XCVIII. ON OVOMUCOID.

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I. THE CARBOHYDRATE CONTENT OF THE OVOMUCOID MOLECULE DURING THE DEVELOPMENT OF THE CHICK.

WHEN the carbohydrate metabolism of the extra-embryonic part of the developing hen's egg was being considered in a previous paper [Needham, 1926], an attempt was made to gain some idea of the movements of the "ovomucoid" fraction by adding the glycogen-glucose to the free glucose and subtracting the value so obtained from the total glucose. The resulting curve (being admittedly only of exploratory significance) gave a peak in the middle of incubation between its initially high values and the minimal ones of the end of incubation. This conflicted with the results of Bywaters [1913], who by means of analyses of coagulable and non-coagulable nitrogen in the egg-white during incubation concluded that there was no preferential absorption of albumin or ovomucoid but that both diminished steadily in the egg-white as development proceeded. Assuming that Bywaters' technique was satisfactory, as may most probably be done, there could be only two explanations of this discrepancy, (a) that the ovomucoid molecule contained more glucose in its prosthetic group at some times than at others during incubation, or (b) that the hump in the ovomucoid curve was due to a temporary increase of mucoprotein in the yolk only, at the time in question, mucoprotein which might or might not possess the same amount of glucose within its molecule as the ovomucoid of the egg-white. The former possibility was made somewhat likely by the recent work of Komori [1926], who considers that the glucose of ovomucoid is united to the protein part in the form of a polysaccharide, but if it were true, the results of Bywaters who found the ratio

$$\frac{\text{g. uncoag. N per 100 g. egg-white}}{\text{g. total glucose per 100 g. egg-white}}$$

to be more or less constant during incubation, would have to be abandoned.

In order to settle this question, therefore, preparations were made of ovomucoid from the egg-white from the 5th to the 18th day of incubation. The method used was as follows. The eggs were candled and pencil marks made on the shells to indicate the clear part where the egg-white lay close to the shell-membrane. They were then opened in that region, and the egg-white was carefully collected, though no attempt was made to recover every trace. The chalazae were included with the white. The mass was then weighed in a

tared bottle, diluted to twice its volume with distilled water and placed on a shaking machine for 2 hours in order to break up the albumin. For this purpose a quantity of broken porcelain was added and a few drops of caprylic alcohol to prevent frothing. The mixture was then further diluted and heated in a boiling water-bath for an hour with acetic acid, the reaction being so adjusted as to remain green to bromocresol purple. In this way the albumin was almost completely removed, subsequent tests with trichloroacetic acid demonstrating the presence of only the slightest traces of albumin. The filtrate was next poured into ten times its volume of 97 % alcohol, and the flocculent white precipitate filtered off, dissolved in as small an amount of hot water as possible, and reprecipitated. It was finally dried well over calcium chloride in a vacuum desiccator, and eventually hydrolysed for 4 hours with 5 % hydrochloric acid, the glucose or glucosamine set free being estimated in the filtrate by the Hagedorn-Jensen method [1923] after precipitation with phosphotungstic acid, which does not remove glucosamine.

Table I. *Experiments on ovomucoid.*

1	2	3	4	5	6	7	8	9	10	11	12
Day	No. of egg- whites	Total wt. g.	Egg-white g. per egg	Col. 4 smoothed	Wt. of egg-white in % of whole egg	Pure ovomucoid isolated mg.	Col. 7 mg. per egg	Col. 8 smoothed	Ovomucoid in mg. % of wet wt. of egg-white	Mg. taken for esti- mation	% glucose
0	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—
5	32	387	12.1	11.8	21.15	3583	112	128	1080	453	11.40
6	31	340	10.9	11.6	21.02	774*	25	125	1080	254	8.44
7	36	418	11.7	11.5	20.95	380*	11	122	1060	380	7.38
8	25	278	11.1	11.3	20.77	3563	143	119	1053	643	13.35
9	19	218	11.5	11.0	20.40	1015	53	117	1065	345	7.99
10	17	180	10.6	10.6	19.74	1427	84	110	1039	447	6.40
11	14	150	10.1	10.0	18.80	960	69	103	1030	310	12.80
12	18	173	9.6	9.3	17.60	2405	134	92	989	785	9.39
13	9	73	8.2	8.3	15.85	584	65	82	988	414	12.90
14	29	335	7.6	7.1	13.68	522	18	70	986	{ 126 176	{ 14.30 13.00
15	3	13	4.3	5.5	10.70	286	95	55	1000	185	11.30
16	44	248	3.02	3.9	7.65	2562	58	40	1026	{ 360 652	{ 14.30 13.00
17	70	337	3.1	2.2	4.36	3166	45	20	909	{ 380 516	{ 14.70 12.60
18	11	12	1.1	0.6	1.20	49	4	4	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—	—

* Considerable losses.

