbour of Hirsholmene, where it grows very gregariously, so that there is here a *Codium*-association. However, it may be found at a greater depth, too, having been met with in down to 11 meters' depth.

The colour of living plants is dark green or more or less bright green. The brighter colour is more pronounced in plants, which are exposed to a rather large amount of sunshine and is particularly characteristic of the young shoots. As the latter are most frequently met with in summer, plants from this season are often rather bright. This species, however, taken at large, too, is lighter than *C. fragile*. The Danish individuals are in that respect different from Cotton's Clare Island plants. Regarding *C. tomentosum* this author writes (1912, p. 114) "Colour dark

green. . . . does not assume a light green colour when growing in sunlight". In contradistinction he says about *C. mucronatum* var. *atlanticum*: "Colour deep green, bright green when growing in sunlight".

The erect part of the thallus consists of branching filaments, of a diameter of about 2—4 mm, the maximum length being up to well over 20 cm. The appearance of the specimens at my disposal is rather varying; nearly all of them are collected in the month of July (a few of them, however, in May, June and August), mainly originating from Hirsholmene. They group round three types, which are to be sure very closely allied.

In one type (plate I), represented by a few plants gathered in May the frond is hardly 20 cm in length by 4 mm in diameter. The ramification is dichotomous, rather scanty. The branches are somewhat squarrose, often curved and bent downwards. The upper segments, of a length of 2.5—6 cm, are rather characteristic, being curved, recurved or incurved. The frond of the greater part of the plant has numerous small warts, often arranged so as to be partly one-sided. On the convex side of the upper segments they have often developed into short lateral shoots, as a rule, however, not exceeding the length of 0.5 cm.

The second type includes a few plants gathered in the month of June. They seem to be consistent with the former except that they have the warts developed into small, short shoots, sometimes attaining the length of more than 1 cm. — The specimen represented in plate II was cast up, but the attached plants looked very much like it, even if the tendency of the upper segments to incurve or recurve is not quite so marked.

The great majority of the plants, however, seem to fall

under the type pictured in plate III. In these plants, mainly gathered in July, the frond reached a length of up to over 20 cm, while the diameter of the branches amounted to about 3 mm. The plants seem to show a distinction between main branches and lateral branches. The former, arisen by dichotomous ramification, are squarrose and curved, recurved or incurved; the lateral branches are mainly found on the convex side. They may, however, be found, too, on the unbranched bottom part of the main shoot. The lateral branches in full-grown plants are generally about the length of 7—10 cm; the ramification is usually regularly dichotomous, in rare cases they develop lateral shoots of their own.

The appearance of a great number of young plants, likewise mainly gathered in July, is similar to that of the latter. The frond of these plants consists of a short main filament, branched or unbranched, often somewhat curved, producing numerous lateral branches particularly on the convex side. In other cases the branching of the young plants is regularly dichotomous throughout the entire plant, without any distinction between main and lateral branches.

The two first mentioned types may presumably be referred to f. *proliferum* KÜTZ. (see VOUK, l. c.). The third may possibly be referred to f. *dichotomum* VOUK because of the comparatively short, not parallel end segments mainly of equal length. Owing to the distinction, however, between main and lateral branches it is not very much like VOUK's fig. 1. of plate 1. In spite of the morphological difference the three types are presumable nevertheless closely related, perhaps representing different stages of development of the same form. Actually, the only difference between types 1 and 3 is that the warts of the former have

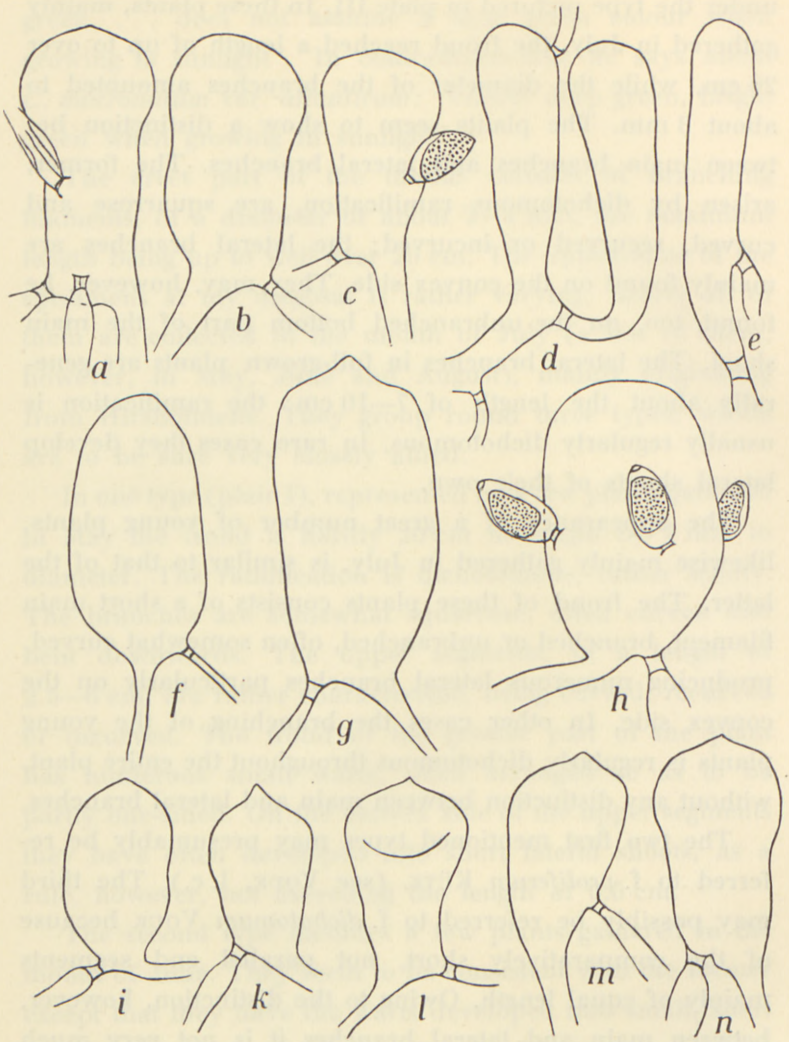

Fig. 1. *Codium dichotomum*. Utricles. *i*, *k*, and *l* from seedlings; *m* and *n* from the apex of a branch. Gametangia with contents are seen in *c* and *h*, a gametangium emptied in *a* and a utricle hair in *d*. — *a-h* and *m*, *n* from Hirsholmene, the harbour; *a-c* and *f-h*, 29-VII-19; *d*, *e*, 23-V-29; *m*, *n*, 10-VII-34. *i*, *k*, from east of Deget, 4-VIII-32. *l* from Holmehavn's Reef, 3-VIII-28. (46:1).

come out as lateral shoots of a considerable length in the latter. The plant shown in plate II represents an intermediate stage. As the three types were gathered in May, June and July, respectively, it is to be presumed that in this species, at the locality mentioned, an abundant production of lateral branches takes place during the summertime.

Nor does the anatomical structure of the three types seem to show any difference. They all have the same types of utricles, and they are subject to the same variation as to the size of the utricles.

As to shape the utricles vary rather much. Vouk (l. c. p. 7) mentions a dimorphism in this species of club-shaped utricles and of utricles of a vesicular shape. A dimorphism like that is not a characteristic feature of the present writer's material, here polymorphism is more pronounced. In addition to club- and bladder-shaped utricles numerous top-shaped and cylindrical utricles appear, and some of still other shapes. In figs. 1, 6, and 7 I have shown different types of utricles.

As to shape the end of the utricles show a fairly great variation. Some of them are rounded, as for instance in Schmidt's figures (1923, figs. 20, 21), others are more or less rounded conical, as appears for instance from van Goor's fig. 1 d, the drawing of which is from a specimen from Hirsholmene. Very often they have, however, a more or less marked point, nay, this type seems even often to be the most frequent. Thus this seems especially to be true in the case of the utricles which occur in the young shoots (fig. 1 m, n). This type, for instance, was very common in the lateral shoots of the specimen shown in plate III. In the upper part utricles like these correspond exactly to the

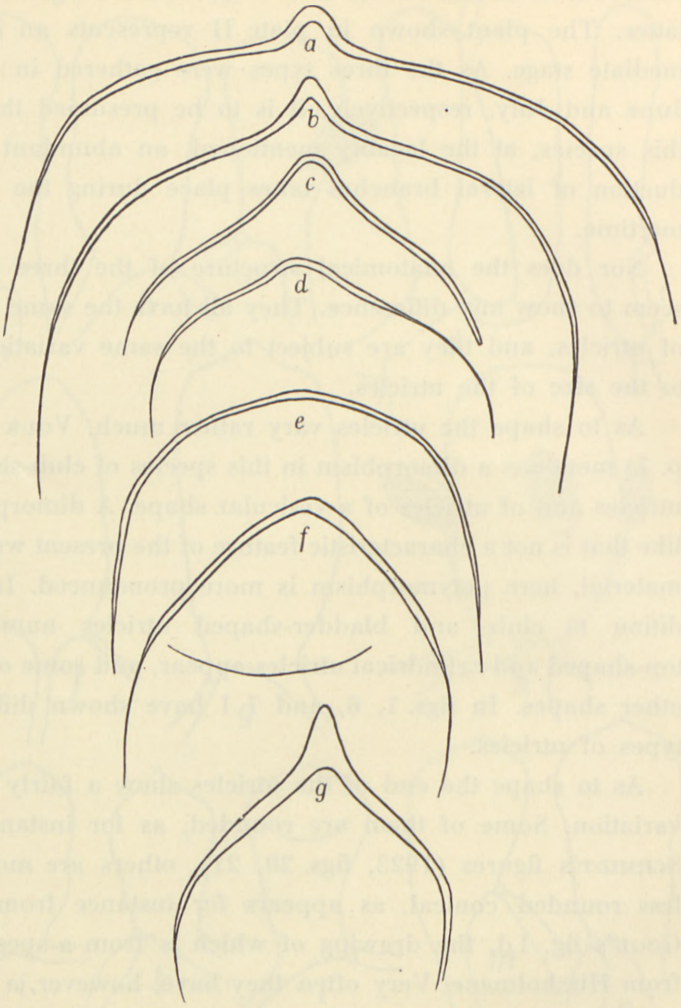

Fig. 2. *Codium dichotomum*. Apices of utricle. *a*, *b*, and *f* from a main filament; *c-e* from a lateral branch, 2 cm from the apex. The apex of utricle in *g* with a marked mucron. — *a-f* from Hirsholmene, the harbour, 29-VII-19; *g* from Hirsholmene 27-VI-19. (173:1).

ends of utricles with point which Suneson (1939) pictured in his fig. 1 A. In figs. 2—4, 7 I have shown different ends

of utricles, rounded ones as well as some with a more or less marked point.

The wall of the utricle is, as a rule, only slightly thickened at the top. Even in utricles with a point the thickness in this place amounts usually to about 4—12 μ only. However, in some cases, the tip may be developed into a more or

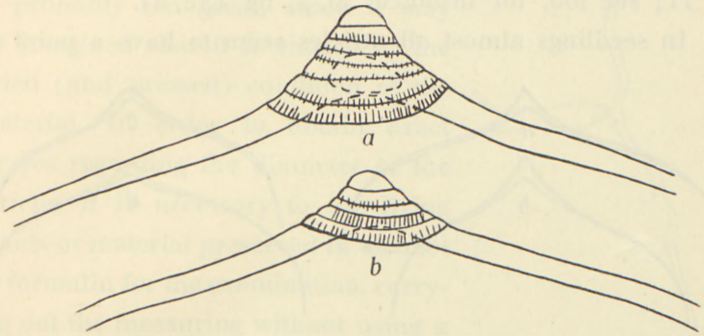

Fig. 3. *Codium dichotomum.* Apices of utricle. Old, thickened apices with longitudinal grooves arranged concentrically in rings (the drawings a little exaggerated, perhaps). In *a* in addition, a cavity in the thickened apex of the utricle is seen. — From Hirsholmene, 27-VI-19. (355:1).

less marked mucron, as it is found in *C. fragile*, and the thickness may then come to 14—21 μ. Nay, in a single case (the case shown in fig. 2g) a utricle was observed, the thickness of the wall of which even amounted to 50 μ, which, however, probably is exceptional.

If the utricles have a mucron, it is usually rounded. At the tip a number of small, longitudinal grooves may sometimes be detected, arranged in concentric rings (fig. 3). The phenomenon completely corresponds to what Schmidt (1935, p. 156) mentioned in *C. fragile*. In addition sometimes in the thickening may be seen cavities (fig. 3a), as also a more or less marked lamination.

Thus it is seen that the ends of the utricle vary rather

much. They may be rounded, or more or less rounded conical; the end may be pointed without any special thickening of the tip or pointed with the tip thickened. The last category leads to *C. fragile*. The fact that *C. dichotomum* may have the tip of the utricle rounded and mucronate as well is evident, too, from the work of Gibson and Auld (l. c. p. 11; see too, for instance, pl. 2, fig. 13c, d).

In seedlings almost all utricles seem to have a point at

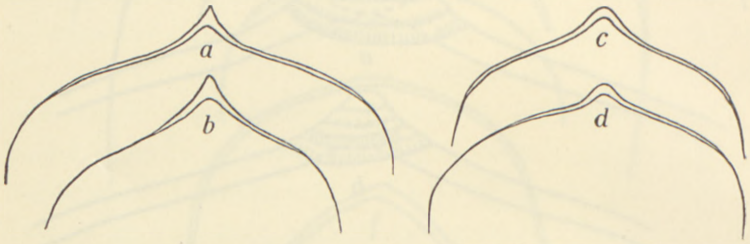

Fig. 4. *Codium dichotomum*. Apices of utricle from seedlings. In *a* and *b* a little mucron is seen. Frederikshavn, the harbour, 28-VII-34. (173:1).

the top (fig. 1 k, l). Generally, however, it is not thickened very much. In some small seedlings, only of a length of 2.5 cm at most, taken by scraping in the harbour of Frederikshavn, most of the tips of the utricles had a small acute mucron, the thickening of which usually amounted to 12—17 μ (fig. 4a, b). These plants, possibly, belong to *C. fragile*. On the other hand, other tips of utricles had no mucron; they were, moreover, only slightly thickened (fig. 4c, d); consequently the tips shown in fig. 4a, b perhaps only denote the variation of the species.

As to size, too, the utricles vary greatly. Suneson states 480—1000 μ × 190—400 μ from his material from the Swedish west coast. These measurements agree fairly well with mine. Only the maximum limits in my material lie still higher, though values over 900—1000 μ are not

very frequent. In bladder-shaped utricles I sometimes noticed a length of up to 1380 μ and a diameter of over 600 μ, nay, in a single case the diameter even came to 670 μ. The latter extreme value of the diameter is probably too great, since it may be imagined that it is caused by the dried (and pressed) condition of the material. In order to obtain exact figures regarding the diameter of the utricles it is necessary to use living plants or material preserved in alcohol or formalin for the examination, carrying out the measuring without using a cover on the slide. The measurements most frequently found seem on an average to be: the length 500—700 μ (—800 μ), the diameter about 300 μ.

The formation of utricles in Danish plants takes place (always?) — as in *C. fragile* — in the manner described e.g. by KÜSTER (1898, p. 171) regarding his plants from the Adriatic. This method by which the utricles develop from the pith-filaments by sympodial growth, is widely recorded in the literature and is mentioned in the introduction of this paper.

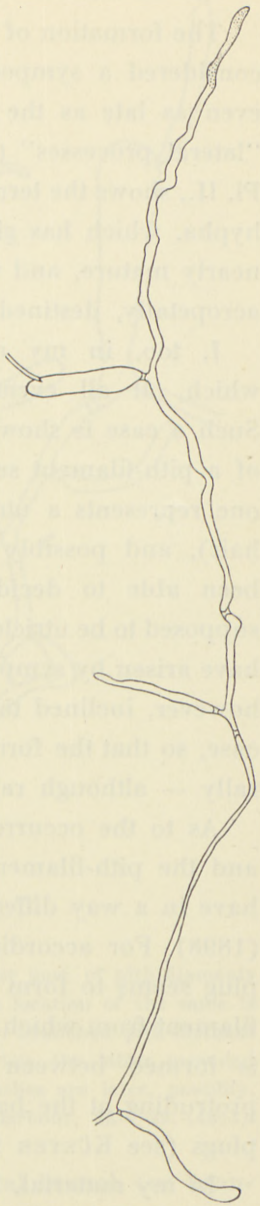

Fig. 5. *Codium dichotomum*. Upper part of a segmented pith-filament with a utricle (carrying a utricle-hair) and two shoots, which possibly will develop into utricles, too. — From Hirsholmene, 29-VII-19. (18:1).

