XXXIX. SCYLLITOL IN SELACHIAN ONTOGENY.

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ONE of the principal aims of chemical embryology must certainly be to find out, in the case of any given egg, exactly what the embryo is provided with by the maternal organism. Anything with which it is not provided at the start must either be absorbed from the environment or synthesised during development, and it is important to know how much of this kind of work the embryo has to perform during its morphogenesis. We know, for example, that the eggs of marine invertebrates are not supplied with enough water or enough ash to make the complete embryo, while those of fishes have enough ash but not enough water. With regard to organic constituents also the same distinction can be made. As far as we know all eggs contain sufficient protein, and in no case does protein have to be manufactured from other nitrogenous bodies. Probably the same holds true of carbohydrate, but undoubtedly certain fishes and gastropods have to synthesise fatty acids during their ontogeny, and there is evidence that in the eggs of birds, for instance, some 15 % of the total cholesterol which the embryo requires has to be formed from other substances. Lipins, on the other hand, judging from the behaviour of ether-soluble phosphorus, are always present in ample amount at fertilisation, though the range of species for which we have information is not large. The two most outstanding instances of substances which are not provided by the maternal organism are pigments—thus no egg contains haemoglobin—and cycloses.

In a previous paper [Needham, 1924] it was shown that the *i*-inositol which is found in all the tissues of the newly hatched chick has to be synthesised by the embryo during the three weeks of its development. This observation had been previously made by Klein [1909] but the course of formation of the cyclose by the chick was followed by an estimation-method, which, though it left much to be desired, was an improvement on any of the techniques which had been at the disposal of the earlier workers.

The inositol of birds and mammals is not the only one which occurs in animal tissues. Of its seven possible stereoisomeric modifications, one other is known, scyllitol, first isolated from elasmobranch fishes by Stadeler and Frerichs [1858]. This body is identical with the quercinitol which has been obtained from acorns, and with the cocositol of the palm-tree [Müller, 1912]. In view of the fact that the cycloses have recently acquired considerable

physiological importance through the identification of *i*-inositol with (Wildier's) Bios I by Eastcott [1928], and for the general embryological reasons already mentioned, it was thought worth while to investigate the origin of scyllitol during the development of a selachian fish. The material, collected at the Millport Marine Biological Station, consisted of eggs and embryos of *Acanthias vulgaris* (the "spur-dog") and *Scyllium canicula* (the "rough-dog").

The method of isolation of the scyllitol embodied the most useful parts of various previous methods. The eggs or minced embryos were preserved in acetone and, when the time came, were extracted repeatedly with acetone. The combined extracts were then freed from acetone by a vacuum distillation and the aqueous solution extracted several times with ether in order to remove pigments and substances of a fatty nature. Most of the pigment of the eggs and some of the pigment from the embryos was thus removed. The solution was freed from ether by evaporation and protein precipitated by boiling with acetic acid at a p_H at which bromocresol purple was just green. A saturated solution of neutral lead acetate was then added after neutralisation and the precipitate, which was usually small in amount, filtered off and discarded. To 100 parts of the filtrate were now added 60 parts of a saturated solution of basic lead acetate, 20 parts of a 40 % solution of cadmium nitrate, and 5 parts of concentrated ammonia, for the cycloses are completely carried down on the precipitate of lead hydroxide and the double salt of cadmium and lead which is formed. The precipitate was then filtered off, washed, suspended in distilled water, and decomposed with hydrogen sulphide. The lead sulphide was next removed by filtration, three or four extractions with water at 70° being required to wash the cyclose completely away from it. A second treatment with hydrogen sulphide was sometimes necessary, but, if not, the solution was evaporated down to about 10 cc. and poured into 20 times its volume of absolute alcohol, when the scyllitol separated in the form of a microcrystalline powder. After being allowed to stand overnight it was filtered off and weighed, a correction being made for whatever ash the product contained, as the cycloses, being insoluble in all organic solvents, are rather difficult to free from salts.

In some cases the simpler method of Rosenheim [1917] was used, in which the original extracts, freed from acetone, and allowed to stand at a low temperature, deposit crystals of scyllitol. A sample of scyllitol prepared in the course of this work melted at 330°. The following results were obtained.

Acanthias vulgaris:

Exp. B₁. 8 yolks of undeveloped eggs (the perivitelline liquid, which was included, is very small in amount):

Total weight, 167 g.; i.e. 20.8 g. each egg wet weight.
 Total dry weight, 53 g.; i.e. 6.63 g. each egg dry weight.
 Percentage of water, 68.2.
 Scyllitol isolated, 2.0 mg.;
 i.e. 0.25 mg. per egg;
 1.2 mg. per 100 g. wet weight;
 4.17 mg. per 100 g. dry weight.