Table I shows the results in general. These are the first figures to be published for the weight of the egg-white at different stages, and it is interesting to note how it disappears altogether by the 18th day. Quantitative expression is thus afforded for what originally was an observation of William Harvey [1653]. Col. 6 shows what percentage of the whole egg is taken up by the white, and is calculated, allowing for the loss of water during incubation, from

the figures of Murray [1925, Fig. 2]. It descends from 21 to 0 %. Col. 7 shows the ovomucoid isolated in g. and cols. 8 and 9 in g. per egg; it falls from 0.128 to 0.004. Although it is not claimed that there was a quantitative recovery of ovomucoid (and indeed these figures are much less than the fragmentary ones of Komori) yet, as the same care was used all through, it is probable that they have some relative significance. When they are compared with those of col. 5 and expressed as percentage of the wet weight of egg-white, as is done in col. 10, it is interesting to note that the amount of ovomucoid in 100 g. of wet egg-white remains constant throughout development at about 1 g. As far as these figures go, then, they confirm the observations of Bywaters and indicate that there is no preferential absorption of ovomucoid from the white, although there may be from the yolk. This latter possibility is made likely by the fact that the hump on the ovomucoid-glucose curve comes just between the two times at which the mucoprotein-glucose inside the embryo is high.

Col. 11 shows the mg. of ovomucoid taken each day for hydrolysis, and col. 12 the values found for the carbohydrate content of the molecule calculated as glucose. The average value is 11.5 %. Probably this represents the most accurate figure so far obtained, for all previous estimations were performed with copper methods and, as Holden [1926] has shown, in no conditions are these more unreliable than when dealing with protein hydrolysates. The values are tabulated as follows:

Investigator	% glucose in ovomucoid
Hofmeister [1898]	15.0
Seemann [1898]	29.4
Langstein [1900]	10.5
Willanen [1906]	19.5 to 22.3
Pavy [1907]	21.7
Samuely [1911]	34.9
Neuberg and Schewket [1912]	24.0
Zeller [1913]	33.7
Average	23.4
Present results	11.5

The most striking thing about col. 12, however, is that when the figures are plotted on a graph, an unmistakable upward trend is seen. The line which is drawn, having regard to all the points, traverses the graph, rising all the time from 7.4 on the 5th day to 15.4 on the 18th. No explanation has been found for this phenomenon. It is at any rate clear that there is no marked increase in the glucose content of ovomucoid at the period when it would be expected in the former of the two views outlined above. The mucoprotein of the yolk is therefore probably much increased at that time, and this will henceforth be used as a working hypothesis.

The gradual trend of the ovomucoid toward a higher glucose content may be regarded as one of those slow intramolecular changes which take place during embryonic development, a good example of which has recently been afforded by Plimmer and Lowndes [1927] in their work on the amino-acid distribution in the proteins of the hen's egg before and after incubation.

II. THE EXISTENCE AND LOCALISATION OF OVOMUCOIDASE IN THE HEN'S EGG.

In 1899 Müller and Masayama [1899, 1900] reported the discovery of an active enzyme in the yolk of unincubated hens' eggs which would hydrolyse starch. This diastase was destroyed by heat and "under favourable conditions" would convert 45 % of a 3 % starch solution into the soluble forms of dextrin and isomaltose in 24 hours at 37°. This put on a sure basis the earlier and rather doubtful results of Krukenberg [1879] and was in turn confirmed by Diamare [1909], Herlitzka [1906] and Roger [1908]. Idzumi [1924] brought forward data which showed that the activity of the extra-embryonic diastase markedly increased as development proceeded, especially after the 15th day (rising from 40 to 640 units). Finally, Komori [1926] demonstrated that most if not all of the glucose in the ovomucoid molecule could be liberated by incubation with diastase prepared from meal. It remained to demonstrate that the egg itself, or certain parts of it, possessed the power of hydrolysing ovomucoid.

