CXXXV. A NOTE ON SELACHIAN YOLK-PROTEINS.

By JOSEPH NEEDHAM.

*From the Biochemical Laboratory, Cambridge, and the Marine
Biological Laboratory, Millport, Scotland.*

(Received November 6th, 1929.)

CONSIDERABLE interest attaches to the proteins of egg-yolk throughout the animal kingdom, but, although those which occur in avian yolk have been fairly well investigated, little has been done with those of the eggs of other groups. As a result of the researches of Bayliss and Plimmer [1906], Plimmer and Scott [1908], Kay and Marshall [1928] and others, we know that only two proteins exist in the egg-yolk of the hen, vitellin, a phosphoprotein, and livetin, a pseudoglobulin, the latter being identical with the so-called Gross's protein [Gross, 1899]. As regards other eggs, however, our information is fragmentary, for while a substance called ichthulin, very similar in its properties and constitution to vitellin, has frequently been found in fish eggs, we do not know whether it is the only protein there, or whether it exists in the eggs of such groups as insects and crustacea.

A supply of the egg-yolk of the selachian *Acanthias vulgaris* (the "spurdog") being available owing to the necessities of other work, it was thought worth while to investigate two points concerning its proteins; (a) the presence of phosphorus in the selachian ichthulin, and (b) the presence of any other protein in the yolk. The first of these arises out of the work of Alsberg and Clark [1908] which forms the only other study of the eggs of this fish. Alsberg and Clark prepared the ichthulin following the classical method of solution in saline and precipitation by dilution, and then, determining the elementary composition of their preparation, found to their surprise that it contained no phosphorus. The eggs of *Acanthias vulgaris*, they concluded, contain no vitellin in the strict sense of the word, but in its place a globulin or a mixture of globulins containing no phosphorus and perhaps no iron. They also put forward the suggestion that this state of affairs, which formed such a striking exception to all other vertebrate eggs, might be connected with the fact that the fish is ovoviviparous.

In the present work the yolk (which is contained within a vitelline membrane forming a sphere about 3 cm. in diameter and flows out as a pale yellow creamy material thinner than avian yolk) was thoroughly mixed with an equal volume of 10 % NaCl and brought to the laboratory in that form. The yolks from 16 eggs were used, *i.e.* 368 g. of raw material. The mixture was now extracted

with ether in a separating funnel until no more pigment and fat passed into the solvent, and then precipitated by dilution with distilled water. When the precipitate had settled it was centrifuged off and again dissolved in 10 % salt solution to be again precipitated by dilution with water, the supernatant liquid being retained for further examination. After five precipitations in this way, the protein was washed with ether-water by successive decantations until no more chloride could be found in the washings, during which time it was observed that the protein settled less and less readily. Then it was transferred to a flask and boiled out with alcohol for 5 days under a reflux condenser, the alcohol being frequently changed. Finally, the material was dried for 6 months *in vacuo* over sulphuric acid.

The protein so obtained was a greyish powder which gave positive results with the biuret, glyoxylic, and Millon reactions. Its solubilities were such as are usually found with vitellins, but no work was done on its coagulation temperature or other physical properties. In Table I are shown the data for elementary composition as determined by Alsberg and Clark and in the present work. The main difference consists in the results for phosphorus, which was determined after a thorough incineration by the colorimetric method of Fiske and Subbarow [1925]. No difficulty was experienced in showing its presence in about the same percentage as is usually found in vitellins and it is therefore difficult to explain their failure to identify it.

Table I.

	C %	H %	N %	P %	S %	
Selachian egg-yolk						
Icthulin	50.49	7.16	16.76	None	0.91	(Alsberg and Clark)
"	48.80	7.58	14.75	0.62	1.17	(Present work)
Avian egg-yolk						
Vitellin	51.24	7.16	16.38	0.94	1.04	[Osborne and Campbell, 1900]

(These figures are corrected for ash, which in the preparations described here amounted to 1.42 %.)

Attention was next directed to the dilute salt solution from which the ictulin had been precipitated, and on evaporating down some 10 or 12 litres of this, it was found that another protein was present. Boiling with acetic acid at a p_H just giving a green colour with bromocresol purple produced a flocculent precipitate and trichloroacetic acid also gave a positive result. After half-saturation with ammonium sulphate, however, and filtration of the resulting precipitate, these reagents no longer showed even a trace of protein. A second half-saturation with ammonium sulphate also gave a negative result. The precipitate, however, gave strong xanthoproteic, biuret, and glyoxylic tests, with weak Millon and weak Molisch reactions, and the obvious probability was that here was a protein in selachian yolk corresponding to avian livetin, just as the ictulin corresponded with the vitellin. In order to purify this material further, it was dissolved in water and reprecipitated four times by half-saturation with ammonium sulphate, after which it was boiled with alcohol under a reflux condenser in the same way as the ictulin. It was then

placed in a collodion sac, brought to p_H 5.0 and dialysed against distilled water for about a week, the water being frequently changed, at the end of which time no more ammonia or sulphate could be found in the dialysate. The protein was then dried *in vacuo* over sulphuric acid, and its elementary composition determined, as shown in Table II, under the suggested name of "thuichthin."

Table II.

	C %	H %	N %	P %	S %	
Selachian egg-yolk						
Thuichthin	45.60	7.39	12.14	0.039	1.96	(Present work)
Avian egg-yolk						
Livetin	—	—	15.35	0.067	1.80	(Kay and Marshall)

(These figures are corrected for ash, which in the preparations described here amounted to 1.16 %.)

The second protein of selachian yolk, therefore, presents a close analogy with the second protein of avian yolk, being low in phosphorus and high in sulphur. The N : P ratio is, according to Kay and Marshall, 1 g. nitrogen to 3.14 mg. phosphorus in the case of livetin, in thuichthin it is 1 g. nitrogen to 3.08 mg. phosphorus.

Unfortunately the preparations described here were not made in a sufficiently quantitative manner to enable any statement to be advanced about the relative proportions of ichthulin and thuichthin in the dogfish egg. The interest of the results lies rather in the remarkable similarity exhibited by the protein distribution in the eggs of such widely different animals as the hen and the dogfish. It may now be definitely stated that in both these cases the protein of the yolk, as far as can be ascertained by the current methods of protein characterisation, divides into two individual substances, one phosphoprotein and one pseudoglobulin. It would be very interesting to examine the eggs of insects, molluscs, echinoderms, etc., from this point of view, for if these two types of protein alone are to be always associated with the raw material of embryonic development, the question arises, why two should be found there, and not one or half-a-dozen.

SUMMARY.

A study of the yolk-proteins of the dogfish, *Acanthias vulgaris*, allows the following conclusions.

1. The main protein present is a phosphoprotein, containing 0.62 % P, and resembling in its general characters the vitellins.
2. A second protein, to which the name "thuichthin" is given, accompanies the ichthulin in this egg. It has the properties of a pseudoglobulin and is analogous to the livetin of avian egg-yolk.

REFERENCES.

- Alsberg and Clark (1908). *J. Biol. Chem.* 5, 243.
 Bayliss and Plimmer (1906). *J. Physiol.* 33, 451.
 Fiske and Subbarow (1925). *J. Biol. Chem.* 66, 375.
 Gross (1899). Inaug. Diss. (Strassburg).
 Kay and Marshall (1928). *Biochem. J.* 22, 1264.
 Osborne and Campbell (1900). *J. Amer. Chem. Soc.* 22, 413.
 Plimmer and Scott (1908). *J. Chem. Soc.* 93, 1699.