Exp. D. 3 yolks of undeveloped eggs:

Total wet weight, 75.7 g.; i.e. 25.2 g. each wet weight.
 Total dry weight, 33 g.; i.e. 11 g. each dry weight.
 Percentage of water, 56.5
 Scyllitol isolated, traces only, insufficient for weighing.

Exp. Am. 2 yolks of 23 cm. embryos:

Total wet weight, 37 g.; i.e. 18.5 g. each wet weight.
 Total dry weight, 12.6 g.; i.e. 6.3 g. each dry weight.
 Percentage of water, 66.
 Scyllitol isolated, 12.0 mg.;
 i.e. 6.0 mg. per egg;
 32.4 mg. per 100 g. wet weight;
 95.1 mg. per 100 g. dry weight.

Exp. E. 2 embryos of 23 cm. length (corresponding to the twentieth month of development out of twenty-one [Ford, 1921]):

Total wet weight, 80 g.; i.e. 40.0 g. each wet weight.
 Total dry weight, 12.5 g.; i.e. 6.25 g. each dry weight.
 Percentage of water, 84.1.
 Scyllitol isolated, 52.5 mg.;
 i.e. 26.25 mg. per embryo;
 65.7 mg. per 100 g. wet weight;
 420.0 mg. per 100 g. dry weight.

Exp. F. 12 embryos of 23 cm. length:

Total wet weight, 496 g.; i.e. 41.3 g. each wet weight.
 Total dry weight, 61 g.; i.e. 5.09 g. each dry weight.
 Percentage of water, 87.6.
 Scyllitol isolated, 292.2 mg.;
 i.e. 24.3 mg. per embryo;
 59.0 mg. per 100 g. wet weight;
 480.0 mg. per 100 g. dry weight.

Exp. G. Adult muscle:

From 47.75 kg. wet weight (9.55 kg. dry weight) 5.75 g. of scyllitol were obtained; this yield was almost exactly the same as that obtained from 50 kg. by Rosenheim [1917] and Müller [1912]: 0.012 mg. per 100 g. wet weight; 0.06 mg. per 100 g. dry weight.

Scyllium canicula:

Exp. C. 4 egg-contents (yolk and jelly):

(As *Acanthias vulgaris* is an ovoviviparous form, retaining its eggs and embryos within the uterus until the end of their development, it might be argued that the supply of scyllitol was derived from the mother. *Scyllium*, however, is oviparous.) Dry weight, 4.36 g.; i.e. 1.09 g. dry weight each egg. Scyllitol isolated; traces only, insufficient for weighing.

The material used in the above experiments was not very abundant, for it is somewhat difficult to collect, but the results are quite concordant and permit of the conclusion that the scyllitol present in the embryos at the end of development has to be synthesised by them and is not provided in the egg at the beginning. The dogfish is thus in exactly the same position as the chick. In Fig. 1 are shown graphically the relations which have been found to hold in the two kinds of eggs. Eastcott [1928] determined the amount of *i*-inositol present in undeveloped hen's eggs by a biological assay dependent on the identification of inositol with Bios I, and, as the diagram shows, her results agree very well with the figures obtained in direct analysis. It is interesting to note that scyllitol itself will not function as Bios I (Eastcott: private communication).

Fig. 1.

One further point which emerges from the data given above may be mentioned. The distribution of solid and water is evidently different in the embryos from what it is in the eggs, and the facts may be written as follows:

	Water	Solid
	g.	g.
<i>Acanthias vulgaris</i> : Undeveloped eggs	14.2	8.8
Finished embryos	35.0	6.0

This is another illustration of the rule that in fishes the egg is not provided with sufficient water to make the embryo, but has to absorb it from the environment, and, as it were, to dilute the raw materials with it. No doubt in the case of *Acanthias*, where the eggs remain within the maternal body, the water required is supplied from the maternal blood, but this dogfish stands low in the Ercolani placenta classification, for it has nothing but pits and inequalities on the maternal side, pressed up against the smooth horny egg-case. Little else than water, therefore, probably passes between maternal blood and the eggs.

The present communication adds further to the evidence that cyclose can be synthesised by animal cells. To the two cases of its formation in the egg may be added its formation by polyuric rats on pure diet, and by the plant, as has recently been shown by Eastcott for yeast. It would be interesting to know whether the eggs of lamellibranchiate gastropods contain enough mytilitol for the needs of the embryo or not.

SUMMARY.

The scyllitol of the undeveloped eggs and fully developed embryos of the selachian fish, *Acanthias vulgaris*, was investigated. It was found that just as the chick has to synthesise during its ontogeny 90 % of the inositol with which it hatches, so the dogfish has to synthesise 90 % of its scyllitol. The cycloses would therefore seem not to be provided in the eggs of birds or fishes by the maternal organism.

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