The formation of utricles, however, has not always been considered a sympodial process. Thus Gibson and Auld even as late as the year of 1900 regarded the utricles as "lateral processes" (p. 11). They write here: "Figure 11, Pl. II., shows the terminal portion of a superficial medullary hypha, which has given off a lateral "palisade cell", now nearly mature, and which is forming two lateral processes acropetally, destined to become "palisade cells"".

I, too, in my material noticed pith-filaments, from which, at all events apparently, lateral utricles project. Such a case is shown in fig. 5, illustrating the upper part of a pith-filament sending out 3 shoots. It is evident that one represents a utricle (which even possesses a utricle-hair), and possibly the others behave alike. I have not been able to decide, whether this utricle and the two, supposed to be utricles, are formed laterally, or whether they have arisen by sympodial growth of the pith-filament. I am, however, inclined to believe that the former was really the case, so that the formation of utricle might take place laterally — although rarely — as well as sympodially.

As to the occurrence of the plugs between the utricles and the pith-filaments the Danish specimens seem to behave in a way different from what is reported by Küster (1898). For according to the description of this writer a plug seems to form first between the utricle and the pith-filament from which the utricle has come out. Later another is formed between the utricle and the lateral branch, protruding at the base. Thus each utricle would have two plugs (see Küster fig. 1 c).

In my material, however, a plug seems not always, by far, to appear between the utricle and the pith-filament that has formed it. On the other hand a plug is always

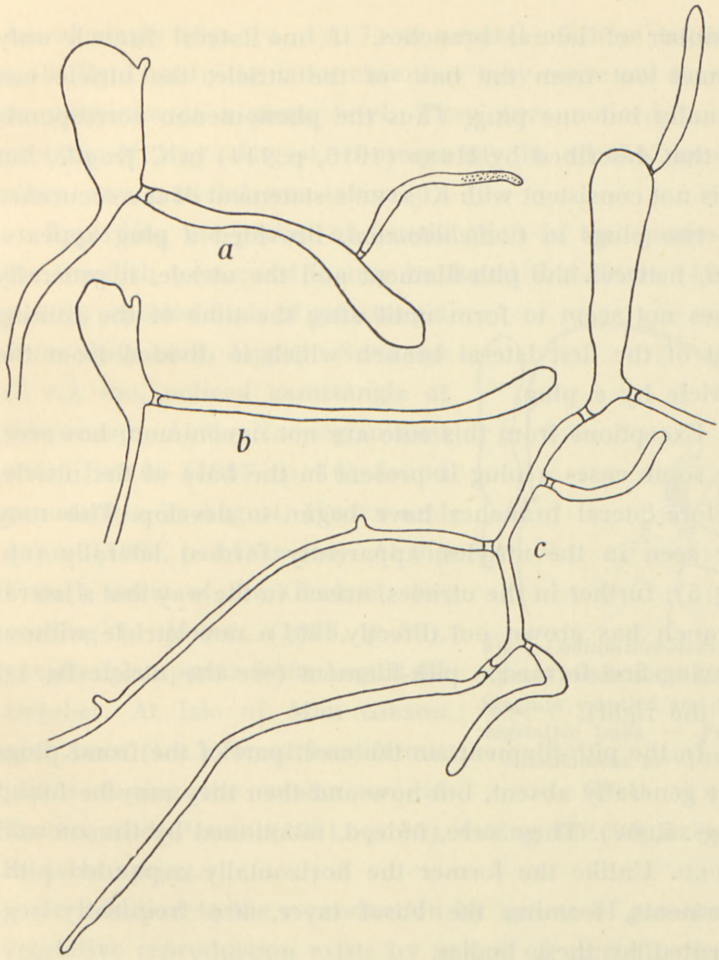

Fig. 6. *Codium dichotomum*. *a* and *b*: the upper part of pith-filaments with utricles, each having one plug only; the location of the same is between the utricle and the lateral branch. *c*: branched pith-filament from the erect frond, showing plugs and a utricle, the latter provided with a utricle hair. The two short lateral branches are later, possibly, developed into utricles. — Hirsholmene, the harbour, 23-V-29. (42:1).

found between the utricle and the lateral branch (or branches) coming out at the base (figs. 1, 6a, 6b). Usually the number of plugs at the base of the utricle is equal to the

number of lateral branches. If one lateral branch only comes out from the base of the utricle, the utricle has usually but one plug. Thus the phenomenon corresponds to that described by Hurd (1916, p. 114) in *C. fragile*, but it is not consistent with Küster's statement of the occurence of two plugs in *C. dichotomum*. Provided a plug appears, too, between the pith-filament and the utricle, it generally does not seem to form until after the time of the coming out of the first lateral branch which is divided from the utricle by a plug.

Exceptions from this rule are not uncommon, however. In some cases a plug is present in the base of the utricle, before lateral branches have begun to develop. This may be seen in the utricles, apparently formed laterally (cp. fig. 5); further in the utricles, arisen in the way that a lateral branch has grown out directly into a new utricle without having first formed a pith-filament (see the utricle fig. 1 d to the right).

In the pith-filaments in the erect part of the frond plugs are generally absent, but now and then they may be found (figs. 5, 6 c). They were, indeed, mentioned by Gibson and Auld. Unlike the former the horizontally expanded pith-filaments, forming the basal layer, are frequently segmented by these bodies.

The utricles in the upper part often bear hairs (figs. 1 d, 5, 6 c), which are distinctly seen to contain chromatophores, even if scanty only. The plugs between the utricles and the hairs either are unilaterally developed, or they may be more or less all-sided.

Gametangia occur laterally on the utricles, most frequently about the middle part. The length usually is 250—285 μ, the diameter being 100—115 μ (—140 μ). They are

found singly (figs. 1 a, 1 c, 7) or several of them together (—3; fig. 1 h). In the latter case they may come out several or only one at a given level. They were noticed in the months of May—August, consequently in all the months when any material was taken.

Regarding the period of fructification in the species at the Swedish west coast it may be stated that KYLIN (1933, p. 391) reported finds of gametangia from July and August. SUNESON (l. c.), too, noticed gametangia at this season. At the Norwegian west coast EKMAN (1857, p. 16) found gametangia in August, HYGEN and JORDE (1934, p. 14) in July. At the French coast of the Channel, according to HAMEL (1928, p. 89), gametangia appear from August till October. At Isle of Man GIBSON and AULD (l. c.) found fructiferous plants during the winter as did KNIGHT and PARKE (1931, p. 55) during the winter and in early spring.

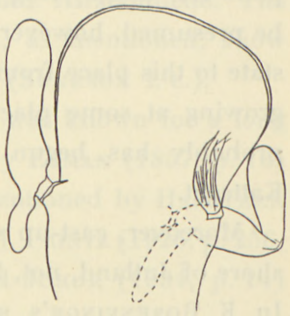

Fig. 7. *Codium dichotomum*. Apex of utricle with a gametangium emptied and two vegetative buds. — From Hirsholmene 27-VI-19. (70:1).

In addition to the reproduction by means of gametes a vegetative reproduction exists by means of vegetative buds coming out on the sides of the utricles (WENT 1889, p. 443, Pl. VII, figs. 3—9; GIBSON and AULD, l. c. p. 15, Pl. II, figs. 4, 6—9, 12). Vegetative buds like these seem to be frequent in the plants which I examined. In fig. 7 is shown a utricle, on which were found two vegetative buds, both on the point of coming out at the base, as well as an evacuated gametangium. Between the vegetative bud and the utricle a plug is seen.

Attached specimens of the species, as mentioned above, are noticed only in the northern Kattegat. But specimens cast up on the shore are found in several places; this was the case at the northern coast of the peninsula of Djursland in the middle part of the Kattegat, where in August 1937 I found a number of cast-up specimens. These plants were consistent with the type from Hirsholmene, pictured in plate III. Considering the abundant occurrence it is not to be presumed, however, that they have drifted in a detached state to this place from Hirsholmene, more likely they were growing at some place near Djursland. Thus the species probably has begun spreading southwards through the Kattegat.

Moreover, cast-up specimens are noticed on the western shore of Jutland, not, however, until after the year of 1929. In K. Rosenvinge's survey on foreign algae cast-up on the shore of west Jutland (1900, p. 83) it is mentioned no more than is the following species.

The presence of the species was first ascertained in Danish waters in 1919 (K. Rosenvinge 1920, p. 129), when it was found, partly in an attached state in the harbour of Hirsholmene and in the neighbourhood, partly cast-up in various places at Hirsholmene. K. Rosenvinge (l. c. p. 130) supposed it to be imported by a ship. In my interpretation it would, however, be as likely to connect its immigration with an importation of drifting, fructiferous specimens deriving from the Norwegian or the British shores. Or is it possible that the vegetative buds be instrumental in its immigration? At any rate there is every reason for presuming that it originates from the same place as the specimens which are cast up on the shore of west Jutland, for these

plants are of the same type as to the utricle as those found at Hirsholmene.

As in the case of Denmark the occurrence of the species in Sweden was ascertained only recently. It was scraped in that country for the first time in 1932 (KYLIN 1933, p. 391), i. e. 13 years after its discovery in this country. The occurrence of the species at the Swedish west coast is possibly due to an immigration from Hirsholmene. The Swedish and the Danish specimens, as mentioned, show much conformity as to the utricles (SUNESON l. c.).

In Norway, on the other hand, it was known for a long time. Thus it is already mentioned by EKMAN (1857, p. 16) from Christianssund. Later it was mentioned by HANSTEEN (1892, p. 355), BOYE (1894—95, p. 45), PRINTZ (1926, p. 256, only drifting, however), HYGEN and JORDE (1934, p. 14) and LEVRING (1937, p. 34).

Regarding the distribution of the species in north west Europe it may be stated, moreover, that it is common at the French, British and Irish coasts. It has not yet been noticed in Holland (VAN GOOR 1923, p. 133) nor at Heligoland (SCHMIDT 1935, p. 157). The northern limit of its range seems to lie at well over 63° north latitude at the Norwegian west coast.

In the following list of localities of the Danish waters the same designations as to the division of the waters are used as by K. ROSENVINGE (1909, p. 19).

Localities. **Ns:** Vorupør, 1 mile north of the pier, 13-VII-30, length 17 cm, cast-up specimen, not intact, with gametangia (S. Lund). — **Sk:** Kandestederne, 22-VII-34, 29 cm, cast-up, not intact, with gametangia (L. K. Rosenvinge); the Nordstrand of Skagen, a little east of the northernmost point, 15-VII-29, 10 cm, cast-up, not intact (S. L.). — **Kn:** Bunken, the beach, 13-VIII-32, cast-up, not intact, with gametangia (H. E. Petersen); Hulsig Stene, seedlings, 25-VII-33 (L. K. R.); Namands-Reef, seedlings

(Boye Petersen); the extreme verge of Hvidsten Reef, seedlings (L. K. R.), Hirsholmene, the harbour, abundant, seedlings and full-grown plants (Boye P., L. K. R. and others); half-way between Kølpen and Hirsholmene, 7.5 m, seedlings (L. K. R.); Holmehavn's Reef, 5.5 m, seedlings (Boye P.); between Maren's Reef and Borrebjerg's Reef, 7.5 m, seedlings (L. K. R.); Maren's Reef, seedlings (H. E. P.); east of Deget, 11 m, seedlings (H. E. P.); at Syvstenen, 5 m, seedlings (L. K. R.); Frederikshavn, the harbour, the small boats' harbour at the end of the southern pier. 1932. In addition, in 1934 seedlings approaching the habits of *C. fragile* (The Marine Biological Summer course); eastern double broom at Nordre Rønner, 10 m, July 1933 (H. E. P.). — **Km:** Fjellerup, the beach, northern coast of Djursland, 23-VIII-37, numerous cast-up specimens with gametangia, up to at least 14 cm (S. L.).

2. Codium fragile (SURINGAR) HARIOT.

var. **typicum** SCHMIDT, 1923, p. 47, figs. 29—31; 1935, p. 156, fig. 3.

Codium mucronatum J. AGARDH 1886, p. 43; HURD 1916, p. 109, pl. 19—24; K. ROSENVINGE 1920, p. 131.

Codium mucronatum var. *Tasmanicum* J. AG., l. c. p. 44, Tab. I, fig. 2; COTTON 1912, pl. 8, fig. 7.

Codium mucronatum var. *Californicum* J AG., l. c. p. 44, Tab. I, fig. 3; COTTON l. c. fig. 8.

Codium mucronatum var. *Novæ Zelandiæ* J. AG., l. c. p. 44; COTTON l. c. fig. 6.

Codium mucronatum var. *atlanticum* COTTON 1912, p. 114, pl. 7, 8, figs. 3—5.

Codium mucronatum var. *tomentosoides* VAN GOOR 1923, p. 136, fig. 1 c.

In the attached state it occurs only in the westernmost and the middle part of Limfjorden. In this water it inhabits the sublittoral region from the upper part to a depth of 11 m. Like the preceding species it is found on stones, wood and shells. The colour is in living plants dark green. The erect frond consists of branching filaments, 3—6 mm in diameter, which may at any rate attain a length of at least 37 cm. Generally the length is not quite so great, though it, however, most frequently seems to be more than 20 cm.

The appearance of the frond does not vary so much as in the preceding species, the branching being almost exclusively dichotomous. Usually the branching of the plants is very regular throughout the entire plant from a little above the basal portion (plate IV). In some cases, however, specimens are found branching richly in the upper part

only. This was the case in some plants, which I secured by scraping at the wharfs at Oddesund Nord on July 4th 1930 (plate V). In these plants the branching was rather scanty in the greater part of the frond. The segments between the levels on which branches developed might even amount to a length of 15 cm. In the upper part, however, dichotomous branching occurred repeatedly, one following the other so closely that the appearance was more or less like a candelabrum.

The different appearance in the two plants pictured is probably due to their environments. In the case of an isolated plant a fairly rich branching begins even a short distance above the basal part. On the other hand, if the plants grow gregariously — as in the case of the plants of Oddesund — the segments become long, giving off few branches only; not until the latter have reached a certain length will the frond branch more abundantly. Such plants, by means of the long segments, secure better conditions of life for a great part of their surface.

In addition to branches, arisen by dichotomous dividing, small lateral branches occur in a few cases. They are, however, not well-grown, but rather like warts only. In a single case such a lateral branch attained a length of 6—7 mm being at the same time very slender.

It is different with a branch that has been injured. Near the injured spot several adventitious branches may project. Some of them become vigorous, continuing the growth of the segments by dichotomous branching.

In cast-up specimens from the Skagerak and the northern Kattegat, however, a rather great number of lateral branches, fairly long, often occur. Not in all the cases, by far, of these lateral branches is it possible to regard their formation as

arising from the injury of the shoot. Their occurrence is perhaps due to an abnormal growth in the plants after their breaking off.

The elongation of the frond, according to Shannon's and Altman's measurements (1930, p. 391) is specially confined to the tips of the branches and to some intercalary zones. In the basal part no growth of length takes place, or it is negligible only, whereas a growth in width goes on.

The basal portion is generally well-grown as in the preceding species. In a specimen, which I gathered at Rønnen, north east of the entrance of Lem Vig in Nissum Bredning, the flattened part measured about 20 cm^2. It was not, however, confined to the shell of a *Mytilus*, which the plant inhabited, it had spread, too, to some pebbles, captured by the byssus filaments of the mussel. It consisted of branching pith-filaments, horizontally expanding and segmented by a rather great number of plugs; at any rate, in many of the pith-filaments chromatophores abounded. The basal portion gave off several vigorous shoots, whereas the pith-filaments frequently had isolated utricles, pointed and mucronate.

Plugs are found in the pith-filaments of the erect frond, too (cp. Hurd l. c.). However, they are less abundant than in the bottom part.

As to the occurrence of plugs between the utricles and the pith-filaments, the phenomenon seems to agree with what is stated by Hurd (l. c. p. 114) and to what is mentioned by me as to *C. dichotomum*. Between the utricles and the pith-filament, having formed the same, not always, by far, a plug is found — or it is not formed until later. On the other hand, between the utricles and the lateral branches plugs are always present.