Experiments with this aim in view were carried out as follows. About fifty eggs were incubated under standard conditions for 5 days. At the end of that time they were broken open and divided into fractions thus: (a) the embryonic body, (b) the blastoderm and yolk-sac, (c) the egg-white, and (d) the yolk. The embryos themselves were placed one by one in distilled water in a small crystallising vessel under a strong beam of light and carefully freed from their amniotic membrane and the small allantois. After having been well washed in distilled water they were ground up with clean quartz sand in a mortar. The blastoderms and yolk-sacs were collected by pouring yolks and whites together into a large volume of water from which the membranes could with care be fished out. They were washed for 6 hours in a strong stream of water and finally ground up with quartz sand. The yolk and white were collected from other eggs with as little contamination as possible and were placed on a shaking machine for 2 hours with broken porcelain in order to mix them well. Then into a series of conical flasks were pipetted 50 cc. each of a solution of pure 5th day ovomucoid previously prepared and so arranged that each trial should contain 400 mg. of ovomucoid. Into a parallel series of flasks were run 50 cc. each of water, and to one of each series were added 50 cc. of embryo extract, yolk, etc., as the case might be. Toluene was added to 10 %. The flasks were plugged with cotton wool, and incubated at 37° for 3 days exactly, being shaken once a day. In view of the preliminary nature of these experiments, the p_H was not controlled by buffers. Glucose liberated was estimated in all the samples by the Hagedorn-Jensen method following precipitation with phosphotungstic acid. Table II gives the results.

As can be seen from Exp. K the ovomucoid preparation itself contained no free glucose at all, but when incubated alone about 2 mg. were split off, perhaps because of the presence of minimal amounts of the enzyme. This auto-digestion effect must be subtracted from the apparent results of col. 6 and the corrected values are shown in col. 7. It will be noted in the first place that the

Table II. *Experiments on ovomucoidase.*

1	2	3	4	5	6	7	8	9	10	11
Exp.	Fraction tested	Substrate added	3 days' incubation at 37°	mg. glucose found	Difference due to ovomucoid hydrolysis	Corrected for Exp. L	Yield % of theoretical	Dry wt. of sample g.	Col. 7 as % dry wt.	p _H of system during incubation
A } B }	Embryo extract	{ - +	{ + +	{ 5.83 6.32	0.49	0.00	0.00	0.351	0	7.0
C } D }	White	{ - +	{ + +	{ 5.04 7.35	2.31	0.44	1.49	6.00	7.3	8.1
E } F }	Yolk	{ - +	{ + +	{ 17.70 28.80	11.10	9.23	31.20	15.00	61.6	5.4
G } H }	Yolk-sac and blastoderm	{ - +	{ + +	{ 0.15 4.13	3.98	2.11	7.13	—	—	6.9
K } L }	Water	{ + +	{ - +	{ 0.00 1.87	—	—	—	—	—	7.1

slight excess of glucose in the embryo preparations is much more than accounted for by the correction of Exp. L, the effect of the egg-white is much reduced, but the yolk and the yolk-sac results still retain considerable magnitude. The conclusion, therefore, is that the enzyme ovomucoidase is not contained in the embryonic tissues themselves and only to a negligible extent in the egg-white, but that the yolk is very rich in it and that the blastoderm and yolk-sac also show a fair activity. Thus the embryo tests yield 0 % of the theoretical, the white 1.49, the yolk 31.2 and the yolk-sac 7.13. This conclusion is not affected by the varying amounts of solid material which must have been contained in the original samples, for as cols. 9 and 10 show, the yolk even so retains its superiority.

These results fit in well with what is already known. Roger, for instance, found more diastatic activity in the yolk than in the white. Bywaters [1913], by comparing the ratio

$$\frac{\text{g. uncoag. N per 100 g. egg-white}}{\text{g. glucose per 100 g. egg-white}},$$

came to the conclusion that the ovomucoid of the egg-white was not split up into protein and sugar moieties before absorption; but he only investigated the white, and for that fraction alone his results do not conflict with those now brought forward. The ovomucoid must be pictured passing from the white to the yolk, and there being split up into free glucose and protein before absorption into the embryo through the vitelline veins. It is interesting that the embryo possesses no ovomucoidase or very little; the hydrolysis must therefore be considered to take place outside it. The passage of ovomucoid from white to yolk is not difficult to imagine, for the existence of a current of water and of free glucose yolkwards has been clearly demonstrated [Aggazzotti, 1919; Needham, 1927]. Idzumi's observations on the increase of activity of the yolk diastase during development fit in perfectly with the increasingly rapid

disappearance of ovomucoid seen in Table I, col. 9. It would be very desirable to extend these observations by investigating the effect of p_H , salt concentration, etc., on the enzyme and by determining whether its activity remains the same in all fractions during development.

SUMMARY.

1. The carbohydrate content of the ovomucoid molecule, calculated as glucose, appears to be on an average 11.5 %. But there is a definite trend upwards as development proceeds, only 7.4 % being present on the fifth day but as much as 15.4 % on the 18th day.

2. An enzyme which hydrolyses ovomucoid appears to exist in the yolk and the yolk-sac of the hen's egg at the 5th day of development, possibly also in the white and the blastoderm, but not in the embryo.

3. Utilisation of ovomucoid by the embryo probably consists in a regular passage of ovomucoid into the yolk, where it is hydrolysed before being absorbed through the vitelline veins at a rate which varies during the incubation period.

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