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In this paper we discuss the origin of eggs phosphorus by making use of labelled (radioactive) phosphate as indicator. As the presence of labelled phosphorus in organic compounds proves that these compounds were synthetised since the administration of the labelled inorganic phosphate we can draw conclusions as to the place and time of formation of the lecithin and other compounds containing phosphorus and present in the egg, by making use of the above mentioned method. In the hope of finding which phosphorus compounds of the blood are responsible for the formation of the lecithin and possibly other phosphorus compounds of the egg we administered labelled sodium phosphate to hens by subcutaneous injection and investigated after some time the yolks removed from the ovary and further the composition of blood and of some of the organs. In other experiments the eggs, layed at different times, were investigated. Finally we carried out also a few experiments in vitro.

Several of the compounds building up the egg contain phosphorus. Lecithin and other phosphatides form about one tenth of the yolk of the hens egg, the ratio of P to that of the other elements present in these compounds being about 1:25. From the phosphoprotein of the yolk vitellin is the most abundant, it contains on the average 0.54 % P. The total of phosphoprotein P present in the yolk is some-

what less than half of the phosphatide P present, while only small amounts of nucleoprotein P are found, as seen in table 1[1].

Table 1.

Phosphorus present in the yolk in percent of the total phosphorus.

Phosphatide P	61.4
Water soluble P	9.5
Phosphoprotein P	27.5
Nucleoprotein P	1.6

The phosphatide P content of the average yolk amounts to 60 mgm and its total P content to about 94 mgm. The total P content of the yolk is thus about 0.6 % of its total fresh weight, while that of the white of the egg is much smaller, amounting to about 0.01 %. The P content per gm of the small yolks found in the ovary is appreciably lower as seen from table 2 and increases with the increasing size of the yolk.

Table 2.

Phosphorus content of yolks.

Weight of yolk in gm	Lecithin P[2] in yolk in mgm	Total P in yolk in mgm	Lecithin P in 1 gm yolk	Total P in 1 gm yolk
0.03	0.03	0.049	1.00 mgm	1.63 mgm
0.1	0.20	0.26	2.00 —	2.67 —
0.694	1.72	3.57	2.49 —	5.18 —
2.51	6.25	13.0	2.49 —	5.2 —
4.63		40.0		8.7 —
7.68		93.75		12.2 —
13.6		125.0		9.2 —

[1] R. H. A. Plimmer and F. H. Scott, Journ. of Physiol. **38**, 247, 1909.

[2] Lecithin plus other phosphatides.

The phosphorus content of the shell of hens eggs is very variable, fluctuating between 0.1 and 0.3 % of the shell weight. It may be of interest to recall that on the average 59 % of the weight of the hens eggs is due to albumin, 30 % to yolk and 11 % to the shell.

According to general experience the yolk is formed while the growing egg is located in the ovary, about half of the white of the egg is formed by the albumin secreting portion of the oviduct, the shell membrane is deposited directly on this; and the more fluid portion of the albumin, constituting the second half of its entire bulk, enters through the shell membrane while the egg is in the isthmus and uterus. It has been found that the egg spends three hours in the glandular portion of the oviduct, one hour in the isthmus, sixteen to seventeen hours in the uterus including the time of laying.

Phosphorus compounds in hens blood.

The concentration of inorganic phosphorus, acid soluble phosphorus, lipoid (phosphatide) phosphorus and also of the total phosphorus present in blood, plasma and cells of chickens determined by HELLER, PAUL and THOMPSON[1] is shown in Fig. 1. The curves seen in the figure were obtained by analysing the blood of a large number of white Leghorn chickens. The analyses were repeated once a month or oftener beginning at the time when the chickens were 1 month of age and continuing through the periods of growth, egg production, and subsequent molting. The results present very instructing data, they show that the phosphatide phosphorus alone, especially that of the plasma, changes very markedly with the age of the chicken, a rapid rise in

[1] V. G. HELLER, H. PAUL and R. B. THOMPSEN, J. Biol. Chem. **106**, 357, 1934.

the latter taking place after the lapse of 5 months at the time of production, this high level being held under the

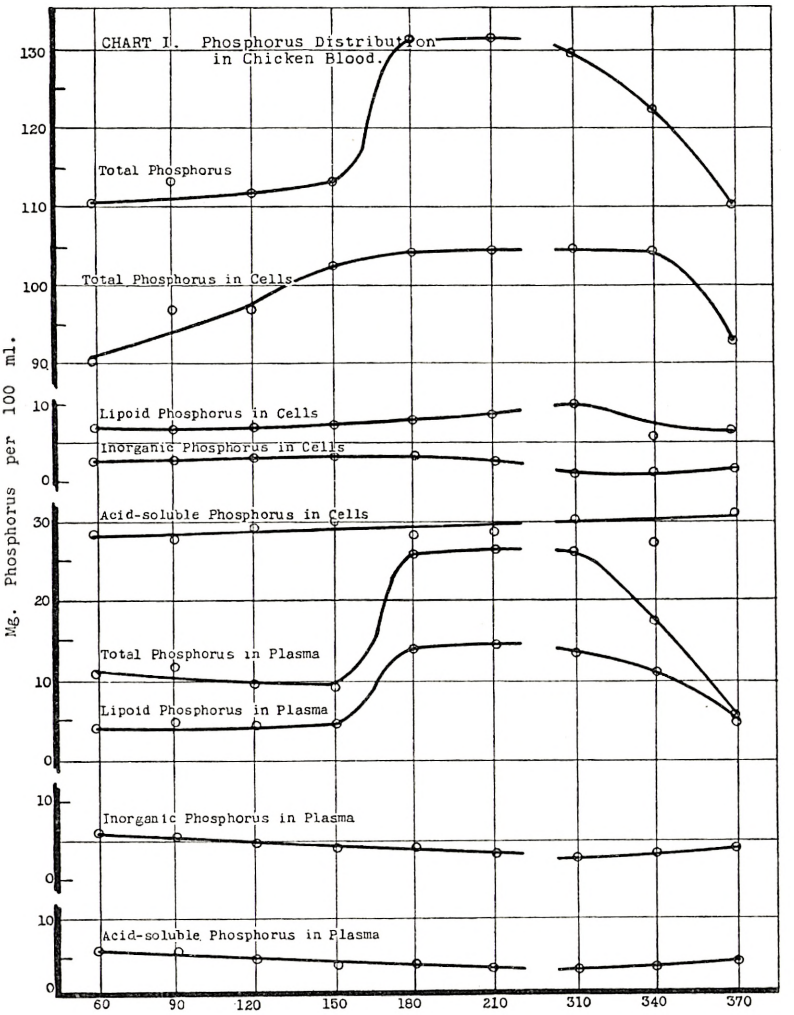

Fig. 1.

entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches.