The utricles are clavate-cylindrical, nearly always unbranched (fig. 8). Branching utricles of an abnormal appearance, however, are noticed, too. Generally the appearance of the apex of old utricles is different from that of those somewhat younger and of young ones. It is more truncate and, in addition, the mucron is less conspicuous (figs. 8h, 9a).

As to shape the utricles seem generally to agree with those in Irish plants, as described by COTTON (l. c. p. 114). The VAN GOOR's type of utricle, too (l. c. p. 134) — as also transitional forms, are, however, rather frequent. Var. *tomentosoides* VAN GOOR, characterised by the form of the utricles and the mucron besides by the size of the utricle, is considered unmaintenable by SCHMIDT (1935, p. 156, footnote).

Regarding plants from the western North America HURD demonstrated (p. 112, 113) that the utricles in the lower part of the frond as a rule were smaller, having their mucrons developed in a way different from those above. The average size of the utricles increased upwards through the frond, the largest utricles appearing from above the middle up to a few cm from the apex of the shoot.

In Danish plants, too, there was a perceptible difference as to size in the lower part of the thallus and the part above. In the lower part of the frond nearly all the utricles were small and tapering in their upper part, having a conspicuous mucron (fig. 9h—k), whereas those from above were considerably larger. The latter measured, on an average, about 800—1050 μ in length and 225—465 μ in diameter. The dimensions, however, might be much greater. Thus in a plant, gathered in January, the utricles of which most often are 900—1050 μ in length to 300—400 μ

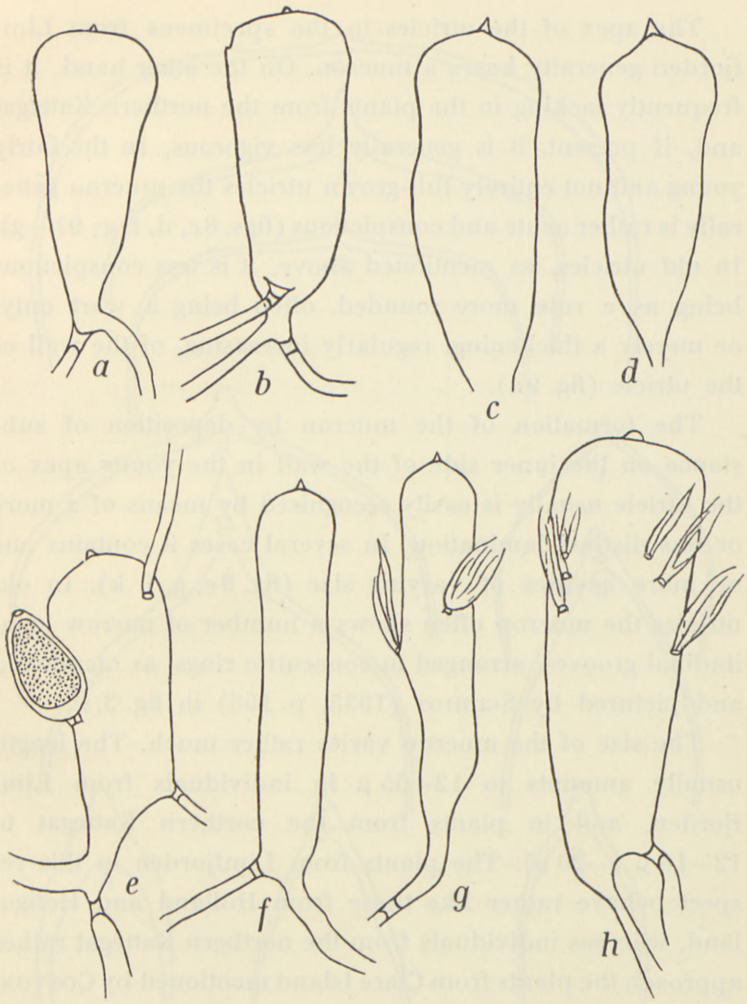

Fig. 8. *Codium fragile.* Utricles. In *h* an old utricle. A gametangium with contents and a utricle hair are seen in *e*, several gametangia emptied in *g* and *h*. — *a* and *c-h* from Limfjorden (*a*, *f*, *g*, from Oddesund Nord, 4-VII-30; *c* and *d* from Rønnen at Lemvig, 18-IX-39; *e* from Thisted, the harbour, 9-VII-30; *h* from "Limfjorden", January 1921); *b* from inside Maren's Reef at Frederikshavn, 21-VII-34. (173:1).

in diameter, several measured 1400—1600 µ in length and up to 600 µ in diameter.

The apex of the utricles in the specimens from Limfjorden generally bears a mucron. On the other hand, it is frequently lacking in the plants from the northern Kattegat and, if present, it is generally less vigorous. In the fairly young and not entirely full-grown utricles the mucron generally is rather acute and conspicuous (figs. 8c, d, f, g; 9b—g). In old utricles, as mentioned above, it is less conspicuous being as a rule more rounded, often being a wart only, or merely a thickening, regularly increasing, of the wall of the utricle (fig. 9a).

The formation of the mucron by deposition of substance on the inner side of the wall in the young apex of the utricle usually is easily recognised by means of a more or less distinct lamination. In several cases it contains one or more cavities of varying size (fig. 9e, g, i, k). In old utricles the mucron often shows a number of narrow longitudinal grooves, arranged in concentric rings, as mentioned and pictured by Schmidt (1935, p. 156) in fig. 3,5.

The size of the mucron varies rather much. The length usually amounts to 12—65 μ in individuals from Limfjorden, and in plants from the northern Kattegat to 12—16 μ (—20 μ). The plants from Limfjorden in this respect behave rather like those from Holland and Heligoland, whereas individuals from the northern Kattegat rather approach the plants from Clare Island mentioned by Cotton, in which the mucron is shorter or absent.

As is well known the variation of the mucron has caused the establishment of various varieties; thus the three varieties of Agardh, mentioned above, and also Cotton's var. *subatlanticum*. While the varieties of Agardh generally are considered unmaintenable, Schmidt (1923, p. 47), however, classifies the variety of Cotton as a subvariety.

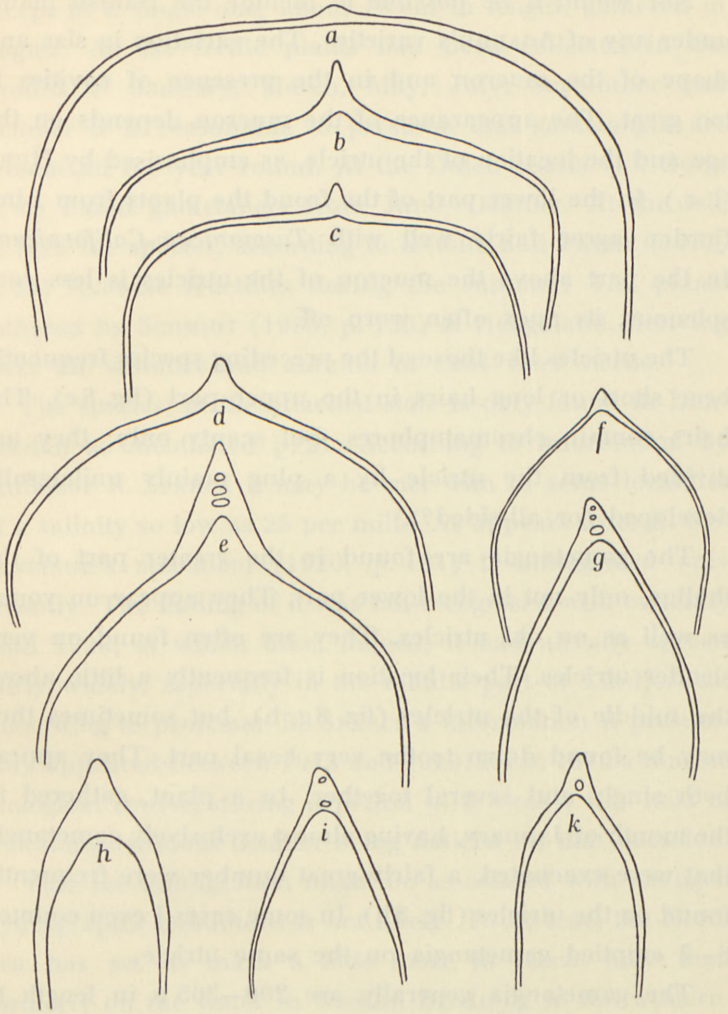

Fig. 9. *Codium fragile.* Apices of utricle from Limfjorden. *a* shows the apex of an old utricle with a mucron not very marked. The apices represented in *b-g* are younger and have a more conspicuous mucron, which in the case of *e* and *g* exhibits cavities. *h-k*: apices of utricle from the basal part of the erect frond. In *i* and *k* cavities are seen in the mucron, which as in *h* is conspicuous. — *a*, *c*, *f*, and *g*, from Oddesund Nord, 4-VII-30; *b*, *d*, *e* and *h-k* from Rønnen at Lemvig, 18-IX-39. (173:1).

Nor would it be possible to include the Danish plants under any of Agardh's varieties. The variation in size and shape of the mucron and in the presence of cavities is too great. The appearance of the mucron depends on the age and the location of the utricle, as emphasised by Hurd (l. c.). In the lower part of the frond the plants from Limfjorden agree fairly well with *Tasmanicum-Californicum*. In the part above the mucron of the utricles is less conspicuous, its apex often worn off.

The utricles like those of the preceding species frequently bear short or long hairs in the upper part (fig. 8e). The hairs contain chromatophores, but scanty only; they are divided from the utricle by a plug mainly unilaterally developed (or all-sided?).

The gametangia are found in the greater part of the thallus, only not in the lower part. They appear on young as well as on old utricles. They are often found on very slender utricles. Their location is frequently a little above the middle of the utricles (fig. 8g, h), but sometimes they may be found down to the very basal part. They appear both singly and several together. In a plant, gathered in the month of January, having almost exclusively gametangia that were evacuated, a fairly great number were frequently found on the utricles (fig. 8h). In some cases I even counted 6—7 emptied gametangia on the same utricle.

The gametangia generally are 200—365 μ in length to 80—160 μ in diameter. Both ♂ and ♀ are demonstrated in the same plant as mentioned earlier by Schmidt (1928, p. 627) regarding Danish plants from Limfjorden. Usually my preparations, however, contained chiefly one kind, either prevailingly ♀ + a few ♂ or prevailingly ♂ + a few ♀.

Gametangia occur in all the specimens at my disposal,

except in a single one, about 18 cm in length, gathered in August. As the fertile plants had been collected in the months of January, March, May, July, September and October it is reasonable to presume that gametangia are present all the year round. At the Dutch coasts VAN GOOR (l. c.) found gametangia from June—October. At the Isle of Man the species, according to KNIGHT and PARKE (1931, p. 55) likewise fructifies during the summer. The plants gathered by SCHMIDT (1935, p. 156) at Heligoland, deriving from the summer and autumn of 1934 were sterile.

The species, in the attached state is only found in Limfjorden as mentioned p. 21. According to information by professor R. SPÄRCK it may be met with in some localities of a salinity so low as 25 per mille. As appears from K. ROSENVINGE'S statement (1920, p. 131) it immigrated only recently. The finding of it was not recognised with certainty until 1920, at which time, indeed, it had already spread fairly widely, especially in the middle part of Limfjorden. According to professor R. SPÄRCK'S information it presumably appeared between 1913 and 1919; for in 1913 a marine biological course, during a period of 6 weeks, was held at Nykøbing, without *Codium* being noticed on that occasion.

That the immigration might be associated with changed hydrographic conditions is not likely. To be true, the North Sea has set its mark a little more in recent time than formerly on the water in Nissum Bredning in the westernmost part of the Limfjord, owing to a widening of the bed of the Thyborøn canal, but the changes have only been small (LUND 1940). Moreover, the species is most widely distributed in the middle part of Limfjorden, and in this place the change is less than in the western part. Consequently, these circumstances do not seem to indicate that

the species prefers North Sea-water to that of Limfjorden. — In this connection, however, it must be stressed that it is not necessary, as a matter of course, that the conditions of immigration coincide completely with the conditions of a continuous occurrence. The water in Nissum Bredning, in recent time more influenced by the North Sea, may possibly have been instrumental in its immigration; once having entered, it may have become acclimatised, and afterwards it may have penetrated farther into Limfjorden. — The writer thinks it likely, indeed, that its presence — like that of other species immigrated in recent time (Lund, l. c.) — is nothing but the result of a natural immigration.

During recent years (since 1933) loose, fertile plants were repeatedly gathered by scraping in the northern Kattegat near Frederikshavn, and fertile plants cast ashore are likewise noticed in this place. Attached plants[1], how-

[1] After having finished this work I succeeded, however, in finding attached plants, too, in this part of the Danish waters. For, during a visit at the harbour of Hirsholmene on the 19th of Juli 1940, several fructifying specimens of this species were found on the pier at the depth of 1 m. They were growing here, partly together with *C. dichotomum*, from which species they were, indeed, easily distinguished as to habitat, owing to the ramification and the coarser thallus as also to the darker colour. These plants, hence the first attached specimens which have been found in the Danish waters outside Limfjorden, were up to 50 cm in length and 8 mm in diameter. In one of these plants, in addition to dichotomous branching, a few lateral branches occurred, too (one of these reaching the length of about 11 cm). The utricles, being perfectly in accordance with the information given above, as to shape and dimensions, on an average had—like the utricles in loose specimens from the northern Kattegat—but a small mucron or the latter was even frequently absent. Attached plants as well as loose ones from the northern Kattegat thus seem to recall Cotton's var. *atlanticum* more than do the plants from Limfjorden.

The finding of attached specimens of *C. fragile* in the harbour of Hirsholmene is interesting by the fact that the time of the arrival of the species may be fixed with fairly great certainty. For in this locality during many years numerous scrapings have been carried out, by

ever, were not yet noticed with certainty in this water, unless the seedlings mentioned p. 12 might actually belong to *C. fragile*. Cast-up plants, moreover, were found a few times on the shore of western Jutland; not until 1930, however.

K. ROSENVINGE, in this case, as well as in that of the preceding species, in 1920 (p. 132) attempted to show a relationship between the immigration and the importation by ships. After the finding, in 1923, of a cast-up specimen at Büsum in Eiderstedt on the shore of western Schleswig-Holstein K. ROSENVINGE admitted in 1925 (p. 417) that there is some probability that the immigration might have taken place "by means of the coastal current, and not . . . by means of shipping".

I, too, suppose that the immigration is due to an importation of drifting plants. This is also indicated by the plants just mentioned, cast up on the western shore of Jutland. Loose and fructifying plants alive, when entering Nissum Bredning in the western part of Limfjorden, exposed to altered hydrographic conditions (during the summer season to an increase of temperature), might be imagined to begin reproduction, forming gametes.

The occurrence of the species at Heligoland seems, moreover, to support the idea of an immigration by means of drifting plants. In this island none but cast-up plants appeared, according to SCHMIDT (1935, p. 156); later, only (1930), attached plants, too, were found.

As to the distribution of the species in Europe it may be stated that it is known from the British coasts (Scotland, the Orkney Islands, the Isle of Man), the Irish west coast

professor ROSENVINGE as well as by the Marine Biological Summer course. As professor ROSENVINGE has been scraping in the harbour so late as 1933 and 1934, the immigration presumably has taken place in the course of these last six years.

and south west coast, Holland, Heligoland as also from the Norwegian south and west coasts. Its northern limit in Europe seems to be at about 60° north latitude on the western coast of Norway, its southern limit at the Dutch and Irish coasts. Whereas its occurrence at the Irish and Scottish coast does not date from recent time (Cotton, l. c. p. 116), the first Dutch specimens were not collected until 1900 (van Goor 1923, p. 133). At Heligoland the species was not noted in the attached state until 1930 (Schmidt 1935, p. 156). On the west side of Sylt Küster (1933, p. 335) found it "namentlich im Sommer 1930 ... überaus reichlich". The plants concerned, however, were probably loose. In Norway the species was first found in the skerries, in 1930, at Mandal by Arwidsson (1936, p. 100); later — in 1935 — near Bergen by Levring (1937, p. 34). In Sweden no finding of the species has been recorded up till now (Suneson 1939, p. 57).

