CXLIV.—*The Proteins of Egg-yolk.*

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IN 1900 T. B. Osborne and G. F. Campbell (*J. Amer. Chem. Soc.*, 1900, **22**, 413) described an alternative method to the one usually employed for preparing vitellin from egg-yolk, which consisted in dissolving the yolk in 10 per cent. sodium chloride solution, extracting the lecithin and fat by shaking with ether, and then dialysing, or pouring the solution into a large volume of water, whereby the vitellin is precipitated. By re-solution in sodium chloride solution and reprecipitation by water, followed by extraction with alcohol and ether to remove lecithin, the vitellin was obtained in a state of comparative purity.

On repeating this process it was observed that, after complete precipitation of the vitellin by water, the aqueous solution gave a very marked biuret reaction, and that, on boiling and slightly acidifying

with acetic acid, a large precipitate of coagulated protein was formed. This was at first regarded as unprecipitated vitellin, but a phosphorus determination at once showed its non-identity with this protein, for it contained 0.1 per cent. of phosphorus compared with 1.0 per cent. in vitellin. It seemed scarcely possible that this coagulable protein was identical with ovalbumin present in the yolk, since ovalbumin is coagulated by ether, and since it contained less nitrogen than does ovalbumin; consequently it was necessary to regard it as a second and as yet undescribed protein in the yolk of eggs.

As it is impossible to decide upon the identity or non-identity of two proteins from the figures obtained by ultimate analysis, and as differentiation by hydrolysis into the constituent amino-acids requires a considerable amount of material, and, moreover, is as yet not sufficiently quantitative in its results to arrive at a decision, recourse was had to the determination of the various forms, in which the nitrogen is contained in the proteins. This method was first carried out by Hausmann, and has been used by several investigators, more especially by Osborne and Harris (*J. Amer. Chem. Soc.*, 1903, **25**, 323) in their study of the vegetable proteins which showed distinct differences. Vitellin and this new protein showed a very close similarity to one another, but they differ considerably from ovalbumin, and it may be concluded that this protein is another constituent of egg-yolk. It is suggested that the protein may be vitellin without the phosphorus-containing portion and that it be provisionally named *livetin*.

The presence of another protein in egg-yolk is mentioned in Hammarsten's *Lehrbuch der physiologischen Chemie*, 6th edition. Gross,* by precipitation with ammonium sulphate, obtained a protein differing widely in composition from vitellin.

EXPERIMENTAL.

I. Twenty-five egg-yolks were mixed with about an equal volume of 10 per cent. sodium chloride solution, and extracted several times with ether until the ether separated without colour. The saline solution was then reddish in colour and almost transparent. It was poured into about twenty volumes of water, and after twenty-four hours the liquid was decanted from the precipitate of vitellin. For purification, this was redissolved in 10 per cent. sodium chloride solution, reprecipitated by water, and the process once more repeated. It was finally dissolved in very dilute sodium hydroxide, filtered and precipitated by acetic acid. After thoroughly washing with water, boiling with alcohol and ether to remove lecithin, and drying over sulphuric acid, the

* *Inaug. Diss.*, to which I have been unable to refer.

vitellin was obtained as an almost white powder; it weighed 31 grams. For analysis the substance was dried at 110° :

0.9242 gave 0.02802 P_2O_5 .^{*} $P = 1.1$.

0.2074 „ 0.03192 N_2 .[†] $N = 15.39$.

0.2322 „ 0.03528 N_2 . $N = 15.19$ per cent.

The aqueous solution from which this vitellin was precipitated gave a very marked biuret reaction, but no further precipitate on dilution; when heated to boiling in the presence of a small quantity of acetic acid, a coagulation occurred. The whole quantity was therefore filtered, and in several portions was heated to boiling, each portion being slightly acidified with acetic acid. The several portions of coagulated protein were collected together on a filter, thoroughly washed with water, boiled with alcohol and ether to remove lecithin, and dried over sulphuric acid, when 30 grams of an almost white powder were obtained. It gave the following results on analysis, for which a portion was dried at 110° .