We determined the blood phosphorus of the laying hen

denoted as I, the result being seen from table 3. The blood phosphorus of another hen is discussed on p. 29.

Table 3.

P-content of hens blood.

	mgm % in plasma	mgm % in corpuscles	mgm % in blood
Phosphatide P	20.0	22.6	20.7
Inorganic P	5.4	—	—
Total acid soluble P	—	53.1	21.3
Rest (Protein) P	9.4	31.8	16.8

In the blood of non-laying hens[1] after 24 hours fasting an average phosphatide P content of 16.8 mg% was found the total plasma P amounting to 13 mgm%, the plasma inorganic P to 4.6 mgm%.

Experimental methods.

The yolk was dryed by adding ice cold aceton, the dry yolk was carefully pulverised and the powder obtained shaken for 15 min with 150 cc ether, the last mentioned procedure being repeated four times using fresh ether. The ether was than carefully evaporated, the residue taken up with dry ether, the latter removed by evaporation, this time in a Kjeldahl flask, and the residue ashed.

The phosphatides of plasma, corpuscles and total blood were extracted by an ether-alcohol mixture after Bloor. The extract was several times carefully evaporated to dryness and taken up with ether or petrol ether. The residue of the first extraction was treated with trichloracetic acid (10 cc of 10 % solution for each cc of blood) and from the filtrate obtained the inorganic P precipitated as ammonium mag-

[1] H. M. Dyer and I. H. Roe, J. of Nutrition, 7, 623, 1934.

nesium phosphate; the esters present in the filtrate were hydrolised and the phosphate produced by the hydrolysis of the esters precipitated as ammonium magnesium phosphate. Though the extraction and the neutralisation of the acid solution were both carried out at —9°, some of the inorganic phosphorus present may be due to decomposition of the esters and we therefore in table gave only the total acid soluble phosphours present in the corpuscles which includes both the inorganic and the ester phosphorus.

The liver was minced, dryed in vacuo at room temperature, pulverised, dryed again in vacuo and extracted with ether-alcohol (1:3), the latter being left to boil for 15 sec. In one case we extracted with ether alone to compare the active P content of the ether soluble phosphatides such as lecithin with that of the total phosphatides. The acid soluble P was extracted from the dryed liver powder by treatment with cold (—10° to —15°) solution of trichloracetic acid, first with a 10 % solution for 10 min and than twice with a 5 % solution each for 5 min. The inorganic and organic constituents of the acid soluble phosphorus were separated as stated above. The P content and activity of the residue obtained after extraction of the phosphatides and the acid soluble P was also investigated.

We determined the phosphorus content of a known fraction of the inorganic phosphate solution obtained in the above described procedures by the colorimetric method of Fiske and Subbarow. The phosphate content of another fraction of the phosphate solution was precipitated in the form of ammonium magnesium phosphate and its activity determined by making use of a Geiger tube counter. Let us say we have administered a hen a labelled phosphate solution containing 1 mg P and showing an activity of 10^6 kicks

per minute. We want to know what percentage of this labelled phosphorus will be found in the yolk lecithin. To arrive at this figure we take from our solution containing the labelled phosphorus as much as corresponds to $^1/_{10000}$ of the amount administered to the hen and precipitate the phosphate, denoting the precipitate obtained as our standard preparation, while we will call the precipitate obtained from the yolk lecithin as lecithin preparation. Before precipitating both the standard and the lecithin preparation we add to the solution a known amount, usually about 80 mgm, of inactive sodium phosphate, by so doing we diminish the amount of labelled phosphate possibly remaining in solution after precipitating with the magnesium citrate reagents and furthermore we obtain a standard and a lecithin preparation of equal weight. The β-rays emitted by the active phosphorus being to an equal extent absorbed in the two preparations the activity of which is to be compared, their weight and thus the thickness of the layers investigated being the same, there is no need to pay attention to the absorbtion of the β-rays in the samples investigated. Nor need the decay of the radioactive P be considered, as both the preparations to be compared, the lecithin and the standard preparation, decay at the same rate. The yolk residue obtained after removal of the lecithin was treated in similar way and also the white of the egg, while the shell was ignited and dissolved after ignition in hydrochloric acid, the solution being treated in the way described above. The samples were placed in small aluminium dishes having a surface area of 1.1 cm^2 and were placed immediately below the aluminium window of the Geiger-counter used.

Before discussing the results obtained we recall some facts about the circulation of labelled phosphorus in the blood.

Sensitivity of labelling.

Let us start from labelled sodium phosphate preparation of such activity that when the later was first put into the blood, 1 mgm. P will show 10000 activity units. As a result of a rapid exchange going on chiefly between bone phosphate and the inorganic phosphate of the blood 1 mgm will soon correspond to less than 10000 activity units. The total inorganic phosphate content of the blood remains constant, except in the case which we will not consider at present where a comparatively large amount is injected, while the individual phosphate ions will very soon be replaced to a large extent by other phosphate ions which were hitherto located in the skeleton or in other organs. After some time we shall find a large part of the labelled phosphate in the organs and the probability that the labelled phosphate leaves the organs and gets back again into the blood will increase, the effect of this reentrance into the blood will be that with increasing time the net rate of decrease of the inorganic labelled phosphate content of the blood will be less and less. Loss of phosphate by excretion and by the formation of organic phosphorus compounds in the blood and in the organs will further complicate the curve representing the labelled P content of the blood as a function of time. We determined the latter experimentally for the blood of different animals and also of human subjects, but not for the hen, (Compare, however, the results given on page 20.) The conclusions drawn in this paper do not necessitate the knowledge of the change of the labelled phosphate content of the hens blood with time, it is for our present purpose sufficient to bear in mind that an initial rapid decrease of the labelled inorganic P content of the plasma occur and becomes slower.