Localities. **Ns:** Vorupør, a few places a little north of the pier, 6-VII-30, cast-up plants, not intact, bearing gametangia; plant of greatest length 33 cm (S. L.). — **Sk:** Tversted, 17-VII-35, 1 very vigorous specimen, intact, about 60 cm in length with gametangia, the utricles having a conspicuous mucron (often 40—70 μ in length) (S. L.). — **Lf:** the western and middle part. Particularly common in the middle part. **Kn:** Hirsholmene, August 1934, about 41 cm in length, loose plant, not intact, with gametangia (Glenstrup); Hirsholmene, the harbour, 19-VII-40, several attached plants with gametangia, about 50 cm in length and up to 8 mm in diameter (S. L.); inside Maren's Reef, about 8 m, 21-VII-34, about 9 cm in length, loose plants, not intact, with gametangia (L. K. R.); north of Hjellen, 3-VIII-33, about 18 cm in length, loose plants, not intact, with gametangia (Boye P.); at Syvstenen, 5 m, 7-VII-34, large loose plants, not intact, with gametangia, up to 33 cm in length (L. K. R.); Strandby, cast up (H. E. P.); Rønnerne at Frederikshavn, 6-VIII-33, about 18 cm in length, cast-up plant, not intact, with gametangia (Glenstrup).

Finally, a brief, diagrammatic survey of the chief characteristics, distinguishing the two species in the Danish waters, is given. The diagram concerns attached plants only.

Survey of the Danish *Codium*-species.

	C. dichotomum	*C. fragile*
Thallus, colour	dark green or a bright green; generally lighter than *C. fragile.*	dark green.
Thallus, ramification	dichotomous. In addition lateral branches generally very numerous. Branching more abundant than in *C. fragile.*	dichotomous. Lateral branches rare, rudimentary as a rule.
Thallus, length	up to well over 20 cm; generally not exceeding 20 cm.	up to 50 cm; generally exceeding 20 cm.
Thallus, diameter...	2—4 mm.	3—8 mm.
Utricles, size.....	generally 500—700 µ (—800 µ) × 300 µ; some larger.	generally 800—1050 µ × 225—465 µ; some larger.
The apices of the utricle, appearance and diameter	rounded (rounded conical) or with a hollow point, 4—12 µ in diameter; mucron may be found, 14—21 µ in diameter, if any.	Utricles from plants from Limfjorden generally with a mucron, 12—65 µ in length; utricles from the northern Kattegat with a short mucron, up to 12—16 µ (-20 µ) in length or mucron absent.— Old utricles often truncate.
Gametangia, number and location	1—a few (—3), on the middle part of the utricle.	1—a few (—7), a little above the middle of the utricle or on the whole of the lower half.
Gametangia, size	250—285 µ × 100—115 µ (—140 µ).	200—365 µ × 80—160 µ.
Vegetative buds.....	frequent.	not noticed.

Summary.

1. A description of Danish specimens of *C. dichotomum* and *C. fragile* is given as also a key for determination. Moreover, the distribution in Danish waters and the adjoining waters is mentioned. The immigration of the species, having taken place recently, presumably has been effected by drifting, fructifying plants.

2. *C. dichotomum* occurs in three types, distinguished by morphological characteristics, nevertheless closely allied, perhaps being stages of development of the same form. As to anatomy they are alike. In *C. fragile* the morphological appearance varies but slightly.

3. The utricles in Danish specimens of *C. dichotomum* in some cases attain considerably larger dimensions than is usually the case in this species. They are rather polymorphous. The ends of the utricles often bear a small point, which is generally, however, but slightly thickened. In some cases, indeed, it may be more thickened, being developed into a mucron, as in *C. fragile*. — The utricles in *C. fragile* generally are cylindrical-clavate. The old utricles are often truncate. In plants from Limfjorden they nearly always have a mucron, rather acute in fairly young and not entirely full-grown utricles, usually fairly rounded in old ones. In plants from the northern Kattegat the mucron is slightly developed only, or absent.

4. In both species the utricles are formed at the apices of the pith-filaments. In more rare cases, too, they may possibly be formed laterally.

5. Plugs occur in both species between the utricles and the pith-filaments, between the utricles and the gametangia, between the utricles and the hairs as also in the pith-

filaments. In *C. dichotomum* between the utricles and the vegetative buds, too. In the pith-filaments they appear mainly in the horizontally expanded basal layer, but they are found, too, in the pith-filaments of the erect frond.

6. The number of plugs at the base of the utricle is in both species as a rule equal to the number of lateral branches coming out from the latter. Between the utricle and the lateral branch(es) a plug is always found, whereas the plug not always, by far, is present in the pith-filament forming the utricle. Provided a plug is present it generally seems to form only after the first lateral branch has come out. In case the plug is formed in the base of the utricle, the utricle still being unbranched, the plugs between the utricles and the lateral branches, if any, as a matter of course do not appear until later.

7. Vegetative buds seem to be common in *C. dichotomum*; they may possibly be of importance for the spreading of the species.

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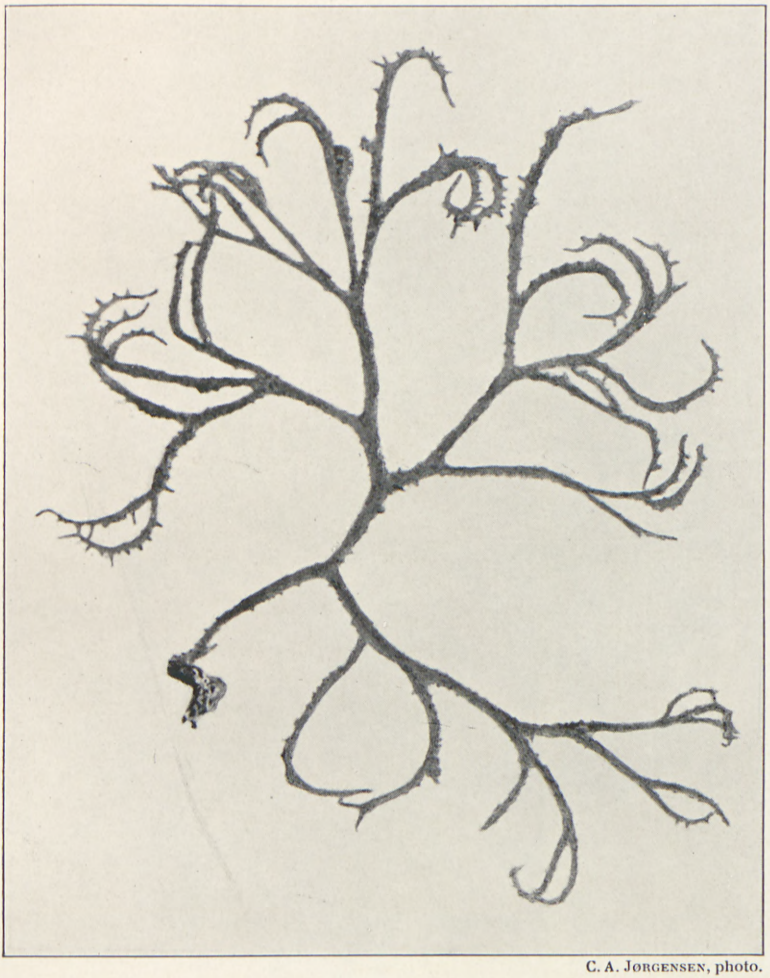
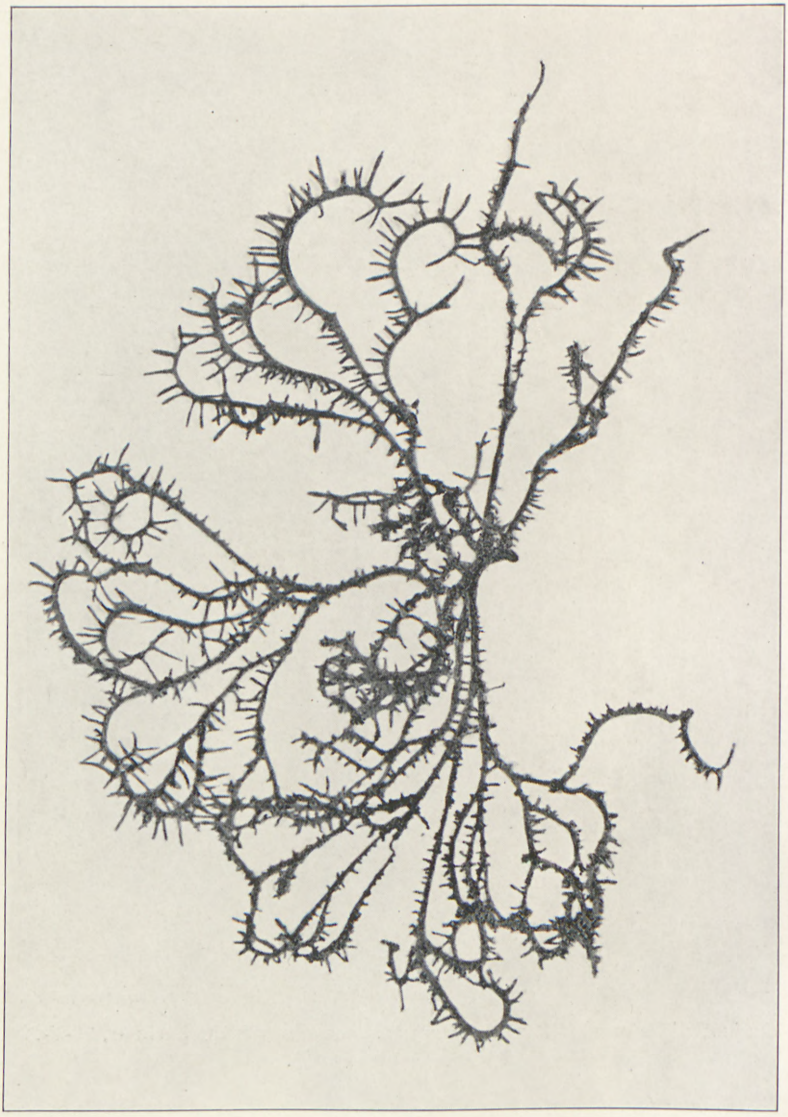


C. A. Jørgensen, photo.

Codium dichotomum (Huds.) Setchell. Hirsholmene, the harbour, 23-V-29 (L. K. R.) (about $^1/_2$).

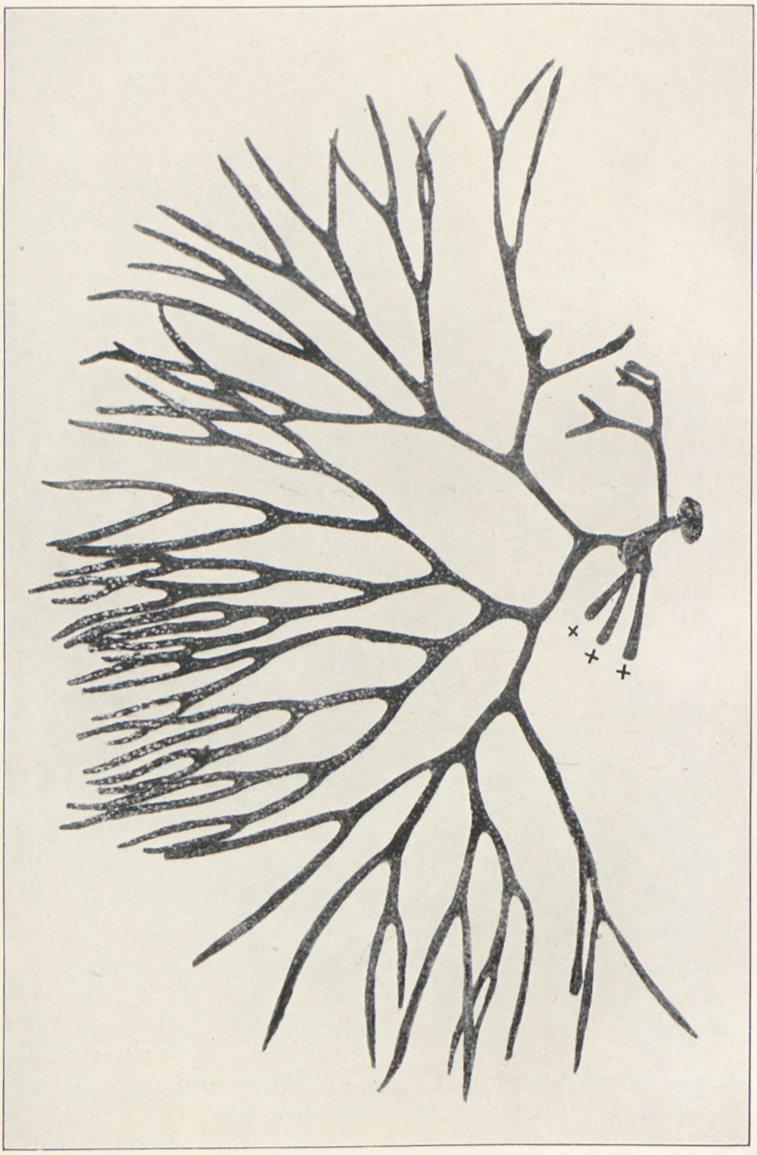


Codium dichotomum (Huds.) Setchell. Cast up at Hirsholmene, 17-VI-19 (Boye P.) (about 1/2).

C. A. Jørgensen, photo.

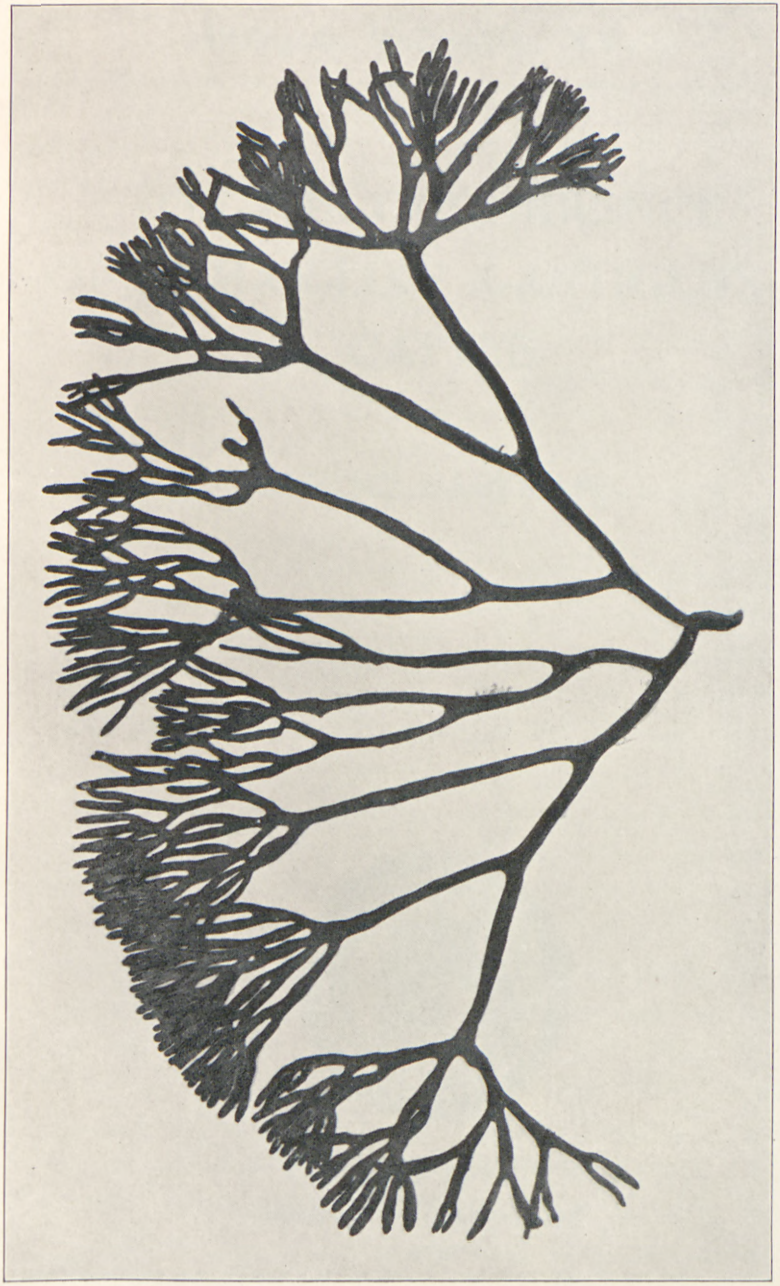
C. A. Jørgensen, photo.

