THE GROWTH OF FISH

I. THE RELATIONSHIP BETWEEN EMBRYO AND YOLK IN *SALMO FARIO*

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(*Received 5th August* 1926.)

(With Four Text-figures.)

In most studies of post-embryonic growth it is customary to accept the observed increment in the total mass of the organism per unit time as an index of growth. Davenport (1897) found, however, that the opening phases of development of the frog were characterised by a very rapid rise in the water content of the embryo whilst the dry weight during this period gradually decreased. He concluded from these facts that the process of early embryonic growth was of a different and more "passive" nature than the more "active" growth which later ensues. Passive growth was regarded by him and by others as some form of imbibition, whilst the subsequent "active" growth involved the conversion of organic substances into living tissue and so involved an increase in dry weight. In the case of terrestrial animals, however, it has been known for some time that with increasing age the percentage of water in the organism falls, and the recent figures of Murray (1926) show that this fall is detectable at a very early age. It must be remembered that Davenport's figures apply to the whole larva which in its early stages is heavily charged with yolk, whereas Murray's figures apply to the embryo alone.

In the case of trout larvae it is a comparatively simple operation to dissect away the embryo from the yolk sac and follow from a fairly early stage the process of conversion of yolk into living tissue.

In the following experiments ten or more alevins from the same batch of eggs were narcotised, and the adherent water removed by filter-paper. They were then weighed in milligrams and the yolk sac dissected off carefully with fine forceps and sharp needles. The embryos were then weighed, and the dry weight of embryo and yolk determined by drying over a water-bath and cooling over strong sulphuric acid until a constant weight was recorded. Owing to the liquid nature of the yolk, the wet weight was taken as the difference between the wet weight of the larva minus the wet weight of the embryo. Direct weighings of wet yolk are unsatisfactory owing to loss of water by evaporation.

The eggs of the Brown Trout (*Salmo fario*) only become satisfactory material for experimental purposes after incubation at 10° C. for about 20 days; prior to this, fertilised eggs are extremely sensitive to mechanical disturbance. This limitation is not of great significance since at the end of this period the embryo only weighs about 0·002 gm. and the error in weighing even comparatively large numbers is considerable. From this point onwards, the eggs are very hardy and can be incubated with little or no mortality. The material used in these experiments was received from the Nailsworth Trout Farm, on about the 25th day after fertilisation and were transferred to hatching trays in Cambridge and incubated at 10° C., which was approximately the temperature maintained at Nailsworth. The date of hatching varies slightly with different batches of eggs but is extraordinarily constant for any one batch. Naturally the date of hatching depends very greatly on the temperature. At 10° C. nearly all eggs hatch between the 42nd and 45th day after fertilisation. All the larvae used in an experiment hatched within 24 hours of each other, the rest were discarded.

The larva hatches at a very early stage in its development, viz. when about 18 per cent. of the wet larva is embryo, and 82 per cent. is yolk. The absolute weight of the larva at hatching varies with the size of the original egg; in one series the wet weight of the newly-hatched larva was 0·099 gm. and the wet weight of the embryo was 0·018 gm. As far as could be determined the process of hatching does not affect the growth of the embryo in any way; the loss of the thin egg-membrane and the perivitelline fluid leads to a loss of about 10 per cent. of the wet weight of the whole egg—otherwise the process of hatching is an uneventful process in the development of the embryo. For a long time prior to hatching the embryo is separated from the yolk by a syncitial membrane, the parablast, and the yolk is conveyed to the embryo in some soluble form by the vitelline circulation. The left vitelline vein is much larger than the right.