In the first experiments described in this paper, in contrast to most of our experiments, we administered large amounts of P, of the order of magnitude of 100 mgm. The very strongly active phosphorus preparation (of a strength of about 10^6 counts) used in these experiments was a generous gift of Professor LAWRENCE and was prepared by the bombardment of few grams of red phosphorus by high speed deuterium ions generated in Professor LAWRENCE's powerful cyclotron. The active P was thus mixed with a comparatively large amount of inactive phosphorus. In the experiments to be described, in contrast to some other experiments, the comparatively large amounts of phosphorus did not interfere, their presence in the active preparation has even the advantage that we can fix exactly the limit within which the sensitivity of our indicator, the number of mgm of total inorganic P indicated by 1 count activity, varied throughout the experiment. The 100 mgm P administered had an activity of 10^6 kicks. If the labelled P had not been diluted by non-labelled P of the organs we should have found after the lapse of 28 hours, the time of the experiment discussed on page 7, a specific activity of the plasma blood inorg. P — activity per mgm P — amounting to about 1 % of the total activity administered. (The amount of inorg. P present in the total plasma is only about 5 mgm and thus much smaller than the 100 mgm P administered.) As seen from Table 9, however, only 0.01 % was found, showing that from the inorganic P atoms present in the blood of the hen after the lapse of 28 hours only 1 % were those actually administered, the rest being ones originating from different organs and partly also from the food taken within that time.

We carried out three types of experiments:

a) Administration of labelled sodium phosphate to a hen and investigation of the eggs layed at different dates.
b) Administration of labelled sodium phosphate, killing the animal, removal of the yolks and investigation of these yolks the blood the liver and other organs.
c) Experiments in vitro in which eggs were placed in labelled sodium phosphate solutions for few days and investigated as to what extent the labelled P penetrated into the egg.

We will first discuss experiments of the type a).

a) Investigation of the labelled phosphorus content of eggs layed at different dates.

We injected radioactive phosphorus as sodium phosphate subcutaneously to hens and investigated the radioactive phosphorus content of the different parts of the eggs layed at different times. The first egg was layed $4^1/_2$ hours after administering the radioactive (labelled) phosphorus. We found the albumin to contain 0.0015 % of the 40 mgm of phosphorus injected, a similar amount 0.0014 % being present in the yolk. As the total phosphorus content of the yolk was found to be 100 mgm and that of the albumin only 4 mgm, the specific activity (active phosphorus per mgm normal phosphorus) was twentyfive times larger in the albumin than in the yolk. We found the lecithin phosphorus to be 53 % of that of the total phosphorus of the yolk and to be entirely inactive. No synthesis of lecithin molecules took place in the yolk therefore within the $4^1/_2$ hours preceding the laying of the egg, as in that case some active

lecithin molecules should have been formed; taking this fact into account the specific activity of the other than lecithin phosphorus present in the yolk works out to be thirteen times smaller than that of the albumin P. As 40 mgm active phosphorus were injected and only 0.0006 mgm are found in the albumen we can conclude that the formation of albumen from inorganic blood phosphorus in the course of the last $4^1/_2$ hours which the egg spent in the oviduct is a very moderate one, even when we take into account that the 0.0006 mgm active phosphorus found in the yolk passed through the albumen into the yolk bringing the amount of labelled phosphorus present at least temporarily in the albumen to 0.0012 mgm and that a large part of the active phosphorus injected gets rapidly replaced in the blood by non active phosphorus present in the skeleton and other organs as discussed on p. 7.

In the shell of the egg we find 10 mgm phosphorus by chemical analysis (colorimetric method of FISKE and SUBBAROW) and 0.1 mgm of the labelled phosphorus administred by radioactive determination (measurements with a Geiger-counter). 1 % of the shell phosphorus originates thus from the labelled phosphorus administred, which got into the shell in the course of the last $4^1/_2$ hours before laying the egg.

The labelled phosphorus content of eggs layed at different time is shown by the figures of the tables 4 to 6.

In what follows we discuss the significance of these figures. That the specific activity of the shell is very much higher after 0,17 days than at a latter date is due to the fact that shortly after the administration of the labelled P the activity of the inorganic P of the plasma is very high and it is the latter which is incorporated into the shell. As found by us

in numerous cases the active P content of the plasma decreases first rapidly and later at a decreasing rate the difference between the specific activity of the plasma and that of the tissues becoming less and less. The specific activity of the shell phosphorus is a measure of that of the inorganic plasma P at the time of formation of the shell and vice versa. The low specific activity of the albumin P in the egg layed after 0.17 days comes possibly for the following reasons. The white of the egg was already to an appreciable extent formed before the administration of the labelled P, the phosphorus compound of the plasma, presumably the plasma protein which mainly enters into the white was at such on early date after the administration of the labelled P active only to a small extent. The synthesis of labelled organic compounds takes some time and therefore shortly after the administration of labelled P the specific activity of the inorganic plasma P is much higher than that of the organic P. On the other hand the labelled organic P disappears at a slower, usually even much slower, rate from the plasma than the labelled P, the latter having an unique opportunity to exchange with the inactive tissue, especially bone tissue P. This fact explains also why except in the already discussed 0.17 day experiment in which special conditions prevailed, the albumin P has a greater specific activity than the shell P, in spite of the fact that albumin and shell are formed at about the same time. An other possible explanation of the low specific activity of the white P few hours after administration of labelled sodium phosphate is discussed on pg. 31.

When comparing the yolk figures with those of the albumin we have to bear into mind that contrary to the albumin which is formed within the day preceeding the

laying of the egg the greater part of the yolk was already present when the active phosphorus was injected and therefore the labelled phosphorus of the yolk was diluted by the unlabelled phosphorus already present in the yolk. With increasing time we should expect the amount of active phosphorus in the yolk to increase.

Labelled P administered at different dates.

In another set of experiments we were interested in producing strongly active egg lecithin to find out whether after feeding the latter as dry yolk to rats, the presence of active

Table 4.

Active phosphorus content of eggs. Hen I.

Egg layed after administration of active phosphorus	Percentage of active phosphorus administered found in: Shell	Albumin	Total yolk	Yolk lecithin
0.17 days	0.24	0.0015	0.0014	0.000
1.0 —	0.052	0.032	0.109	0.014
3.0 —	0.036	0.030	0.42	0.17
4.5 —	0.026	0.027	0.95	0.34
6.5 —	0.022	0.020	0.85	0.35

lecithin in the blood of the rats can be ascertained. This was found not to be the case. In these experiments we administered to the hen on several days active phosphorus which made the interpretation of the activity measurement of the yolk removed from the ovary rather difficult. A comparison of the activity of the shell of the yolk with its fluid interior revealed large differences. The semi-solid yolk shell formed from very active blood was found in one case to be

Table 5.

Hen I.

Percentage active phosphorus administered found in 1 mgm egg phosphorus x 10^3. (Specific activity x 10^3).

Egg layed after administration of active phosphorus	Shell	Albumin	Yolk after removal of lecithin	Yolk lecithin
0.17 days	24.0	0.38	0.03	0
1.0 —	5.2	8.0	2.0	0.26
3.0 —	3.6	7.5	5.1	3.3
4.5 —	2.6	6.8	12.6	6.4
6.5 —	2.2	5.0	10.4	7.0

Table 6.