0.5676 gave 0.001395 P_2O_5 . $P = 0.11$.

0.2514 „ 0.03808 N_2 . $N = 15.15$.

0.2876 „ 0.0434 N_2 . $N = 15.09$ per cent.

This coagulated protein thus contains about the same amount of nitrogen as does vitellin, but the amount of phosphorus is very different. It must therefore be regarded as not identical with vitellin.

It has recently been found by Kaas (*Monats.*, 1906, 27, 403) and by Willcock and Hardy (*Abstr.*, 1907, i, 366) that ovalbumin contains 0.13 per cent. of phosphorus, and I have specially confirmed this with a specimen of crystallised ovalbumin prepared by Hopkins' method:

0.9356 gave 0.00228 P_2O_5 . $P = 0.11$ per cent.

In spite of this similarity in the phosphorus content, it seemed scarcely possible that these two proteins could be identical, since ovalbumin is coagulated by ether, whereas livetin is not coagulated. The following experiment with carefully washed yolks was therefore undertaken to determine this, and in order to confirm the above result.

II. Thirty-two egg-yolks were singly thoroughly washed free from adherent egg-white by allowing a gentle stream of water to flow over them when held in the palm of the hand. They weighed 548 grams, and were mixed with an equal volume of 10 per cent. sodium chloride solution. As before, this saline solution was extracted with ether

* The estimations of phosphorus were all made by Neumann's method as modified by Plimmer and Bayliss (*J. Physiol.*, 1906, 33, 441).

† All the nitrogen estimations described in this paper were carried out by Kjeldahl's method.

until the ether was colourless, but instead of being poured into a large volume of water it was dialysed for twenty-four hours; at the end of this time a large precipitate of vitellin formed; this was separated from the aqueous solution which was again dialysed for twenty-four hours, whereby the last traces of vitellin were thrown down. The precipitate of vitellin was purified as in Experiment I, and 42 grams of lecithin-free material were obtained. The analysis was carried out with a portion dried at 110° :

0.4636 gave 0.01445 P_2O_5 . $P = 1.14$.

0.2900 „ 0.04354 N_2 . $N = 15.01$.

0.2070 „ 0.03094 N_2 . $N = 14.94$ per cent.

As before the aqueous solution was filtered and heated in several portions to 100° , when coagulated protein separated in the form of flakes, commencing at about 60° , and being complete at about 90° . The collected portions were washed, boiled with alcohol and ether, and dried over sulphuric acid; the yield of livetin in this case was 10 grams, and just as in the first experiment it contained 0.1 per cent. of phosphorus, as shown by the analysis:

0.8750 gave 0.00203 P_2O_5 . $P = 0.10$.

0.3730 „ 0.05544 N_2 . $N = 14.86$.

0.3050 „ 0.04592 N_2 . $N = 15.05$ per cent.

III. A third experiment was carried out exactly as described above with twenty egg-yolks; 16 grams of vitellin and 11 grams of livetin were obtained, which gave the following results on analysis:

Vitellin III.

0.5900 gave 0.0142 P_2O_5 . $P = 1.05$.

0.2486 „ 0.03752 N_2 . $N = 15.09$.

0.1972 „ 0.02926 N_2 . $N = 14.84$ per cent.

Livetin III.

0.8956 gave 0.00216 P_2O_5 . $P = 0.11$.

0.3294 „ 0.04886 N_2 . $N = 14.83$.

0.2116 „ 0.03136 N_2 . $N = 14.82$ per cent.

IV. It was now necessary to determine whether livetin existed in egg-yolks as such or whether it resulted by the action of sodium chloride on vitellin. Fourteen washed egg-yolks (= 254 grams) were therefore extracted with fresh quantities of ether until the ether separated almost without colour. The residue was then extracted three times with about 2 litres of water; the second extract only contained a small quantity of protein, whilst the third extract was free from protein. The first aqueous extract was filtered and heated to boiling in slightly acid solution, when a coagulum was formed. This was washed with water, boiled with alcohol and ether to remove lecithin,

and dried over sulphuric acid (yield = 6 grams). Analysis was carried out with material dried at 110° :

0.9376 gave 0.001395 P_2O_5 . $P = 0.65$.