Codium dichotomum (Huds.) Setchell. Hirsholmene, the harbour, 27-VII-21 (L. K. R). (about $^{2}/_{5}$).



C. A. Jørgensen, photo.

Codium fragile (Sur.) Hariot. Rønnen at Lem Vig, Limfjorden, 19-V-40 (S. L.). (about $^2/_5$).



Codium fragile (Sur.) Hariot. Oddesund Nord, Limfjorden, 4-VII-30 (S. L.). (about $^2/_5$).

C. A. Jørgensen, photo.

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ON THE OCCURRENCE OF HUMAN-LIKE A-ANTIGENS IN CATTLE

BY

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1. Introduction.

It is a well-known fact that antigens closely related to the A-antigen of the human organism are found in the organs and secretions of several of our domestic animals.

Thus A-antigen occurs in the saliva of horses (BRAHN and SCHIFF, LANDSTEINER, FRIEDENREICH and THYSSEN), in commercial pepsine, i. e. in the stomach wall of pigs, (SCHIFF and WEILER, BRAHN, SCHIFF and WEINMANN) and in peptone prepared by maceration of the stomachs of pigs (OTTENSOOSER). Further WITEBSKY and ZEIZIG have shown that alcoholic extracts of the duodenum and the abomasus from cattle exhibit large amounts of antigens reacting with immune anti-A. The secretions from cattle also, however, contain A-antigen, as established by FRIEDENREICH. The latter author examined saliva from 85 cows 49 % of which were found to react with human iso-serum anti-A. It should further be noted that cattle, although not belonging to the group of animals possessing the Forssman heterogenetic antigen, would seem to contain A-antigen in their blood corpuscles (WITEBSKY). This author found that blood corpuscles from about 25 % of the cows examined reacted with human immune anti-A sera while the remaining cows exhibited, in their sera, an antibody capable of agglutinating human A blood corpuscles with an intensity far greater than blood corpuscles of other groups.

The aim of the present investigation has been to examine, in some details, the content of A-antigen in the saliva and

organs of cattle in order to form an idea of the quantitative distribution and genetics of this antigen.

2. The Methods used for the Determination of the Antigen Concentrations.

For the examination of secretions (saliva) and aqueous extracts the agglutination-inhibition method was employed in the following form: Two series of small test tubes were used in the determination of the antigen content of each sample. In the first tube of each series 0.1 cc of the original antigen solution was introduced, (concentration 1). In the second tube of each series 0.1 cc of a concentration $^1/_2$, in the third 0.1 cc of a concentration $^1/_4 = \frac{1}{2^2}$, in the fourth 0.1 cc of a concentration $^1/_8 = \frac{1}{2^3}$, etc. Thus in tube number n 0.1 cc of an antigen solution of concentration $\frac{1}{2^{n-1}}$ was introduced. Hereafter 0.1 cc of a serum anti-A was introduced into all the tubes of one of the two series while 0.1 cc of a serum anti-B was added to the contents of all the tubes of the other series. Thus after this operation the antigen concentrations in the tubes of each of the two series were $\frac{1}{2^1}, \frac{1}{2^2}, \frac{1}{2^3}, \ldots\ldots \frac{1}{2^n}$. In the following experiments the same iso-anti-A (Ulla $^1/_{16}$) was employed throughout all the investigations. This serum proved fairly constant in strength. Different iso-anti-B sera were employed, but no reaction with anti-B was ever found. After the introduction of serum the test tubes were kept at about 20° C. for an hour when washed blood corpuscles of group A_1 and B were added, the A_1 blood corpuscles to the test tubes with serum iso-anti-A, the B blood corpuscles to the test tubes

with serum iso-anti-B. After two hours the tubes were shaken. In one of the two series agglutination is seen to take place in all the tubes (generally the series with anti-B), while in the other, agglutination occurs only in the tubes following a certain number n (the series with anti-A). The number n is taken as a measure of the antigen concentration in the original antigen solution and is termed the titer reading for this solution. It may be noted that n is the power of 2 in the expression for the antigen concentration in tube number n after the addition of serum.

For the determination of the antigen content in alcoholic extracts the complement fixation method (with sheeps' blood as test blood corpuscles) was employed. The technique adopted for this method is chiefly the same as that of the agglutination-inhibition method. The main difference is that with the former method the serum, instead of the antigen, is titrated in a series of tubes according to the scheme indicated above. The antigen concentration or the "titer reading" is the number of the last tube in which complete inhibition of the hemolysis of the sheeps' blood corpuscles still takes place. As antibody was employed an immune serum anti-A produced by immunisation of rabbits with human blood corpuscles of group A_1. The serum was used in a dilution $^1/_{10}$ and absorbed with blood corpuscles of groups B- and O-MN.

If we consider a large number of samples from different subjects and want to know the range within which a certain quality, characteristic of these samples, varies, i. e. what is here termed the indefiniteness of this quality, it is first of all necessary to obtain an idea of the uncertainty of the method used for the measurement of the quality in question. In order to achieve this knowledge of the un-

certainty with the two methods indicated above, 10 titer readings with the same antigen, the same serum, the same test blood corpuscles and, in the case of the complement fixation method, the same complement and the same amboceptor were taken. The observations were further taken on the same day.

So the fluctuations in the results must be solely due to the uncertainty of the method. As a measure of this uncertainty the standard deviation μ for the ten observations was chosen. Two instances of these tests are given below, the one corresponding to the agglutination-inhibition method and the other to the complement fixation method. In both cases the standard deviation amounts to about half a titer.

Table I.

Repeated Observations on the Inhibition of the Isoagglutination by Saliva from Cow D. 11.

Exp. 22-7-39	⟨ Titers *n* ⟩									
Saliva from ...	2	3	4	5	6	7	8	9	10	11
Cow D. 11 ...	0	0	0	0	0	(+)	+	+++	+++	+++
— ...	0	0	0	0	0	0	+	+(+)	++	+++
— ...	0	0	0	0	0	0	+	++	+++	+++
— ...	0	0	0	0	0	+	+(+)	++	++	+++
— ...	0	0	0	0	0	0	+	+++	+++	+++
— ...	0	0	0	0	0	(+)	+	++	++	+++
— ...	0	0	0	0	0	0	+	++	+++	+++
— ...	0	0	0	0	0	(+)	+	++	+++	+++
— ...	0	0	0	0	0	0	(+)	+	++	+++
— ...	0	0	0	0	0	0	+	+(+)	++	+++

Average Titer: 6.6
Standard Deviation μ: 0.52

Table II.

Repeated Observations on the Inhibition of the Hemolysis of Sheeps' Bloods by Complement Fixation with an Alcoholic Extract of Duodenum from Cow C. 3.

Exp. 7-11-39	Titers *n*						
Alcoholic Extract of	1	2	3	4	5	6	7
Duodenum from Cow C. 3 ..	0	0	0	10	100	100	100
— ..	0	0	0	10	30	100	100
— ..	0	0	0	0	20	100	100
— ..	0	0	0	0	80	100	100
— ..	0	0	0	100	100	100	100
— ..	0	0	0	0	60	100	100
— ..	0	0	0	10	80	100	100
— ..	0	0	0	10	80	100	100
— ..	0	0	0	20	80	100	100
— ..	0	0	0	0	10	80	100
	Average Titer: 3.4 Standard Deviation μ: 0,52						

So the two methods are judged to be fairly satisfactory and suited for the quantitative investigations here dealt with.

It may be noted that two similar tests on the agglutination inhibition method were performed and gave $\mu = 0.53$ and 0.88 respectively. Quite obviously the close agreement between the values for μ derived from Table I and Table II is accidental.

3. The Concentration of A-Antigen in Saliva from Cattle.

A considerable number (562) of saliva samples from cows was examined in order to elucidate the fluctuations

in the concentration of the A-antigen within a random group of cows. Before the individual fluctuations or "the sample to sample indefiniteness" could be derived, it was, however, necessary to find out how large might be the normal fluctuations in the course of time for the saliva from a single cow. The latter fluctuations were determined from observations on four cows, the observations extending over one week and the samples being collected at different times of the day. The samples were boiled immediately after collection and then kept in a cool place to avoid destruction of the antigen. All the samples thus collected were tested at the same time by the agglutination-inhibition test, identically the same serum being employed in all the tests.

Table III shows the titer readings for all the samples in question. From the observations on "cow 23" and "cow 1" were derived the standard deviations μ_R; they were found to be 0.88 and 0.70 respectively. The observations on "cow 6" and "cow 18" were less suited for the determination of the indefiniteness, seeing that they include zero readings, i. e. readings covering all values below titer 1. Now, what has here been determined is not solely the fluctuations in the course of time or the pure "time to time indefiniteness". The fluctuations also reflect the uncertainty μ_M of the method. The latter was found to be $\mu_M = 0.53$ titers expressed in terms of the standard deviation. The actual time to time indefiniteness μ_T is determined by:

$$\mu_R^2 = \mu_T^2 + \mu_M^2 .$$

Introducing $\mu_M = 0.53$ and $\mu_R = \frac{0.88 + 0.70}{2} = 0.79$ we find $\mu_T = 0.59$ titers. This figure will be used below in

Table III.

Fluctuations of the Antigen Content in the Course of Time for Salivas from 4 Cows.

Cow 23 Nøruplund		Cow 6 Nøruplund		Cow 18 Nøruplund		Cow 1 Nøruplund	
Sample taken:	Titer	Sample taken:	Titer	Sample taken:	Titer	Sample taken:	Titer
2—8. 11^{30} a.m.	4	2—8. 11^{30} a.m.	0	2—8. 11^{30} a.m.	0	2—8. 11^{30} a.m.	1
— 5 p.m.	2	3—8. 5 a.m.	1	3—8. 5 a.m.	0	3—8. 5 a.m.	1
3—8. 5 a.m.	5	4—8. 5 p.m.	1	4—8. 5 p.m.	1	4—8. 5 p.m.	3
— 5^{30} p.m.	4	5—8. 5 a.m.	1	5—8. 5 a.m.	0	5—8. 5 a.m.	1
4—8. 5 a.m.	5	— 5 p.m.	1	— 5 p.m.	2	— 5 p.m.	2
— 4 p.m.	4	6—8. 5^{30} a.m.	2	6—8. 5^{30} a.m.	1	6—8. 5^{30} a.m.	2
5—8. 5 a.m.	4	7—8. 5 a.m.	1	7—8. 5 a.m.	1	7—8. 5 a.m.	2
— 5 p.m.	5	— 5^{30} p.m.	1	— 5^{30} a.m.	2	— 5^{30} p.m.	1
6—8. 5^{30} a.m.	4	8—8. 6 a.m.	2	8—8 6 a.m.	1	8—8 6 a.m.	2
7—8. 5 a.m.	3	4^{30} p.m.	3	— 4^{30} p.m.	2	— 4^{30} p.m.	1
— 5^{30} p.m.	5						
8—8 6 a.m.	5						
— 4^{30} p.m.	4						
Average Titer: 4.2		Average Titer: 1.3		Average Titer: 1.0		Average Titer: 1.6	
Standard Deviation:		Maximum Deviation:		Maximum Deviation:		Standard Deviation:	
μ_R = 0.88 Titers		1.7 Titers		1.0 Titers		0.70 Titers	

the determination of the actual sample to sample indefiniteness.

We now proceed to consider the main results comprising the 562 observations on the random group of cows referred to above. The material was obtained from cattle in "Kødbyen" in Copenhagen. (The Municipal Slaughter Houses, Copenhagen). The saliva was collected with a spoon immediately after the animal was killed. The cows were of different breeds, most of them adult animals, but the material also included some not quite young bull calves. The salivas were boiled, centrifuged and then titrated for

their inhibition of the isoagglutination. The results are represented in Table IV and in the distribution diagram fig. 1 where the percentages are plotted against the corresponding titer readings.

Judging from the diagram it would seem that A-antigen is a constituent in all cattle, in contradistinction to the

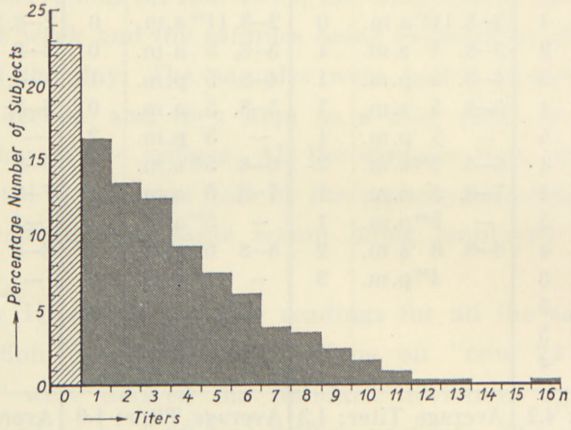

Fig. 1. Distribution Diagram for the Concentration of A-Antigen in Saliva from Cattle. (562 Samples from different Subjects).

opinion held by former authors. It should here be noted that the titer reading 0 does not indicate an absolute lack of A-antigen but only a concentration lower than the concentration corresponding to the titer reading 1. It appears further from fig. 1, that in relatively few cows only there is a higher amount of A-antigen. The shape of the block diagram suggests that the actual distribution curve for the antigen concentration may have the form shown in fig. 2.

The curve below titer 1 has been drawn in such a way that the area between this curve and the axis of abscissa is about 22.6 % of the whole area under the curve, corresponding to the relative number of the titer readings 0,

and otherwise drawn as simply as possible. The fictive titers 0, —1, and —2 would correspond to antigen solutions produced from the original solution in tube 1 by evaporising the latter (without serum) to $^1/_2$, $^1/_4$ and $^1/_8$ of its original volume. The curve thus obtained greatly resembles other distribution curves for biological qualities when a logarithmic scale is used for the quality. Such curves are very often characterised by a steep rise to a maximum followed by a slower fall in the direction of higher values for the quality in question. This is true for instance for the distribution of antigen concentrations in saliva from man (the secreter type). From fig. 2 the standard deviation μ_R' was found to be 2.6 titers. This, however, is not, as will now be understood, a true measure of the individual fluctuations, or the "sample to sample indefiniteness" μ_S of the antigen concentration within a random group of cows, seeing that μ_R' also includes the "time to time inde-

Table IV.
Titer Distribution for 562 Saliva Samples from Cattle.

Titer n	Saliva Samples	
	Absolute Number	Percentage Number
0	127	22.6
1	92	16.4
2	75	13.3
3	69	12.3
4	52	9.2
5	41	7.3
6	34	6.0
7	22	3.9
8	20	3.6
9	13	2.3
10	9	1.6
11	5	0.9
12	1	0.2
13	1	0.2
14	0	..
15	0	..
16	1	0.2

finiteness" μ_T for the single subject and further the indefiniteness μ_M due to the uncertainty of the method. The resultant μ_R of the latter two indefinitenesses was found

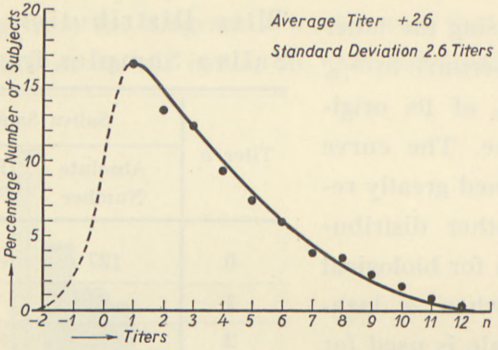

Fig. 2. Presumed Distribution of the Concentration of A-Antigen in Salivas from Cattle.

to be 0.79 titers. Thus the actual "sample to sample indefiniteness" μ_S is to be determined from:

$$\mu_R'^2 = \mu_S^2 + \mu_R^2 \qquad \text{or}$$

$$\mu_S = \sqrt{2.6^2 - 0.79^2} = 2.48 \quad \text{or 2.5 titers,}$$

which means that μ_R' may practically be taken as a measure of the "sample to sample indefiniteness" for the group in question.

4. The Heredity of the A-Antigen in Cattle.

The simple shape of the curve in fig. 2 suggests several possibilities with regard to the genetics of the A-antigen in cattle. In the first instance the possibility obtains that the faculty of developing A-antigen depends on genes present in all subjects. The whole interval of fluctuations in fig. 2

would in that case simply represent the width of the interval of indefiniteness for these genes, and family investigations would not then probably disclose any relation between the antigen concentration in parents and offspring.