Distribution of the active phosphorus administered between different parts of the egg.

Egg layed after administration of active phosphorus	Shell	Albumin	Yolk after extraction of lecithin	Yolk lecithin
		Hen I.		
0.17 days	98.9 %	0.6 %	0.5 %	0.0 %
1.0 —	27.0 -	17.0 -	48.8 -	7.2 -
3.0 —	7.0 -	6.0 -	50.9 -	36.1 -
4.5 —	2.6 -	2.7 -	60.5 -	34.2 -
6.5 —	2.5 -	2.2 -	56.0 -	39.3 -
		Hen II.		
0.5 days	46.1 %	35.0 %	18.9 %	
3 —	30.5 -	6.8 -	38.2	24.5
4 —	15.0 -	6.2 -	56.4	32.4
6 —	7.2 -	2.8 -	58.5	31.5

seven times more active than the fluid interior of the yolk, and five times in another case. With decreasing size of the yolk the difference between the specific activity of the yolk

phosphorus originating from the inner and the outer part of the yolk diminished and finally vanished.

b) Specific activity of yolk phosphorus.

We administered to a hen 100 mgm P as sodium-phosphate showing an activity of 10^6 kicks and killed the hen after the lapse of 28 hours. From the ovary 34 yolks were removed and from the ovidicut one egg. The weights of these are recorded in Table 7.

Table 7.

Weight of yolk	Specimens present
About 30 mgm	20
— 100 —	9
— 700 —	1
— 2500 —	1
— 5000 —	1
— 7500 —	1
— 13000 —	1
(Egg) 18000 —	1

The specimens of 700 mgm and more were treated separately while averages of the 30 mgm and the 100 mgm yolks were taken. The lecithin was extracted by ether and the residue brought into solution as described above. The results obtained are seen in Table 8.

The specific activity of the total P shows a maximum in the case of the 2500 mgm yolk. This result, puzzling at first sight, can be easily understood after considering Fig. 2 taken from a paper of H. GERHARTZ[1], in which the daily increase in weight of the yolks of a hen is recorded. The yolk grown from active blood and thus active will be diluted by the

[1] H. GERHARTZ, Arch. f. d. gesamt. Physiol., 156, 215, 1914.

Table 9.

Specific Activity of yolk phosphorus.

(Percentage of the activity administered present in 1 mgm P)

Weight of yolk	Lecithin P	Non Lecithin P	Total P
30—100 mgm	0.00055 %	0.018 %	0.0073
700 —	0.00814 -	0.0173 -	0.0129
2500 —	0.0147 -	0.0186 -	0.0164
4600 —	—	—	0.0090
7700 —	—	—	0.0055
13600 —	—	—	0.0044

non active yolk already present and this dilution will lead to a decrease of the specific activity of its P content. The dilution being least in the case of the 2500 mgm yolk, (comp. Fig. 2) its specific activity is bound to be highest. It takes some time after administration of the labelled sodium phosphate until labelled lecithin is transported into the plasma whereas inorganic P of very high activity is present almost at once after injecting the active sodium phosphate. The non lecithin P of the yolk is partly inorganic P which gets into the yolk in the early stage of the experiment when its specific activity is very high (comp. pg. 10,); we must therefore consider the lecithin P and not the non lecithin or total P content as a proper measure ot the growth of the yolk. From the fact that the lecithin P of the 30—100 mgm yolks became active only to a very slight degree we must conclude for example that they had hardly grown within the last 28 hours. When discussing the labelled lecithin P present in blood and in some of the organs we shall find definite evidence that the yolk lecithin is drawn from the plasma lecithin.

It is of interest to remark that the ratio of the total active

lecithin content of small yolks, such as would require 10 days or more to attain completion, is a quantitative measure of their relative growth since the administration of the labelled P. When, however, comparing the lecithin P activity of a small yolk which increases its weight in the course of

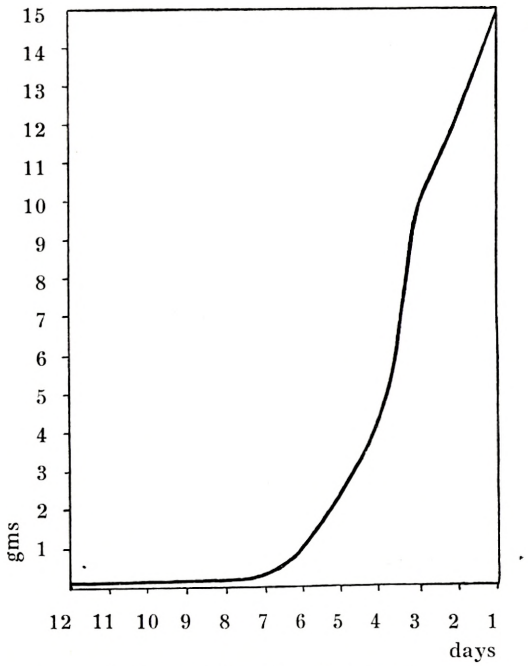

Fig. 2. Increament of the weight of yolks in the course of 12 days before completion of the yolk according to Gerhartz.

a day only to a slight extent with that of a large yolk growing at a rate of few gms per day the ratio of the total activities will not always be a correct measure of the growth since the administration of the labelled P. It may happen (comp. Fig. 2) that the growth of yolk per hour is larger at the end than in the beginning of the experiment the latter process determining thus to a larger extent the total growth within the time of experiment. From which it follows, that if at

the beginning of the experiment the specific activity of blood lecithin happens to be greater than at the end, we underestimate the growth of the large yolk.

It is, however, the determination of the slow rate of growth of the small and tiny yolks, often present in a very large number in the ovary, which can be of special interest and which can hardly be determined by any method other than that outlined above.

Investigation of blood phosphorus.

Plasma and corpuscles of the hens blood were separately investigated using the experimental method described on page 7. The results obtained are seen from Table 9 which contains data on the specific activity (activity per mgm P in percent of that injected) and also the total phosphorus present in the hens blood under the assumption that the volume of blood of the hen amounted to 150 cc and the volume of the blood plasma to 100 cc.

Table 9.

Specific activity and total phosphorus content of the hens blood.

Fraction	Specific activity	Total phosphorus content
Plasma inorganic	0.0104	5.4
Plasma phosphatide	0.0125	20.0
Corpuscles phosphatide	0.0046	11.3
Corpuscles acid soluble	0.0036	26.5
Corpuscles protein	0.0031	15.9

That the specific activity of the plasma phosphatide P is greater after 28 hours than that of the inorganic P is due, as discussed on page 14, to the rapid disappearance of the individual inorganic P atoms from the plasma. In the

experiment discussed on page 29, in which the hen was killed only 5 hours after the administration of the labelled P, the specific activity of the phosphatide P was found to be only 42 % of that of the inorganic P.