0.3354 „ 0.05026 N_2 . $N = 14.98$.

0.3620 „ 0.05404 N_2 . $N = 14.93$ per cent.

The residue was dissolved in dilute sodium hydroxide, filtered, and precipitated by acetic acid. This process was repeated, and 12 grams of vitellin were obtained after boiling with alcohol and ether, and drying over sulphuric acid. For analysis, a portion was dried at 110° :

0.6470 gave 0.01268 P_2O_5 . $P = 0.85$.

0.3028 „ 0.04648 N_2 . $N = 15.35$.

0.3530 „ 0.05404 N_2 . $N = 15.31$ per cent.

V. Twenty-one washed egg-yolks (= 436 grams) were treated exactly as in experiment IV. Ten grams of vitellin and 7 grams of livetin were obtained of which the following are the analyses:

Vitellin V.

0.3664 gave 0.0085 P_2O_5 . $P = 0.99$.

0.3284 „ 0.05068 N_2 . $N = 15.43$.

0.2764 „ 0.04242 N_2 . $N = 15.35$ per cent.

Livetin V.

1.0316 gave 0.0014 P_2O_5 . $P = 0.59$.

0.3338 „ 0.05082 N_2 . $N = 15.22$.

0.3196 „ 0.04802 N_2 . $N = 15.02$ per cent.

The percentage of phosphorus in the preparations of livetin IV and V is considerably greater than in the preparations I, II, III. This may be due to a slight solubility of vitellin in water in the presence of livetin, which would consequently increase the amount of phosphorus in these preparations; it is scarcely possible that vitellin is separated into a phosphorus-containing portion and a phosphorus-free portion by the action of 10 per cent. sodium chloride solution as used in the first preparations, for under these conditions the amount of phosphorus in vitellin would be greater than 1 per cent., and, further, when the vitellin is purified by re-solution in sodium chloride solution and re-precipitated, no protein coagulable by heat is found in the water. The former method is therefore the better one for separating the two proteins.

The yields of vitellin and livetin obtained in the various preparations fluctuated considerably; in the first preparation, they were respectively 31 and 30 grams or almost equal amounts; in the other preparations, the yield of livetin was considerably less than that of vitellin. This difference suggests a close relationship between the two

proteins in the yolk of eggs, and it is emphasised by the preparations containing almost exactly the same amount of nitrogen, which is very different from that in ovalbumin, and also excludes the identity of livetin with ovalbumin. Ovalbumin, according to Osborne and Harris, contains 15.51 per cent. of nitrogen.

In order to determine the relationship of these two proteins in the yolk of egg more closely, the preparations were hydrolysed into their constituent amino-acids, and the various forms in which the nitrogen is contained—namely, amide-nitrogen, humin-nitrogen, diamino-nitrogen, monoamino-nitrogen—was estimated by Hausmann's method in the manner described by Osborne and Harris in their study of the vegetable proteins.

About one gram of each preparation was hydrolysed by boiling with 20 c.c. of concentrated hydrochloric acid for seven to ten hours. The solution was evaporated in a vacuum at 40° to remove free hydrochloric acid, the residue treated with about 300 c.c. of water, and a slight excess of magnesia, freed from ammonia by boiling, was added. This mixture was distilled in a vacuum at 40° and the ammonia (= amide-nitrogen) estimated. The solution was then filtered; the residue was thoroughly washed with water, and the nitrogen contained in it (humin-nitrogen) estimated by Kjeldahl's method. The filtered solutions and washings were concentrated on the water-bath to 100 c.c., allowed to cool, and precipitated with 30 c.c. of phosphotungstic acid solution (20 grams of phosphotungstic acid and 5 grams of sulphuric acid per 100 c.c. of water). The precipitate was filtered off after twenty-four hours and washed three times with a solution of phosphotungstic acid containing 2.5 grams of phosphotungstic acid and 5 grams of sulphuric acid in 100 c.c. of water, the washing being effected by rinsing the precipitate from the filter into a beaker and returning to the filter paper three times, each portion of the wash water being allowed to drain away completely before the next was added. The nitrogen was determined in the precipitate (= diamino-nitrogen) by Kjeldahl's method and the nitrogen in the filtrate (= monoamino-nitrogen) calculated by difference of these values from the total nitrogen of the preparations. Two experiments were carried out in each case, and for comparison two experiments were performed with caseinogen (Merck), the other well-defined phosphoprotein. The results are embodied in the following table, in which are also given the values obtained by Osborne and Harris for ovalbumin.