Because of the great variation of the antigen concentration in the different subjects it would perhaps seem more likely that the development of antigen depends on multiple factors just as many other qualities which, within a group of subjects, exhibit a continuous quantitative variation. In that case family investigations should as a rule disclose an offspring with an antigen concentration between those of the parents.

Finally the possibility obtains that the heredity of the A-antigen in cattle is the same as that of blood groups in man, i. e. a heredity depending on multiple allelomorphs similar to the genes of the A_1, A_2, A_3, A_4 system of man. (THOMSEN, FRIEDENREICH and WORSAAE, FRIEDENREICH, GAMMELGAARD and MARCUSSEN). The objection might be raised that in the latter case a distribution curve with several maxima might be expected. In this connection it must, however, be noted that distribution curves may overlap to such a degree as to render impossible or at least difficult the distinction between them. This is for instance true in the case of curves representing the distribution of the antigen concentration in saliva from A_1 and A_2 persons (secreters). Here the joint distribution curve for the two groups has only one maximum, although each group may be shown to possess a separate curve, the two curves, however, lying very close to each other.

In order to find out which of the three genetical theories is the most likely a number of family investigations was carried out. Three of the most common races in Denmark

were examined. None of these three races differed from the others with regard to the antigen content, as will be seen from Table V.

Table V.

Family Investigation on the Concentration of A-Antigen in Salivas from Cattle.

Farm	Parents				Offspring				Race
	Bull	Titer	Cow	Titer	Cow	Titer	Calf	Titer	
Langø	Terp	8	149	8	..	..	T 149	10	
(Lundstein)	Jan	0	—	—	..	..	J 149	4	
	Sten	..	116	0	143	1	..	..	
	Terp	8	143	1	..	..	T 143	9	Guernsey
	—	—	91	0	20	3	T 91	6	
	—	—	20	3	..	..	T 20	3	
	—	—	1	7	..	..	T 1 a	11	
							T 1 b	16	
	Jan	0	117	11	..	..	J 117	1	
	—	0	96	1	22	9x	..	..	
	Terp	8	9	0	21	0	T 9	5	
	Jan	0	—	—	23	1	J 9	3	
	Sten	..	—	—	89	0	..	..	
	Terp	8	21	0	..	..	T 21	3	
	Jan	0	38	4	..	..	J 38	3	
	Terp	8	87	2	137	3	T 87	4	
	—	—	137	3	164	3	T 137	5	
	Sten	..	—	—	170	1	..	..	
	Terp	8	164	3	..	..	T 164	8	
	—	—	97	9	..	..	T 97	10	
	Sten	..	—	—	168	10	..	..	
	Jan	0	168	10	..	..	J 168	7	
Gydegaard	Bonde	1	36	6	..	..	B 36	4	Guernsey
(Bonde)	—	—	37	5	..	..	B 37	15	(Breed:
Drammelstrup	—	—	77	5	..	..	B 77	4	Højager)
Skovly	Poul	7	1	1	..	..	P 1	9	
(Hytting	—	—	2	0	..	..	P 2	6	Guernsey
Petersen)	—	—	3	6	..	..	P 3	6	(Breed:
Tirstrup	—	—	4	0	..	..	P 4	5	Højager)
	—	—	5	4	..	..	P 6	6	

Table V (continued).

Farm	Parents				Offspring				Race
	Bull	Titer	Cow	Titer	Cow	Titer	Calf	Titer	
Bertel Lassen, Tversted	Bertel	9	64	5	..	..	B 64	3	Cross between: Guernsey and Jerseys
	—	—	54	1	..	..	B 54	1	
	—	—	66	4	..	..	B 66	9	
Kallehave	Tyr	8	17	1	..	..	T 17	2	Jersey
Herman Andersen, Raabjerg	Peter	2	12	1	..	..	P 12	10x	Jersey
	—	—	27	1	..	..	P 27	1	
	—	—	26	2	..	..	P 26	2	
Mandrup Andersen, Raabjerg	Mandrup	2	14	1	..	..	M 14	1	Jersey
	—	—	16	8	..	..	M 16	13	
Christen Winther, Tversted	Kræn	0	1	7	..	..	K 1	10	Holstein
	—	—	7	2	..	..	K 2	2	
	—	—	8	2	..	..	K 8	2	
	—	—	5	3	..	..	K 5	3	
	—	—	21	2	..	..	K 21	2	
	—	—	66	4	..	..	K 66	8	
Mrs. Graff, Raabjerg	Graff	1	7	2	..	..	G 7	3	Jersey
	—	—	9	4	..	..	G 9	5	
	—	—	75	8	..	..	G 75	2	

In this table 49 families are considered, each comprising the parents and at least one of the offspring. The latter was in most cases a very young calf sometimes not more than a few days old. Within the Langø-stock only, adult subjects were found among the offspring. These subjects are indicated in the table. The denomination "calf" in the table also covers some cow-calves. For instance T 1 a was a cow-calf, while T 1 b was only five days old. Summing up the results of the investigations it was found that out of 49 of the offspring 14 had the same antigen concentration

as one of the parents; in seven of the cases the same as the strongest of the parents, in the other seven cases the same as the weakest of the parents. Again, 19 had a concentration lying between the parents and 16, i. e. about 33 %, had a concentration higher than the strongest of the parents. None of the calves exhibited a lower antigen concentration than the weakest of the parents. This fact would seem to indicate that the antigen concentration of the young subject lies on a higher level than that of the adults. This difference in concentration is most clearly illustrated in the block diagram fig. 3 a—b representing separately the distribution curves for the calves and their parents. The average titer for the calves would seem to be displaced by about two titers upwards relatively to that for the parents.

Now, however, no definite answer as to the question of the heredity of the A-antigen can be derived from the investigations considered above, simply because of the difference in the levels of antigen concentrations of "infants" and adults. Unfortunately it proves practically impossible to obtain a material of parents and adult offspring. It would seem, however, that a certain quantitative relationship obtains between the antigen concentrations of parents and offspring. Two exceptions only, indicated by a cross in the table, were found in which two parents with practically no antigen content had offspring with a high antigen content. In one of the cases, that of the Langø stock, there was, however, some doubt with regard to the paternity in so far as the period of gestation was rather long. The stock was bred on a little isolated Danish island and at the time of conception two bulls were found on the island, "Jan", the assumed father, and "Sten" a bull killed before the

investigation took place. Examination of "Sten's" descendants did not, however, suggest any greater antigen concentration for this bull, (compare Table V). In the second case, that of the Herman Andersen stock, there was no

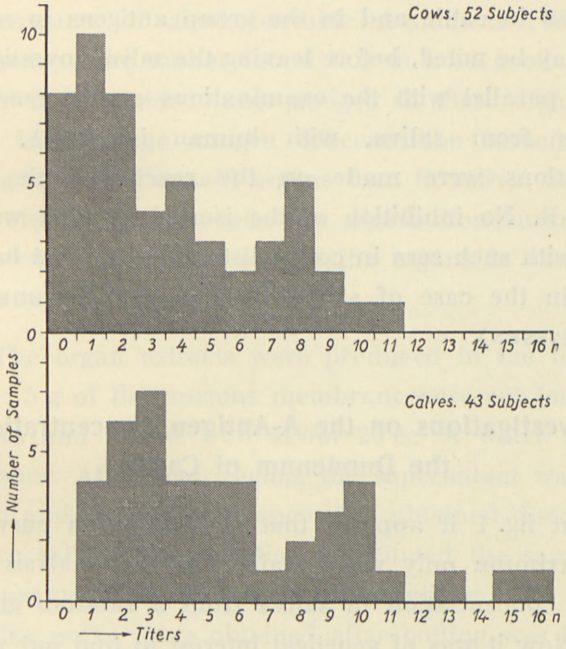

Fig. 3. Distribution Diagrams for the Concentration of A-Antigen in Salivas from Cattle.

other reason for suspicion as to the paternity than the fact that the neighbour's bull, also a Jersey bull, was considered by Mr. Andersen a better breeding animal than his own, and then perhaps that the pastures of the two stocks were adjacent to each other.

The experience that a certain quality, here the antigen concentration, was more pronounced at an early age than later on was found by the author in the group antigens

in saliva from man. Here the group antigen of the so-called non-secreters is as a rule distinctly developed at the time of birth, and disappears in the course of the first year[1]. Thus, on this point, the family investigations disclosed an interesting analogy between conditions as revealed in the A-antigen in cattle and in the group antigens in man.

It may be noted, before leaving the saliva investigations, that in parallel with the examinations on the reaction of antigens from saliva, with human iso-anti-A, similar observations were made on the reaction with human iso-anti-B. No inhibition of the isoagglutination was ever found with such sera in contradistinction to what has been found in the case of saliva from horses (Friedenreich and Thyssen).

5. Investigations on the A-Antigen Concentration in the Duodenum of Cattle.

From fig. 1 it appears that a distribution curve with one maximum only was obtained in the analysis of the antigen concentration in saliva from a random group of cattle. Now it was of genetical interest to find out whether this curve is made up of two or more distribution curves slightly displaced with regard to each other, or whether it constitutes one single distribution curve only. In this connection the following experience of the author should be recalled. Between the two human types "secreters" and "non-secreters", with regard to group antigens, there is only a quantitative difference in the antigen content. A particular organ thus exhibits two distribution curves, one

[1] The Paper on this subject has not yet been published.

for "non-secreters" and another for "secreters". Now, if the antigen content is comparatively high the two curves are distinctly separated, while in the case of a comparatively low concentration they will almost coincide. It is known that the duodenum and the abomasus in cattle contain great amounts of alcohol-soluble A-antigens (WITEBSKY and ZEIZIG). The author found this to hold good also for the content of water-soluble antigen in these organs, and further the average antigen concentration turned out to be far greater in the said organs than in saliva. So it was found wise to examine also the distribution curve of the antigen concentration in one of the organs in cattle. The duodenum was chosen.

The organ extracts were produced in the following way: 5 g of the mucous membrane were cut into small pieces and boiled with about 20 cc of water for half an hour. After centrifuging, the supernatant was vaporised and the dry substance thus obtained dissolved in 2.5 cc saline. This solution constituted the sample for the examination of water-soluble antigen.

The organ mass obtained after boiling was kept for ten days in 55 cc of 99 % alcohol when the organ mass was removed. The remainder formed the extract containing the alcohol-soluble antigens. Before the test the alcoholic extract was vaporised and the dry substance suspended in a volume of saline equal to that of the original alcoholic extract.

The investigation comprised 150 aqueous extracts and 105 alcoholic extracts from the duodenum. In Table VI the titers obtained with the different extracts are given.

Table VI.

Concentrations of A-Antigen in the Duodenum from a random Group of Cows. In the Titer Columns the left Hand Figures correspond to aqueous Extracts, the right Hand Figures to alcoholic Extracts.

Duodenum No.	Titer n		Duodenum No.	Titer n		Duodenum No.	Titer n		Duodenum No.	Titer n	
K. 3	13	4	C. 1	4	3	H. 10	1	3	J. 12	12	2
K. 6	12	3	C. 2	6	2	I. 1	16	3	J. 13	15	0
K. 71	17	4	C. 3	17	4	I. 2	13	3	J. 14	7	1
K. 83	13	3	F. 1	6	5	I. 3	17	4	J. 15	13	4
U. 1	15	3	F. 2	17	5	I. 4	19	5	J. 16	16	0
U. 2	15	5	F. 3	8	1	I. 5	9	2	J. 17	8	2
U. 3	15	4	F. 4	6	0	I. 6	14	5	J. 18	10	1
U. 4	6	4	F. 5	19	5	I. 7	7	4	J. 19	16	1
U. 12	15	3	F. 6	6	6	I. 8	10	1	J. 20	18	2
U. 13	1	3	F. 7	20	4	I. 9	16	3	L. 1	0	
U. 14	3	3	F. 8	5	6	I. 10	20	0	L. 2	7	
U. 15	3	0	F. 9	11	3	I. 11	9	4	L. 3	11	
U. 16	6	0	F. 10	5	3	I. 12	17	0	L. 4	10	
U. 17	14	4	G. 1	4	0	I. 13	5	5	L. 5	3	
U. 18	4	5	G. 2	10	3	I. 14	5	3	L. 6	4	
U. 19	16	4	G. 3	3	1	I. 15	14	5	L. 7	10	
U. 20	4	0	G. 4	5	1	I. 16	13	0	L. 8	3	
U. 21	5	5	G. 5	18	2	I. 17	9	0	L. 9	14	
U. 22	3	5	G. 6	5	1	I. 18	15	3	L. 10	5	
U. 23	14	5	G. 7	9	0	I. 19	13	2	L. 11	2	
B. 1	9	4	G. 8	4	0	I. 20	12	3	L. 12	3	
B. 2	6	4	G. 9	19	3	J. 1	15	5	L. 13	7	
B. 3	16	5	G. 10	1	0	J. 2	5	0	L. 14	2	
B. 4	7	4	H. 1	4	3	J. 3	16	0	L. 15	0	
B. 5	3	1	H. 2	13	3	J. 4	4	4	L. 16	12	
B. 6	14	4	H. 3	10	4	J. 5	11	2	L. 17	2	
B. 7	4	2	H. 4	5	0	J. 6	6	2	L. 18	12	
B. 8	4	1	H. 5	4	0	J. 7	14	3	L. 19	11	
B. 9	3	0	H. 6	7	4	J. 8	5	0	L. 20	8	
B. 10	2	1	H. 7	5	0	J. 9	9	0	L. 21	2	
B. 11	14	4	H. 8	2	2	J. 10	18	3	L. 22	11	
B. 12	10	2	H. 9	3	2	J. 11	8	1	L. 23	16	

Table VI (continued).

Duodenum No.	Titer *n*	Duodenum No.	Titer *n*	Duodenum No.	Titer *n*	Duodenum No.	Titer *n*
L. 24	4	L. 33	15	L. 39	12	L. 42	2
L. 25	7	L. 34	17	L. 27	1	L. 43	0
L. 26	3	L. 35	6	L. 28	2	L. 44	12
L. 30	3	L. 36	4	L. 29	11	L. 45	7
L. 31	9	L. 37	14	L. 40	13	L. 46	3
L. 32	3	L. 38	5	L. 41	4		

Both the titers for the aqueous extracts and for the alcoholic extracts are stated, the first in the column to the left, the last in the column to the right. Considering the aqueous extracts, i. e. the titers measuring the content of water-soluble antigen in the duodenum, it should be noted that scarcely any samples, at least only 3 out of 150, show the titer 0. This is in good agreement with the conception stated above that all cows contain A-antigen. A block diagram and a smoothed-out distribution curve was drawn for the titers of the aqueous extracts. In contradistinction to the block diagram drawn for the saliva samples the duodenum diagram shows a rather irregular shape suggesting that it is, in fact, to be considered the resultant of two or perhaps three curves with maxima at about titer 4, (titer 9), and titer 14. This is indicated in the smoothed-out curve above the block diagram. Thus the suspicion that several quantitatively different A-types obtain in cattle would seem justified.