It is of great interest that the specific activity of the plasma phosphatide P is several times larger than that of the corpuscle phosphatide which shows that a much smaller percentage of the corpuscle phosphatide than of the plasma phosphatide is renewed in the course of the experiment. This is an interesting result as it definitely disposes of the often discussed possibility that the blood phosphatide is synthetised in the corpuscles. Some of the corpuscles being formed during the experiment from labelled plasma are bound to contain labelled phosphatides; labelled phosphatides can furthermore easily get into the shell of the corpuscles which are partly composed of lecithin.

A very suggesting change in the phosphatide content of hens blood at the time of production was ascertained by HELLER, PAUL, and THOMPSON (comp Fig. 1). The most interesting feature of the curves recorded by them is a gradual increase in the total P of the blood at the time of production, this high level being held during the entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches. The increase is due to that of the lipoid P and is much more conspicious in the case of the plasma than in that of the corpuscles; the lipoid P content of the plasma is higher all through than that of the corpuscles, at the peak of production the former value being nearly three times higher than the last mentioned one. As about $^2/_3$ of the blood volume is composed of plasma it follows, that from the total lipoid P present in the blood $^5/_6$ are to be found in the plasma. The predominance of

phosphatide P in the plasma found for laying hens is entirely unique as seen from the figures of Table 10, but understandable if we envisage the great strain put on the organism of a hen as to lecithin supply. A hen laying daily has to produce about 60 mgm lecithin[1] P a day; taking a total plasma volume amounting to 100 cc the total lecithin P of the plasma works out to be 20 mgm. If the lecithin found in the yolk is, as suggested from our results, taken from the plasma lecithin then the plasma has to give off three

Table 10.
Phosphatide P in plasma and cells of different animals.

	mgm % P in		
	Plasma	Cell	Ratio $\frac{\text{cell}}{\text{plasma}}$
Rat	2.6	10	3.8
Rabbit	3.3	12	3.6
Man	9	19	2.1
Dog	14	14	1
Laying hen	14—20	8—23	0.87

times its total lecithin content in the course of a day thus putting an appreciable strain on the lecithin circulation. A strain which would be still more pronounced in the case of a lower plasma lecithin content.

Protein phosphorus in the hens blood.

After removal of the phosphatides and the acid soluble phosphorus, the remaining P is generally assumed to be

[1] Lecithin plus other phosphatides.

present as protein P. The protein P content of 31.8 mgm$^0/_0$ found in the corpuscles of the hen in the 28 hours experiment is much higher than in the corpuscles of the blood of other animals, the corpuscles of the rabbit containing for example, as found by Mr. ATEN, 4.4 mgm$^0/_0$. The same considerations apply to the protein P content of the plasma, which was found to amount to 9.4 mgm$^0/_0$ for the blood of the hen in question and of 7 mgm$^0/_0$ in the case of the hen discussed on page 29 while the plasma of a rabbit, for example, was found to contain only 0.03 mgm$^0/_0$ protein P. From the high value of the specific activity of the protein P in the 5 hours experiment it follows that the protein phosphorus compounds present in the plasma were renewed even at a higher rate than those of the phosphatides. This result suggests a great participation of the plasma phosphorus protein in the formation of the egg. To arrive at a final conclusion as to the relation between the phosphorus protein compounds of the plasma and those of the yolk and white is very difficult because of the fact that we lack simple methods of separation of the protein compounds. Vitelin, for example, can only be isolated by very tedious and lengthy processes and the isolation and separation of the blood protein phosphorus compounds are still more difficult, partly because only small quantities of these substances can be secured in the experiment. The simple fact that we have to base our conclusions on the amount of phosphorus present in the residue, remaining after extraction of the phosphatides and the acid soluble phosphorus compounds makes the result obtained less trustworthy than those arrived at when investigating the phosphatides, for example. The high value for the protein phosphorus of the corpuscles found by us, which may to some extent be due to an incomplete separation of the

phosphatides and acid soluble P, is supported by the data obtained by Heller, Paul, and Thompson. They find for the total P present in the cells of laying hens about 100 mgm $^0/_0$, but only about 40 mgm $^0/_0$ for the sum of inorganic acid soluble and lecithin P. The discrepancy suggests the presence of a further not investigated P fraction, which might be protein P. In the case of the plasma phosphorus the curves of Heller and his colleagues show the anomaly mentioned only to a smaller degree; the total phosphorus found by them is not very much larger than the sum of the acid soluble and phosphatide P.

The high protein P content of the blood plasma of a laying hen has presumably the same biological significance as the high phosphatide P content, namely a reduction of the strain put on the protein resp. phosphatide producing and carrying system in the organism of laying hens.

Phosphatide content of the liver.

We extracted the total phosphatide content of the liver of a hen 28 hours after the administration of the labelled P, using the method described on page 7. Since we were interested in seeing whether lecithin soluble in ether shows the same specific activity as the total phosphatides we extracted another part of the liver tissue with ether alone. We found no marked difference, as seen from Tables 11 and 12, which also contain data on the specific activity of inorganic and acid soluble (other than inorganic) P of the liver.

As seen from Table 11 the specific activity of the liver phosphatide P is 56 $^0/_0$ of that of the inorganic P, from which it follows that about one half of the phosphatide

Table 11.
Specific activity of the liver P.
(Activity per mgm P)

Fraction	Specific activity
Total phosphatides (ether-alcohol extract)	0.0152
Lecithin (ether extract)	0.0158
Inorganic	0.0272
Acid soluble, other than inorganic	0.0224

molecules are labelled and thus formed after administration of the labelled sodium phosphate. This result must, however, be interpreted with great caution. As already mentioned on page 10 in the early stages of the experiment the specific activity of P of the plasma is much higher than in the latter stages and the inorganic P of the liver was also more active at the early stage. This change of the specific activity with time would not affect our results if the specific activity of the phosphatide P should decrease with time at the same rate as does that of the inorganic P. That is, however, not the case. The phosphatide molecules can only escape from the circulation at an appreciable rate into the yolk, while

Table 12.
Percentage of labelled P administered found in plasma, corpuscles and liver.