	Total nitrogen.	Amide- nitrogen.	Humin- nitrogen.	Diamino- nitrogen.	Monoamino- nitrogen (by difference).
Livetin I...	15.15 } 15.12 15.09 }	0.78 } 0.75 0.71 }	0.37 } 0.32 0.26 }	3.26 } 3.29 3.32 }	10.76
Vitellin I..	15.39 } 15.29 15.19 }	0.86 } 0.84 0.81 }	0.23 } 0.25 0.28 }	3.85 } 3.84 3.84 }	10.36
Livetin II.	14.86 } 14.96 15.05 }	0.68 } 0.64 0.59 }	0.44 } 0.39 0.34 }	3.17 } 3.32 3.46 }	10.61
Vitellin II.	15.01 } 14.98 14.94 }	0.78 } 0.84 0.89 }	0.29 } 0.28 0.27 }	3.48 } 3.48 3.49 }	10.38
Livetin III	14.83 } 14.83 14.82 }	0.87 } 0.82 0.76 }	0.32 } 0.29 0.27 }	3.23 } 3.20 3.17 }	10.52
Vitellin III	15.09 } 14.97 14.84 }	0.89 } 0.91 0.93 }	0.26 } 0.26 0.26 }	3.35 } 3.34 3.34 }	10.46
Livetin IV.	14.98 } 14.96 14.93 }	0.66 } 0.68 0.69 }	0.28 } 0.26 0.25 }	3.09 } 3.12 3.15 }	10.90
Vitellin IV	15.35 } 15.33 15.31 }	0.69 } 0.75 0.80 }	0.26 } 0.25 0.25 }	3.78 } 3.77 3.76 }	10.56
Livetin V..	15.22 } 15.12 15.02 }	0.67 } 0.68 0.68 }	0.27 } 0.26 0.24 }	3.12 } 3.31 3.50 }	10.87
Vitellin V.	15.43 } 15.39 15.35 }	0.76 } 0.79 0.83 }	0.40 } 0.32 0.25 }	3.82 } 3.78 3.74 }	10.50
Caseinogen.	15.25 } 15.30 15.36 }	1.55 } 1.52 1.49 }	0.20 } 0.22 0.23 }	3.16 } 3.20 3.23 }	10.36
Ovalbumin (Osborne & Harris).	15.51	1.34	0.29	3.30	10.58

As can be seen from the table, distinct differences are noticeable between the values obtained for vitellin and livetin for the same preparation, although they are very close to one another. These differences are most marked in the monoamino-nitrogen figures, which are considerably higher for livetin than for vitellin. The opposite is the case for the amide-nitrogen and the diamino-nitrogen figures.

The several preparations of both vitellin and livetin do not give exactly the same figures; this is probably due to the impossibility of preparing a protein in a state of purity, in consequence of which some investigators, such as E. Fischer, consider a protein as made up of a mixture of complex substances which cannot be separated. If the complexity of a protein be considered, the same protein may not always be built up by the cells of amino-acids in exactly the same proportions. In the case of eggs, it must be remembered that greater or smaller changes in development occur before they are available for examination. This may also account for the different yields which were obtained, and it is possible that both vitellin and livetin represent stages in the change of a single original protein in the egg-yolk.

It may, however, be concluded that egg-yolk contains two proteins, which are fairly closely related to one another, the one, vitellin, containing 1 per cent. of phosphorus, and the other, livetin, containing 0.1 per cent. of phosphorus.

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