These investigations, however, were carried out with aqueous extracts, i. e. on antigens in the same, water-soluble form as the antigens found in saliva, while WITEBSKY'S observations on A-antigen in the duodenum were made on alcoholic extracts. Now, passing on to the alcoholic

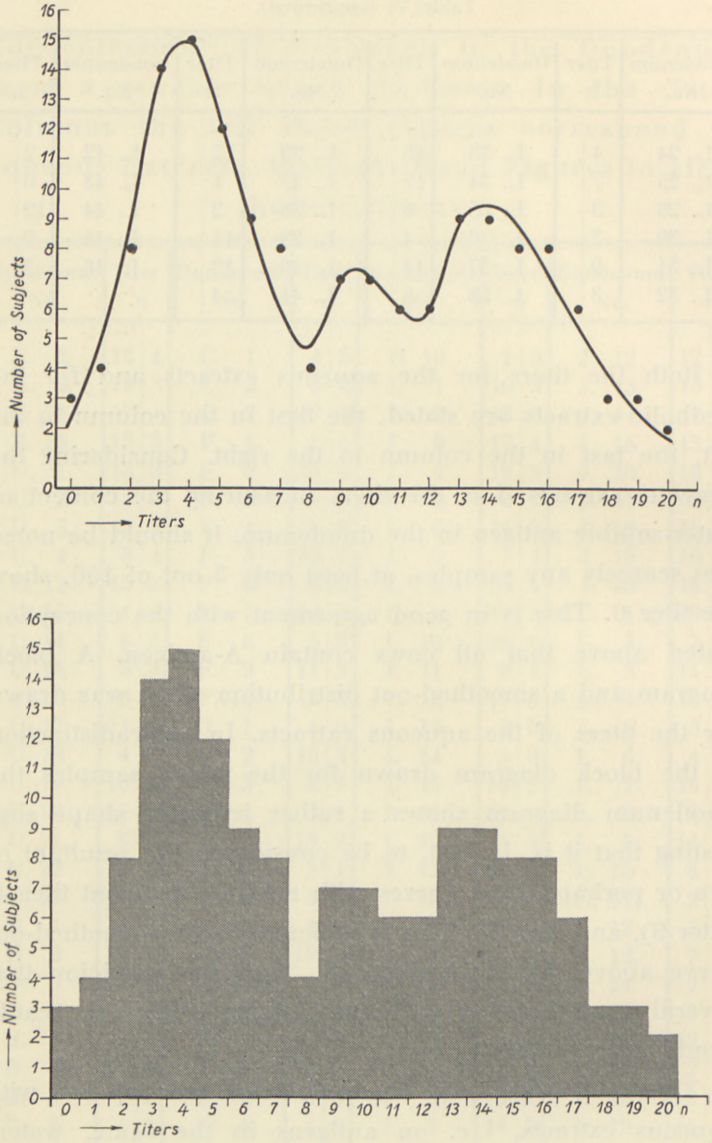

Fig. 4. Distribution Diagrams for the Concentrations of A-Antigen in aqueous Extracts of the Duodenum from Cattle. (Samples from 150 Subjects).

extracts of the present investigation, it appears from Table VI and from the block diagram fig. 5 that the titers are here much more uniform and do not suggest a division into several groups. It is particularly to be noted that no relation at all would seem to obtain between the water-soluble and the alcohol-soluble antigens with regard to their concentrations. As in the case of the saliva samples here also it may be maintained that all the extracts contain a certain amount of A-antigen, seeing that titer 0 stands for all concentrations below titer 1. It was stated that the alcoholic extracts were made from the organ mass after the production of the aqueous extracts. This would seem the right thing to do in so far as it rendered possible a comparison of two extracts from the same sample. Otherwise the objection might be urged that some of the alcohol-soluble antigens may be added to the aqueous extracts as a result of the boiling, or that a certain amount of water-soluble antigens left in the organ mass may be extracted by the 99 % alcohol. It was, however, established beyond doubt that these two possibilities can be neglected. Three samples of duodenum with high antigen concentrations were boiled 6 to 7 times with fresh water until all traces of water-soluble antigens were removed. After this procedure alcoholic extracts were made from the remaining organ mass. These organ extracts turned out to be just as powerful

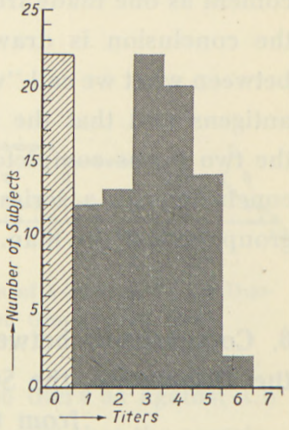

Fig. 5. Distribution Diagram for the Concentration of A-Antigen in alcoholic Extracts of the Duodenum from a Random Group of 105 Cows.

as, or even a little more so than, extracts produced from fresh organ samples. Again, the inverse experiment was performed. After the production of an alcoholic extract the mass left was used for the production of an aqueous extract. This extract likewise showed the same antigen content as one made from fresh organ. From all these facts the conclusion is drawn that a sharp distinction obtains between what we call "water-soluble" and "alcohol-soluble" antigens and that the two methods of extraction separate the two forms completely. The author was led to the same conclusions in a series of investigations on the content of group antigen in man.

6. Comparisons between the Antigen Concentrations in the Duodenum, the Submaxillary Gland and the Saliva from the same Subjects.

It may be asked whether there is any correlation between the antigen concentrations in the duodenum and the saliva—in spite of the distribution curves being rather different. In order to settle this question the duodenum and a saliva sample from each of 50 subjects were compared. The result of the comparison is represented in fig. 6.

The samples are arranged according to their "duodenum titer", so that the highest values are plotted to the left above the axis of abscissa. It appears that no very distinct correlation obtains between the titer values for the two substances. It would, however, seem safe to say that the average antigen concentration in saliva from subjects with high concentrations in the duodenum is distinctly higher than the corresponding value from subjects with a low concentration in the duodenum. If titer 9 is taken as the border

titer between high and low values for the antigen concentration in the duodenum, the average titer for saliva within

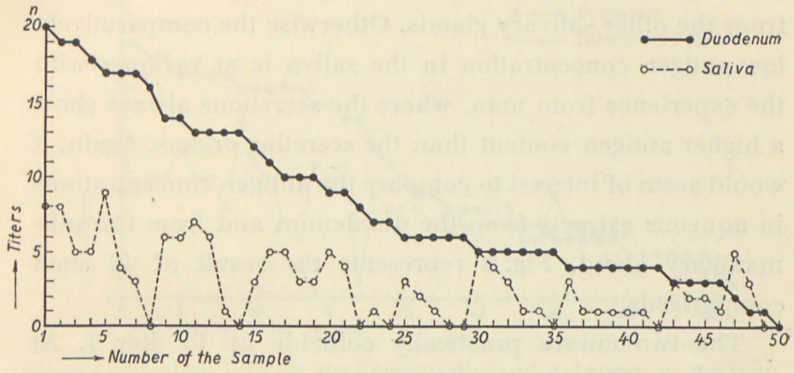

Fig. 6. Comparison between the Concentrations of A-Antigen in the Duodenum and in the Saliva from a Random Group of 50 Cows.

the "strong" group is found to be 4.6 titers as against 1.6 titers in the "weak" group. It was thought that the correlation might prove more distinct in the case of a comparison between saliva and an extract from a salivary gland, e. g. the submaxillary gland. So such a comparison was made and the results plotted, fig. 7, in a similar way to that shown in fig. 6.

Quite obviously the agreement is much more pronounced than in the case of the duodenum and the saliva.

As a rule saliva would seem to exhibit a lower antigen content than the corresponding gland. This may

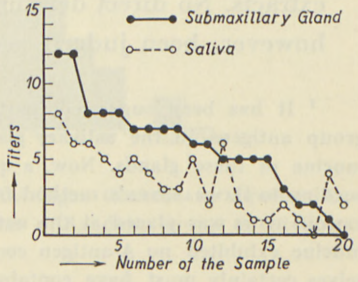

Fig. 7. Comparison between the Concentrations of A-Antigen in the Submaxillary Gland and in the Saliva from a Random Group of 20 Cows.

be due to the complex character of the saliva which undoubtedly contains secretions both from the parotid gland—which gland has a rather low antigen concentration—and from the other salivary glands. Otherwise the comparatively low antigen concentration in the saliva is at variance with the experience from man, where the secretions always show a higher antigen content than the secreting organs. Again, it would seem of interest to compare the antigen concentrations in aqueous extracts from the duodenum and from the submaxillary gland. Fig. 8 represents the result of 42 such comparisons.

The two curves practically coincide up to titer 7. At higher titers they deviate from each other owing to the fact that the submaxillary gland is characterised by a much more uniform antigen concentration than the duodenum. In accordance herewith a distribution curve with one maximum only is found for the submaxillary gland, fig. 9[1].

In our discussion of the indefiniteness and uncertainty, (comp. paragraph 2), we had no occasion to consider the uncertainty due to the production of the extracts. No direct determination of this uncertainty has, however, been judged necessary, because investigations

[1] It has been suggested, particularly by Tasiro, that the human group antigens in the salivary glands might form a constituent of the mucine of these glands. Now, a quantity of pure mucine produced according to Hammarsten's method from 2 kg of submaxillary glands from various cows was placed at the author's disposal. It turned out that the mucine exhibited no A-antigen content at all, though the glands themselves certainly must have contained an ample amount of this antigen. In view of the agreement between the human group antigen A and the A-antigens in cows this experience would seem to be of considerable interest. In this connection it should be emphasised that it is precluded that the application of the Hammarsten method can have had any destructive effect on the A-antigens.

on human organs showed that this uncertainty is of minor importance or practically negligible.

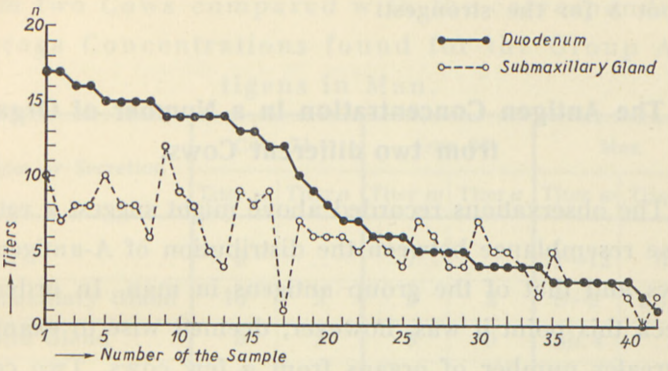

Fig. 8. Comparison between the Concentrations of A-Antigen in the Duodenum and in the Submaxillary Gland from a Random Group of 42 Cows.

Alcoholic extracts from 36 of the submaxillary glands considered above were examined together with the aqueous extracts. The results were of much the same character as those found for the duodenum extracts. In particular it

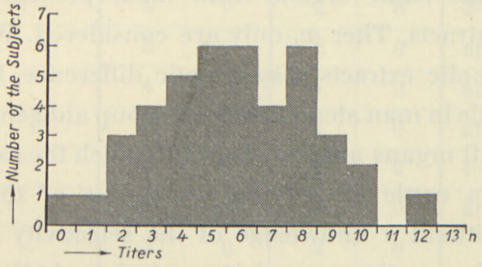

Fig. 9. Distribution Diagram for the A-Antigen Content in aqueous Extracts of the Submaxillary Gland from a Random Group of 42 Cows.

should be noted that no correlation whatever could be traced between the concentrations of water-soluble and of alcohol-soluble antigens. Further it may be remarked that 12 extracts, i. e. about 33 % of the material, showed no

inhibition of the hemolysis. The other extracts exhibited inhibition though of various degrees, the titer readings being about 5 for the strongest.

7. The Antigen Concentration in a Number of Organs from two different Cows.

The observations recorded above might suggest a rather close resemblance between the distribution of A-antigen in cows and that of the group antigens in man. In order to check this point it was, however, deemed wise to examine a greater number of organs from a few cows. Two cows were chosen which, judging from the amount of A-antigen in their salivas, might be expected also to exhibit a measurable amount in the organs; Table VII contains the results.

It appears that a remarkably good agreement obtains between the content of A-antigen in the various organs of the cows and the average concentrations of group antigens found in the same organs from man, provided that the aqueous extracts, Titer *w*, only are considered. With regard to the alcoholic extracts a systematic difference, however, is found. While in man alcohol-soluble group antigens are found in nearly all organs and developed to much the same degree, they are in cattle only found in organs of the digestive system. Without a knowledge of the selectivity of the two methods of extraction one might a priori doubt the reliability of the observations and suspect a transmission of water-soluble antigens to the alcoholic extracts, seeing that the organs of the digestive system are particularly rich in the water-soluble antigen. Now, however, the investigations described in paragraph 5 showed rather clearly that the water-soluble antigens were not transmitted to the alcoholic

Table VII.

The Concentrations of A-Antigen in various Organs from two Cows compared with the corresponding average Concentrations found for the Group Antigens in Man.

Organ or Secretion	Cow 71		Cow 83		Man	
	Titer *w*	Titer *a*	Titer *w*	Titer *a*	Titer *w*	Titer *a*
Saliva	9	..	7	..	11—12	0
Submaxillary Gland	10	3	9	2	8—9	ab. 3
Parotid Gland	5	3	3	0	ab. 4	..
Pancreas	7	2	8	1	8—7	ab. 3
Rumen	5	3	3	3	..	..
Reticulum	8	3	0	2	..	..
Abomasus	16	4	12	4	11—12	4—5
Duodenum	17	3	13	2	10—11	ab. 4
Liver	3	0	3	0	ab. 4	3—4
Gall Bladder	6	0	5	0	8—9	ab. 3
Colon	3	0	2	0	2—3	..
Lung	5	0	2	0	ab. 3	ab. 4
Kidney	4	0	1	0	ab. 4	4—5
Myocardium	0	0	2	0	ab. 4	ab. 4
Muscle	3	0	3	0	4—5	ab. 3
Fat	4	0	3	0	ab. 4	..
Thymus	2	0	2	0	..	..
Spleen	3	0	1	0	ab. 2	4—5
Blood	0	0	0	0	0	4—5

extracts. So there is in fact no reason for doubting the reliability of the observations considered above. As to the organs outside the digestive system no trace at all of a content of alcohol-soluble antigens could be established. This

holds good also for the extracts of the blood corpuscles, a peculiar fact to which we shall return in the next paragraph.

8. Research on A-Antigens in the Blood Corpuscles of Cattle.

In 1927 Witebsky published some investigations on A-antigens in blood from cattle. He found that about $^1/_4$ of all cows exhibited a certain amount of A-antigen in their blood corpuscles. He arrived at this result by showing that the blood corpuscles from these cows were hemolysed by the addition of complement and immune anti-A serum produced by immunising rabbits with human blood corpuscles of group A, i. e. a serum similar to that used in our investigations on alcoholic extracts. He likewise found that alcoholic extracts of blood from "A-cows" were active in a complement fixation test. This observation was not borne out by our experience, at least not in the case of the two cows examined. The disagreement might be accidental, though it would seem rather strange that cows exhibiting an ample amount of A-antigen, should not also have A-antigen in their blood. In order to settle the question 9 fresh alcoholic extracts of blood from different cows were produced. However, these extracts, too, gave no reaction in a complement fixation test. Four different immune sera were employed in the experiments. Hereafter natural blood corpuscles (about a score of samples) were examined in the way indicated by Witebsky. These too failed to give any reaction, at least when they were quite fresh. After having been kept for one or two days in the icebox they sometimes gave a faint hemolysis, but this reaction was not distinct and was certainly of a "non-specific" character.

Finally some experiments were made on the ability of the blood corpuscles to inhibit the agglutination of human A-blood corpuscles by anti-A sera. Washed blood corpuscles (samples from 8 cows) were added to two different sera, an immune serum and an iso-serum, the blood volumes being $^1/_2$, 1, $^3/_2$ and twice that of the serum in question. Even in the case of absorption with two volumes of blood corpuscles no trace of A-antigen could be detected in any of the blood samples. So it would seem safe to say that alcohol-soluble A-antigen is not present in cattle outside the digestive system.

9. A Correlation in Cattle between the Anti-A in the Serum and A-Antigen in the Saliva.

The paper by WITEBSKY mentioned above also included some observations on serum antibody in cattle. WITEBSKY found that serum from cows, diagnosticated as belonging to group O, agglutinated A-blood corpuscles from man to a higher degree than blood corpuscles of other groups, in contradistinction to the serum from cows belonging to group A. At first sight it would seem strange that such a discontinuity should prevail in the amount of anti-A in the serum, while the amount of A-antigen in the organism shows a continuous variation in strength within a random group of cows. FRIEDENREICH has subjected the question as to the serum antibodies to a thorough examination. By the courtesy of Dr. FRIEDENREICH these investigations, so far unpublished, have been placed at my disposal. FRIEDENREICH examined corresponding samples of saliva and serum from 69 cows with regard to their content of A-antigen and anti-A respectively. The amount of A-antigen was determined

in the way indicated above, so that these titers could be directly compared to those found by the author. The serum anti-A was determined in the following way. After absorption with blood corpuscles of group O and B in the ratios $^1/_3$—$^1/_2$, the serum was examined for its abilitity to agglutinate blood corpuscles of group A_1. This ability was read on a scale of serum concentrations $^1/_2{}^0$—$^1/_2{}^1$—$^1/_2{}^2$—$^1/_2{}^3$ - - - - $^1/_2{}^n$. The strength or ability was indicated as n, when n was the last tube in which agglutination of the blood corpuscles still took place. The readings were made both at 20° C. and at 37° C. Also the absorptions were generally carried out at both of these temperatures. The readings were as a rule the same at the two temperatures, in four cases only was a reduced titer found at 37° C. (indicated by crosses in the table). This anomaly was, however, undoubtedly due to the fact that in these four cases the sera were rather old. The complete material from Friedenreich's experiments is given in Table VIII.