Fraction	Total Plasma (100 gm)	Total Corpuscles (50 gm)	Total Liver (44 gm)
Phosphatide P	0.25 %	0.052 %	0.608 %
Inorganic P	0.056 -	— -	
Total acid soluble P	— -	0.100 -	1.643 -
Protein P	0.176 -	0.050 -	
Total P	0.482 -	0.202 -	2.251 -

the individual inorganic P atoms present in the plasma can rapidly exchange with such present in the skeleton, the latter being a much faster process in view of the huge extent of the skeleton. Therefore, when drawing conclusions from the comparison of the specific activities of the phosphatide P and the inorganic P as to what extent the phosphatide molecules got labelled we are apt to get values which are possibly too high. A trustworthy value could be obtained by keeping the specific activity of the inorganic P of the plasma constant by continuous injection of labelled phosphate of varying concentration and by thus avoiding a decrease in the specific activity of the inorganic P of the plasma, which is used for the synthesis of the phosphatide molecules in the liver and elsewhere. In the above case we can, however, conclude that a very appreciable part of the liver phosphatide molecules must have been renewed within the 28 hours of the experiment. In experiments on isolated livers in which the skeleton and other organs are not present it is easy to calculate from the ratio of the specific activities of inorganic P and phosphatide P the amount of newly formed phosphatides. In an isolated liver of a cat in the course of 2.5 hours about 1 % of the phosphatide molecules present are newly formed. If in the course of 2.5 hours in an isolated liver of a cat about 1 % of the phosphatide content is renewed there can be hardly any doubt that in the liver of a living hen in the course of 28 hours a large part of the phosphatide found is synthetised during that interval; in the liver of a living animal the enzymatic and other actions necessary for the synthesis of phosphatides are certainly as abundant as in an isolated liver and the phosphatide formation in the liver of a laying hen could hardly be less than in that of a cat. We are led to the same conclusion by the following

consideration. The daily amount of phosphatide P transfered from the plasma into the ovary is, in the case of the hen in question, which was laying one egg every other day, about 50 mgm. The main source of phosphatide production is, as we will see, the liver, and an amount not very far from 60 mgm must therefore have been produced daily in the liver of the hen. Since the latter containing 38 mgm of phosphatide P, a large amount of the liver phosphatide must have been renewed during the experiment. A similar conclusion applies to the plasma phosphatides, the 50 mgm phosphatide P being carried by the plasma, the total content of which is 20 mgm, the plasma phosphatide molecules must have been renewed to a large extent.

We are thus led to the result that the main source of phosphatides in a laying hen is the liver and that more than one half of the phosphatide molecules present in the hens liver were newly formed during the 28 hours preceeding the administration of labelled phosphate. That the greatest part of the phosphatides is formed in the liver of a laying hen and reach the ovary through the plasma is very clearly shown in an experiment in which the hen was killed only 5 hours after administration of the labelled phosphate. Before discussing the result of this experiment we want to mention the difference between the specific activity of the inorganic P extracted from the liver and that extracted from the plasma.

As seen from Table 6 the specific activity of the inorganic P isolated from the liver is found to be nearly three times as high as that found in the plasma. The low value of the latter at the end of the experiment was explained mainly by the exchange interaction between the skeleton and the plasma inorganic phosphate. Now one would expect the

liver inorganic phosphate to be in exchange equilibrium with the plasma phosphate and the fact that such a marked difference in the specific activities is actually found, shows that the interaction takes sometime. The above mentioned difference could also be wholly or partly explained by the assumption that what we isolated as inorganic phosphate was not wholly present as such in the liver but is freed through decomposition after the animal is killed either before or during the extraction process.

The acid soluble P of the liver, other than inorganic, mainly derived from P ester, shows, as seen in Table 11, a higher specific activity than the phosphatide P present in the liver.

Experiment on a hen killed after five hours.

3.8 cc of physiological sodium chloride solution containing 10 mgm labelled sodium phosphate were injected subcutaneously into a hen which weighed 1800 gms. The hen, which layed previously one egg daily weighing about 45 gm was killed after the lapse of 5 hours. The hen was then dissected by Dr. Marie Krogh, whom we wish to thank. Mr. A. H. W. Aten jun. extracted with great care the phosphatides of the liver, plasma, ovary and yolk. The procedure used will be described by him together with some other experiments. The results obtained are seen in Table 13 and 14. Two separate determinations were carried out, the values found and also their average are given.

As seen from Table 13 the specific activity of the phosphatide P, which is a measure of newly formed phosphatides, is by far the greatest in the liver and markedly higher than that of the plasma phosphatide P. Contrary to the 28 hours

experiment, where the percentage of newly formed phosphatide molecules in the plasma nearly reached that found in the liver, in the 5 hours experiment the concentration

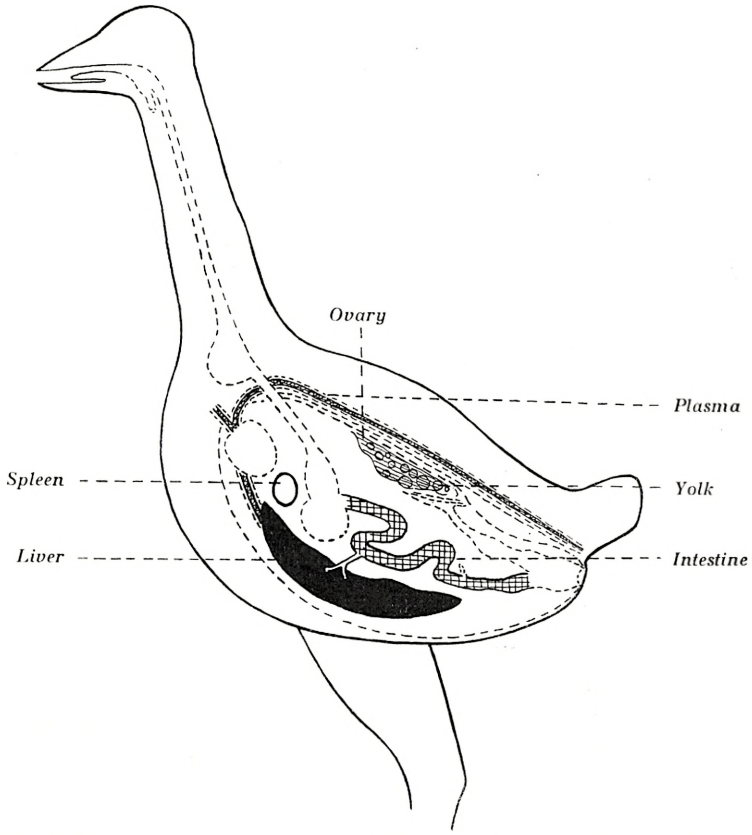

Fig. 3. The heaviness of the shading indicates the specific activity of the lecithin P and thus the percentage of the phosphatide molecules formed within the last five hours in the total phosphatides of the organ in question.

gradient in the flow of labelled phosphatides directed from the liver into the plasma is very clearly shown (comp. Fig. 3). The percentage of labelled molecules in the ovary phosphatide is, on the other hand, much smaller than in

Table 13.

Specific Activity of Phosphatide P.

Organ	Specific activity (% of activity given, found in 1 mgm P)		Relative specific activity; that of the inorganic plasma P taken = 1
	Single values	Average	
Liver	0.094 0.082	0.088	0.54
Plasma	0.069 0.069	0.069	0.43
Ovary	0.0064 0.0064	0.0064	0.039
Yolk	0.0053 0.0075	0.0064	0.035
Intestine	0.018 0.018	0.018	0.11
Spleen	< 0.02	< 0.02	< 0.1

the plasma phosphatides. From this it follows that the labelled phosphatide molecules present in the ovary were within 5 hours only partly replaced by ones present in the plasma. We investigated a yolk weighing 1.0 gms. The figures obtained are given in Table 13. A second yolk investigated weighed 2.7 gms. and its lecithin P had a specific activity of 0.0050. The specific activity of the yolk lecithin of the first mentioned yolk was found to be about $^1/_{11}$ of that of the plasma lecithin. From these figures it follows that about $^1/_{11}$ of the 1.0 gm, i. e., 0.09 gm, of yolk were grown within 5 hours. The actual growth was, however, presumably greater than 0.09 gm, since in the early stages of the experiment the plasma phosphatide was only very slightly active and so was the yolk tissue formed in this phase of its development. The fact that the specific activity of the ovary phosphatide was found to be low, as low as that of the yolk, proves definitely that the role of

the ovary is not production of phosphatides but their extraction from the blood plasma together with other suitable constituents. The combination of phosphatides with proteins giving the characteristic composition and consistency of the yolk, is evidently one of its principal functions. In the experiment described above the specific activity of the P of the yolk soluble in trichloracetic acid was found to be 0.035, thus $^1/_{4.5}$ part of that of the inorganic P of the plasma, the latter being 0.16. Making the assumption that most of the acid soluble P originates from the inorganic P of the plasma we find a growth of the yolk amounting to $^1/_{4.5}$ part of its weight of 1.0 gm during the experiment. While the above mentioned figure of $^1/_{11}$ was, as already mentioned, a lower limit of the part of the yolk newly formed within 5 hours, the figure of $^1/_{4.5}$ is a higher limit. A part of the acid soluble yolk phosphorus was formed at an earlier stage when the specific activity of the plasma inorganic P was appreciably higher than at the end of the experiment, and as our calculation is based on the specific activity of the plasma inorganic P at the end of the experiment it gives too high a value for the amount of yolk formed during the experiment.

The phosphorus of the white of the egg removed from the oviduct had a low specific activity, namely 0.0013. This is an interesting result in view of the strong activity shown by the phosphorus compounds present in the plasma (comp. Table 14). A possible explanation of this result is that some of the phosphorus present in the protein or other compounds of the oviduct tissue is utilised to produce the phosphorus compounds present in the white of the egg. In the course of five hours perhaps the compounds present in the tissue of the oviduct get labelled only to a slight extent. An other explanation is that while the average

plasma protein P has a high specific activity 0.15 after the lapse of five hours, the specific activity of the phosphorus of one of the components of the protein mixture might be low. If the P of the white originates from the protein or acid soluble fraction of the blood it must come from a less abundant component of the latter (comp. also pg. 14). We are now engaged in the investigation of the origin of the phosphorus present in the white of the egg.

Table 14.
Specific Activity of Plasma Phosporus.

Fraction	Specificactivity
Inorganic P	0.16
Lecithin P	0.069
Protein P	0.14

c) Experiments in vitro.

We placed eggs in a neutral physiological sodiumphosphate solution containing 30 mgm P for 24 hours and investigated the activity of the different parts of the eggs, the results being seen in Tables 15 and 16.

The comparatively high labelled P content of the shell is due to phosphate exchange processes between the large

Table 15.
Ratio of the specific activity of egg P and solution P.

	Shell	Albumin	Yolk
Egg I (Total P)	1.8×10^{-1}	1.4×10^{-3}	1.9×10^{-5}
Egg II (Total P)	2.0×10^{-1}	1.5×10^{-3}	4.0×10^{-5}

Table 16.

Distribution of the active phosphorus taken up between the different parts of the egg.

	Shell	Albumin	Yolk
Egg I	99.46 %	0.44 %	0.10 %
Egg II	99.40 -	0.41 -	0.19 -

shell surface and the solution and possible also to the formation slight amounts of calcium phosphate from the carbonate of the shell. An investigation of the activity of the lecithin extracted from the yolk gave an entirely negative result, this in agreement with the observation recorded on p. 13 that after the egg left the ovary no more lecithin formation takes place.

Discussion.

We saw that by investigating the labelled phosphorus content of eggs or yolks we could draw conclusions as to the growth of the egg or yolk since the date of administration of the labelled P. It is, for example, possible to show that while the egg is in the oviduct not only is no more yolk formed but also no new lecithin molecules are synthetised. Should suitable enzymes be present, new and thus labelled lecithin molecules could be formed without any growth of the yolk. The ovary of a laying hen contains numerous tiny yolks growing at a slow rate; by comparing the incorporation of labelled P by such yolks we get a quantitative measure of their relative growth since the administration of the labelled P. When comparing the growth of small yolks with large ones we can usually not obtain strictly quantitative results as to the relative growth because of the much more

rapid relative growth of large yolks compared with that of small ones.

Placing eggs in a solution containing labelled P for some days we find the shell to contain an appreciable part of labelled P, while the amount shown by the white and especially by the yolk is very small, though easily measureable, even in the case of the yolk. No formation of labelled lecithin is, however, found in the yolk.