In order to see whether a more or less simple correlation obtains between the amounts of antigen and antibody a diagram, fig. 10, comprising all the corresponding samples was drawn in the same manner as those of figs. 6—8. Quite obviously there is a tendency for the antibody to increase at decreasing content of the antigen. Otherwise it appears that rather large fluctuations obtain. Thus for instance a subject exhibiting no antigen in its saliva may at the same time show a complete lack of anti-A in its serum (compare subjects Nos. 32 and 33).

Disregarding for a moment these anomalies we may try to produce a curve showing the average variation of the content of antigen in the saliva with the concentration of anti-A in the serum. The experimental material (saliva)

Table VIII.

Comparisons between the Concentrations of A-Antigen in Saliva from a Random Group of Cows and the Concentrations of Anti-A in the Serum from the same Subjects. (Friedenreich).

Number of the Cow	Saliva Titer *n*	Serum Anti-A Content	Number of the Cow	Saliva Titer *n*	Serum Anti-A Content
11	0	3	50	6	0
12	0	5	51	0	3
13	0	7	52	0	4
14	6	1	53	0	5
15 a	0	1	54	0	5
15 b	7	3	55	5	1
16	9	0	56	0	6
17	6	0	57	0	5
18	0	7	58	0	3
19	0	4	59	0	5
(x) 20	5	4	60	0	5
21	0	2	61	0	7
22	10	0	62	6	2
(x) 23	4	5	63	2	7
24	0	2	71	9	1
25	5	0	72	2	1
(x) 26	2	4	73	0	0
(x) 27	0	4	74	0	2
28	0	6	75	10	0
29	0	6	76	0	1
30	6	1	77	0	0
31	7	3	78	4	2
32	0	4	79	0	5
33	0	7	80	9	1
34	0	3	82	0	2
41	2	3	83	8	0
42	3	0	84	2	6
43	4	2	85	0	6
44	7	1	86	5	1
45	9	2	87	0	7
46	5	2	88	0	2
47	0	7	89	6	3
48	0	2	90	0	8
49	11	2	91	0	7

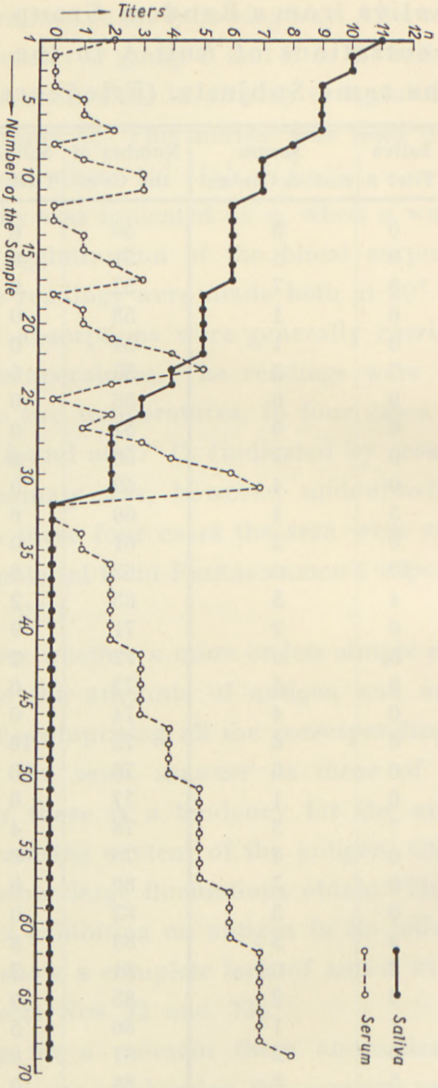

Fig. 10. Comparisons between the Concentrations of A-Antigen in Saliva from a Random Group of Cows and the Concentrations of Anti-A in the Serum from the same Subjects. (FRIEDENREICH).

was for this purpose divided into groups each covering two titers. Of each group the average antibody concentration was calculated. The results are stated in Table IX and further represented in fig. 11 where the abscissa is the average concentration of the antibody and the ordinate the average concentration of the corresponding antigen.

In spite of the scanty material within the groups containing the higher antigen concentrations the curve is rather regular, showing a smooth decrease of the concentration of A-antigen with increasing concentration of the anti-A. In fact the curve may with fairly great exactitude be represented by a hyperbola. From the results now stated it is seen that the presence of A-antigen in cows does not at all, as in man, preclude the presence of anti-A in the serum. Only it would seem that a large content of A-antigen in some degree hampers the development of anti-A.

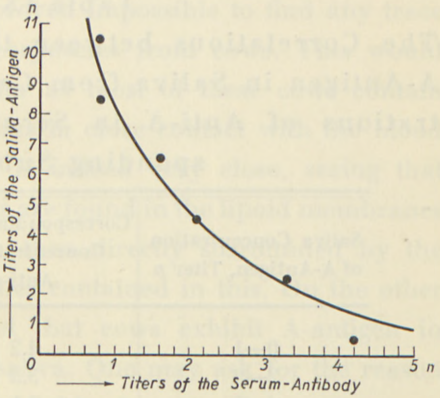

Fig. 11. The Correlation between the Concentration of A-Antigen in Saliva from Cattle and the Concentrations of Anti-A in Serum from the corresponding Subjects.

Now this result seemed so remarkable that it was deemed necessary to subject the antibody in question to a closer investigation. As appears from figs. 10 and 11, a considerable number of cows exhibited both a relatively large content of A-antigen in the saliva and a measurable content of antibody in the serum. Now the investigation referred to gave the highly interesting result that this antibody

actually reacted with the saliva antigen from the same animal in an agglutination-inhibition test. Again the reactions with salivas from cow and man of 1) a strong anti-A from a cow and 2) the ordinary iso-anti-A were compared. An absorbed anti-A serum from a cow in a dilution of $^1/_8$

Table IX.

The Correlations between the Concentration of A-Antigen in Saliva from Cattle and the Concentrations of Anti-A in Serum from the Corresponding Subjects.

Saliva Concentration of A-Antigen, Titer *n*	Corresponding Serum Concentration of Anti-A	Number of Observations
0—1	4.2	38
2—3	3.3	7
4—5	2.1	7
6—7	1.6	9
8—9	0.8	5
10—11	0.8	3

showed the same power to agglutinate human A_1 blood corpuscles as the iso-anti-A, Ulla $^1/_{16}$, employed in the present research. The titers of 8 saliva samples from different individuals and 13 saliva samples from different cows were read on two titer scales, employing the two sera respectively. In Table X a—b the results of the comparison are given. It is seen that no difference whatever could be traced between the quantitative effects of the two antibodies. So, from this twin experiment at least, no difference between man and cattle could be established either with regard to the anti-A or with regard to the antigen in the saliva.

Now if it be true that the same organism may contain a preformed antibody together with the corresponding preformed antigen, this fact will throw light on some peculiarities found in the distribution of the A-antigen within this organism. In order to understand what is meant the following points must be recalled. In the first place it will be remembered that it proved impossible to find any trace of A-antigen in blood corpuscles from cows. This would seem intelligible in so far as most of these cows contain anti-A in their serum, thus in close contact with the blood corpuscles. The contact is indeed very close, seeing that in man the group antigens are found in the lipoid membranes of the blood corpuscles, thus directly surrounded by the plasma and the anti-bodies contained in this. On the other hand, it was established that cows exhibit A-antigen in their organs and in the saliva. One may ask for the reason of this difference between blood corpuscles and organs (saliva). Most probably the explanation is to be found in the fact that the antigens in organs (saliva) are isolated from the corresponding antibodies by a barrier or wall built of various cells. This wall should not of course be considered as an absolute barrier between the antigen and the antibody. On the contrary the relation found between the concentrations of antigen and antibody in the same organism shows that some interaction must take place between the two. The ability of the serum antibodies to pass cell walls is perhaps best known from the maternal iso-antibodies which will pass in small quantities through the single layer of cells separating the maternal and the fetal circulations in the placenta. A similar limited permeability may exist, within the cells of the organism proper.

Table

Comparisons between the quantitative Effects of a) Cow Cow Serum, and of b) Human Saliva on Human Iso-anti-A of the Ag-

a.

Saliva from Cow...	2	3	4	5	6	7	8	9
Calf Terp	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Cow Calf	0	0	0	0	0	(+)	+	+(+)
Terp 1	0	0	0	0	(+)	+	+(+)	++
Cow 117	0	(+)	+	+(+)	++	+++	+++	+++
	0	0	(+)	+	+(+)	++	+++	+++
Cow Calf	0	0	(+)	+	+	+(+)	++	+++
Terp 97	0	0	0	(+)	+	+(+)	++	++
Calf.....	0	0	(+)	+	+(+)	++	+++	+++
Terp 149.....	0	0	0	(+)	+	+(+)	+(+)	++
Calf 1 C. W.	0	0	0	(+)	+	+(+)	+(+)	++
	0	0	0	0	0	(+)	+(+)	++
Calf 12 H. A.....	0	0	0	0	0	(+)	+	+(+)
	0	0	0	0	+	+	++	+++
Cow 97	0	0	(+)	+	+(+)	++	+++	+++
	0	0	0	(+)	+(+)	++	+++	+++
Bull Terp.....	0	(+)	+	++	+++	+++	+++	+++
	0	0	0	(+)	+	+(+)	++	+++
Cow Calf	0	0	(+)	+	+(+)	++	+++	+++
Terp 164	0	0	0	(+)	+	+(+)	++	++
Calf 66 B. L.....	0	0	0	0	(+)	+	++	+++
	0	0	0	0	+	+(+)	++	+++
Bull	0	0	0	0	(+)	+	++	+++
Kallehave.....	0	0	0	(+)	+	+(+)	+++	+++
Cow 75	0	0	0	(+)	+	+(+)	++	+++
	0	0	0	(+)	+	+(+)	++	+++

X a.

Saliva on Human Iso-anti-A and on Anti-A preformed in and on Anti-A preformed in Cow Serum. Method: Inhibition glutination.

Titer n											Serum
10	11	12	13	14	15	16	17	18	19	20	
0	0	0	0	0	0	0	0	(+)	+	+(+)	Ulla $\frac{1}{16}$
0	0	0	0	0	0	0	(+)	(+)	+	+(+)	Cow2 $\frac{1}{8}$
++	+++	+++	..	..	..	..	..	..	..	..	Ulla
+++	+++	+++	..	..	..	..	..	..	..	..	Cow 2
..	..	..	..	..	..	..	..	..	..	..	Ulla
..	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
++	+++	+++	..	..	..	..	..	..	..	..	Ulla
+++	+++	+++	..	..	..	..	..	..	..	..	Cow 2
..	..	..	..	..	..	..	..	..	..	..	Ulla
..	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	+++	..	..	..	..	..	..	..	..	..	Ulla
+++	+++	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2
+++	..	..	..	..	..	..	..	..	..	..	Ulla
+++	..	..	..	..	..	..	..	..	..	..	Cow 2

b. Table

Human Saliva from:	5	6	7	8	9	10	11
S. Group A_1	0	0	0	0	0	0	(+)
	0	0	0	0	0	(+)	(+)
K. Group A_1.....	0	0	0	(+)	+	+(+)	++
	0	0	0	(+)	+	+(+)	(+)
L. Group A_1.....	0	0	0	0	0	(+)	+
	0	0	0	0	0	+	+(+)
K. Group A_1.....	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
M. S. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
H. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	(+)
V. F. Group A_1	0	0	0	0	0	0	(+)
	0	0	0	0	0	0	+
K. B. Group A_1	0	0	0	0	0	0	0
	0	0	0	0	0	0	(+)

Otherwise, the assumption of a barrier between the two substances is perhaps not necessary at all. In fact, HEIDELBERGER and coworkers, working with the purified specific polysaccharide of Type III pneumococcus as antigen, found that when certain assumptions were made the law of mass action might be applied to the antigen-antibody reaction in a precipitation test. Here it should be noted that in our case as in that of HEIDELBERGER the antigen forms a constituent of a solution so that ionic processes are not precluded. Otherwise there is no reason for believing that the antigen-antibody reaction should take place in an essentially different way in vivo than in the precipitation. Unfortunately, however, it is not feasible with the methods

X b.

Titer *n*							Serum
12	13	14	15	16	17	18	
+	+(+)	++	+++	+++	..	..	Ulla
+	+(+)	++	+++	+++	..	..	Cow 2
+++	+++	+++	..	..	..	..	Ulla
++	+++	+++	..	..	..	..	Cow 2
+(+)	+++	+++	+++	..	..	..	Ulla
+(+)	++	+++	+++	..	..	..	Cow 2
(+)	+	+(+)	++	+++	+++	+++	Ulla
0	(+)	+	+(+)	(+)	++	+++	Cow 2
0	0	0	0	0	(+)	+(+)	Ulla
0	0	0	0	0	0	+	Cow 2
+(+)	+(+)	++	+++	+++	..	..	Ulla
+	+(+)	+++	+++	+++	..	..	Cow 2
+	++	++	+++	+++	..	..	Ulla
+(+)	+(+)	+++	+++	+++	..	..	Cow 2
0	(+)	+	++	+++	+++	..	Ulla
+	+	++	+++	+++	+++	..	Cow 2

available and the unknown quantities of antigen and antibody in the initial materials to settle the question whether the "hyperbolic" relation found between these two substances is derivable from HEIDELBERGER's theory or if other conceptions, such as that of a barrier, must be introduced.

10. Discussion and Summary.

The investigations on the A-antigen in cattle gave as the main result that the antigen was to be considered as a species character of these animals. Further a number of analogies were disclosed between man and cows with regard to the distribution of this antigen. These analogies,

however, exist only in the case of water-soluble antigen, while the distribution of alcohol-soluble antigen exhibited remarkable differences from that found in man. The analogies with regard to the water-soluble A-antigen are:

1) If a cow has in its saliva an amount of A-antigen comparable to that found in the saliva from a human A_1 secreter, then the distribution of the A-antigen in the various organs of this cow is also found to agree quantitatively with the distribution in the same organs from the human A_1 secreter.

2) The concentration of antigen in saliva from calves is higher than that found for the adult animals. This corresponds to the phenomenon found for non-secreter babies of group A and B, who would seem to have, at least as a rule, a certain amount of antigen in their saliva, this antigen, however, disappearing during the first year.

3) From investigations on aqueous extracts of duodenum the impression is gained that the A-antigen in cows consists of two or three quantitatively different types. This is an analogy to the $A_1 - A_2 - A_3 - (A_4)$ system of the human group antigen A.

On the basis of these analogies it may be considered highly probable that the genetics of the water-soluble antigen in cows are essentially the same as those of the A-antigen in man. That is to say, we may expect the development of the A-antigen to depend on multiple allelomorphs similar to those determining the A_1, A_2 and A_3 qualities in man. It should here be noted that this conclusion could not be drawn with certainty on the basis of the genetic investigations on saliva, owing to the complications arising from the particular conditions in calves. That is to say,

the possibility is not precluded that the heredity might depend on a limited number of multiple factors, seeing that such an heredity could certainly give rise to quantitatively distinguishable A-types. We pass on to consider the differences between the A-antigen in man and in cattle. These differences chiefly concern the distribution of the alcohol-soluble antigens throughout the organism. While in man alcohol-soluble antigens are found in most of the organs and particularly in the blood corpuscles, they are in cattle found only in organs belonging to the digestive system and no trace at all can be demonstrated in the blood corpuscles. A similar difference is found with regard to the contents of anti-A in serum from man and cattle. In man either A-antigen or anti-A obtain, the two substances never existing together in the same subject. Otherwise in cattle, where the two substances are found in the same subject. This curious difference was explained by the assumption of a barrier or wall of cells separating the two substances in cattle. It proved impossible to distinguish between the anti-A in cattle and the iso-anti-A in man with regard to their effects on saliva from man and cows. This would seem to be a fact of considerable interest and the more so since a distinct difference obtains in the reactions of cow saliva and human saliva with human immune anti-A. (FRIEDENREICH). While human saliva scarcely reacts with immune serum produced by immunisation of rabbits with human A_1 blood corpuscles, the reaction of cow saliva is as pronounced with such an immune serum as with human iso-sera.

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(From the Institute of Forensic Medicine,
University of Copenhagen.
Chief: Professor Knud Sand).

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