As to the formation of lecithin in the growing yolk, we arrive at the following result: The phosphatides found in the yolk are synthetised at least to a large extent in the liver and are transported through the plasma to the ovary which extracts the phosphatides. This is most clearly seen in the experiment in which the hen was killed only 5 hours after the administration of the labelled sodium phosphate. In this experiment the specific activity of the liver phosphatide P reached 54 % of that of the plasma inorganic P, while the specific activity of the plasma phosphatide P was appreciably smaller, amounting to only 43 %; that of the ovary was very much smaller, namely 3.9 %, and about as large as that of the strongest yolk phosphatide P. In the 28 hours experiment, as to be expected, the difference in the specific activities was much smaller, the specific activity of the liver phosphatides being only somewhat higher than that of the plasma phosphatides. In the 28 hours experiment on the hen which used to lay one egg every other day the amount of phosphatides passing through the plasma on the way into the ovary was, in the course of the experiment, about twice the amount of phosphatides present in the plasma. In the 5 hours experiment, in which the hen experimented on was laying one egg daily, the amount of phosphatide passing the plasma on the way into the ovary

was about half the amount present in the plasma. From the low specific activity of the phosphatide P, that is from the low percentage of newly formed phosphatide, in the ovary it follows that in this organ only an insignificant amount of phosphatide can be formed. We have also to consider that a part of the labelled phosphatides found in the ovary is due to the presence of blood containing the latter. The specific activity of the plasma phosphatide P being appreciably smaller than that of the liver the labelled phosphatides must have come from the liver into the blood and not vice versa. By carrying out experiments in vitro with blood containing labelled sodium phosphate we found only a slight formation of labelled phosphatides, which is in accordance with the above conclusion.

The formation of phosphatides in the intestinal mucose by using radioactive phosphorus as indicator was first shown by ARTOM, PERRIER, SANTAGELO, SARZANA and SEGRE[1]. They found in an experiment carried out on a rat, that after injecting labelled sodium phosphate the phosphatides extracted from the gut after a few days showed a specific activity only exceeded by that of the liver phosphatide P, the ratio of the specific activities being 1.2. The phosphatide production in a laying hen is larger than in any other animal of similar size, as the amount produced daily to be incorporated in the yolk is as much as about 2 times that present in the liver which contains more phosphatide than any other organ. The laying hen is, therefore, a very suitable animal for studing phosphatide formation. In our 5 hours experiment the specific activity of the intestinal phosphatide P is much smaller than that of the liver phosphatide P and also than the plasma phosphatide P. The bulk of the labelled

[1] Nature, 139, 836, 1937.

P present in the plasma can, therefore, not originate from the intestinal phosphatide and the latter can not be the chief source of the yolk phosphatide. The phosphatides formed in the intestine can, however, have and presumably actually do have a role in the supply of the plasma phosphatides. The presence of phosphatides in the intestinal lymph was repeatedly shown[1] in experiments on dogs. The amount of phosphatides reaching the hens circulation by the influx of intestinal lymph could be ascertained by measuring the amount of intestinal lymph produced and also its phosphatide content. In figure 3 we show the specific activities of the phosphatide P in the organs of the hen killed 5 hours after the administration of the labelled sodium phosphate. The heaviness of the shading indicates the specific activity.

A hen laying daily deposits about 60 mgm phosphatide P in the yolk or about 3 times as much phosphatide as present in the plasma. In the course of a day the phosphatide content of the plasma of a laying hen must therefore be replenished three times. In view of this great strain on the phosphatide circulation in the plasma it is very significant that the plasma phosphatide content of a laying hen is higher than in most other animals. If the laying hens plasma should show such a low phosphatide content as does a rabbit or a rat (per cc) the plasma lecithin would have to be replenished as much as 17—22 times a day. It is significant that the high phosphatide content is maintained only during the laying period and that the cells contain less phosphatide than the plasma, a behaviour not shown by the blood of any other animal investigated. We find furthermore that in the course of 28 hours taken by the experiment a much

[1] H. E. Hamerich, Americ. J. of Physiol. 114, 342. 1934; S. Freeman and A. C. Joy l. c. 110, 132, 1935.

greater part of the phosphatides found in the plasma is labelled than of that contained in the corpuscles. This is a significant result as it demonstrates clearly that lecithin is carried to the ovary by the blood plasma and not the blood cells which obtain their lecithin in various ways. Labelled phosphatide could be taken up by the cell membrane, possibly diffuse through the cell membrane; labelled inorganic phosphorus which was found by us to diffuse at a moderate speed into the corpuscle could lead to the formation of labelled phosphatide phosphorus inside the latter, finally the lecithin could get into the corpuscles at their birth. If they are formed from labelled plasma the newly formed corpuscles should become labelled as well. As to the formation of labelled phosphatide from labelled inorganic P in blood, we found in experiments in vitro that such a formation actually takes place, though only on very minute scale. As to the rate of formation of blood corpuscles, some information on this point could be obtained by injecting labelled plasma and investigating the radioactivity of the phosphorus compounds isolated from the corpuscles after the lapse of some time. If after the lapse of a day, for example, only 1 % of the corpuscle phosphatides were found to be labelled we could conclude that the rate of formation of the corpuscles per day is less than 1 % of the total corpuscles present.

As to the white of the egg, we find that at least a large part of its phosphorus content is drawn from organic phosphorus compounds, possibly from protein phosphorus. We arrived at this result by comparing the specific activity of the phosphorus of the white of the egg with that extracted from the shell. The latter derives its phosphate content from the inorganic P of the blood plasma and is accordingly a

convenient measure of the activity of the latter. The shell is formed at about the same time as the white of the egg, the great discrepancy between the specific activity of the shell P and albumin P exclude the possibility that they are of common origin.

Summary.

By administering labelled sodium phosphate to laying hens the share of the labelled phosphorus administered in the formation of the yolk, albumin and shell of the egg can be followed by aid of radioactive measurements. The comparison of the specific activity (activity per mgm P) of the phosphorus extracted from blood plasma phosphatides with that extracted from the liver, the ovary, and the yolk phosphatides leads to the result that the bulk of the phosphatides of the yolk originate in the liver. It gets from the liver into the plasma and is then taken by the latter to the ovary.

No formation of phosphatides takes place in the ovary. After the egg leaves the ovary no more active phosphatide is formed. No formation of labelled phosphatide in the yolk can be ascertained in experiments in which an egg is placed for a day in a labelled sodium phosphate solution. In the last mentioned experiment in vitro slight amounts of labelled phosphorus are found in the yolk, appreciable quantities in the white, and large amounts in the shell.

The specific activity of the phosphatides extracted from the blood corpuscles was found to be only $^1/_3$ of that extracted from the plasma. Therefore, we conclude that the

phosphatides formed in the liver and other organs are carried to the ovary by the plasma rather than by the corpuscles. The latter apparently play no important role in this process.

Some of the labelled phosphorus used in our experiments was prepared by us from sulphur under the action of neutrons emitted from a radium-beryllium mixture most kindly put at our disposal by Prof. NIELS BOHR, and some of it was a generous gift from Prof. LAWRENCE of the University of California. The hens and yolks were kindly given us by Prof. A. KROGH and Dr. DAM. We wish to express our sincere thanks to those named above and also to Mr. A. H. W. ATEN and Miss HILDE LEVI for their assistance in this work.

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Færdig fra Trykkeriet den 23. Marts 1938.

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