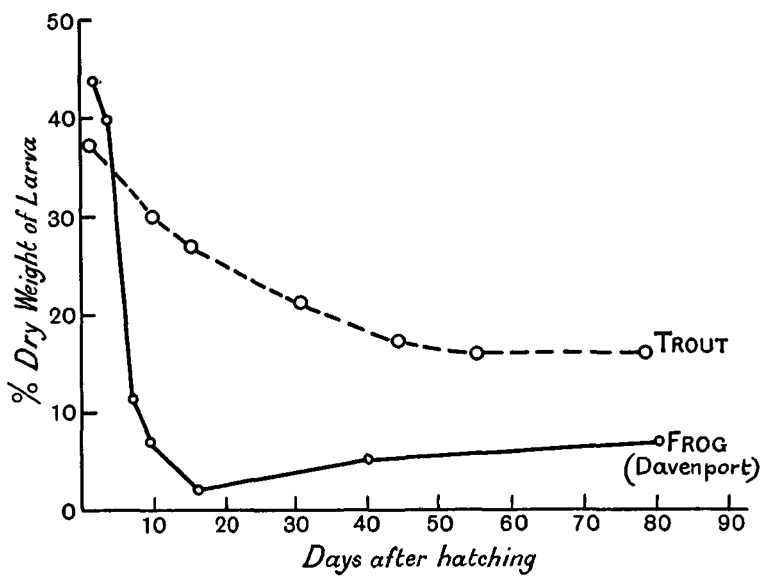

Diagram illustrating change in water content of Larvae

Fig. 1.

The percentage of dry solids in the larva, the embryo, and in the yolk are shown in Table I and Figs. 1 and 2, which represent the results of two different experiments.

Table I illustrates the following points:

(i) Between the time of fertilisation and of hatching the percentage dry weight of the egg falls slowly from 38 to 35·5 per cent.

(ii) The act of hatching increased the percentage dry weight by about 1·5 per cent. This is due to the sudden loss of the perivitelline fluid.

(iii) After hatching, the percentage dry weight of the larva falls from 37 to 16 per cent.

(iv) During the whole of this phase of development the percentage dry weight of the embryo alone is approximately constant at 16 per cent., and that of the yolk at 41 per cent.

Table I.

The eggs hatched on 38th day having previously been incubated at about 11·5° C.

Age in days	Percentage dry solids in		
	Larva	Embryo	Yolk
0	38·0	—	—
34	35·5	—	—
38	35·5	—	—
38 (H.)	37·0	—	—
43	—	14·8	41·0
46	37·2	—	—
48	34·0	16·0	—
55	29·8	16·4	—
60	27·2	16·5	40·4
64	28·0	17·0	—
68	24·3	16·6	—
75	22·0	15·7	42·0
82	19·0	—	—
91	17·4	—	—
100	16·0	16·0	—

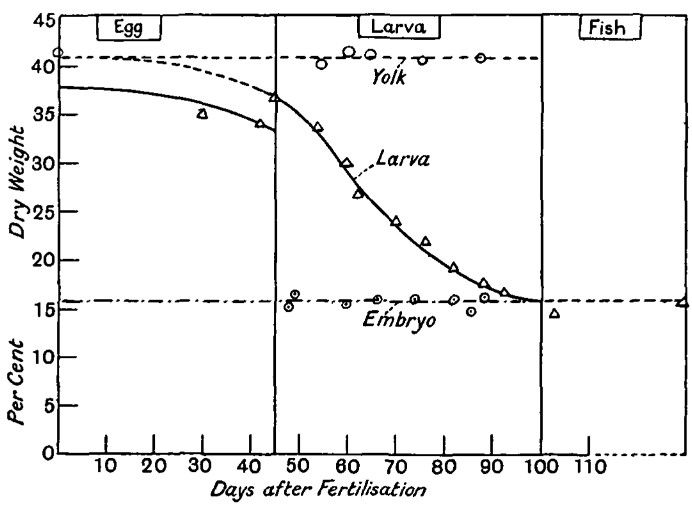

Fig. 2. Diagram illustrating the percentage dry weight of larva, embryo and yolk at different stages of development. Hatched on 45th day.

In Fig. 2 the continuous curve of the percentage dry weight of the larva is the calculated curve obtained from Fig. 3 (which shows the relative masses of embryo and yolk at different ages) on the basis that the dry weight of the embryo remains constant at 16 per cent. and that of the yolk at 41 per cent. It is clear that the fall in the percentage dry weight of the whole larva is entirely due to the development of the embryo, which itself undergoes no appreciable change in water content. In other words, as yolk is converted into embryo water is added from the external environment; the yolk itself may be regarded as more or less desiccated nutriment. From the figures given by Fauré-Fremiet (1925) it seems certain that a similar explanation applies to the development of the frog's egg, and we can therefore conclude that the concepts of "passive" and "active" growth have no foundation in fact.

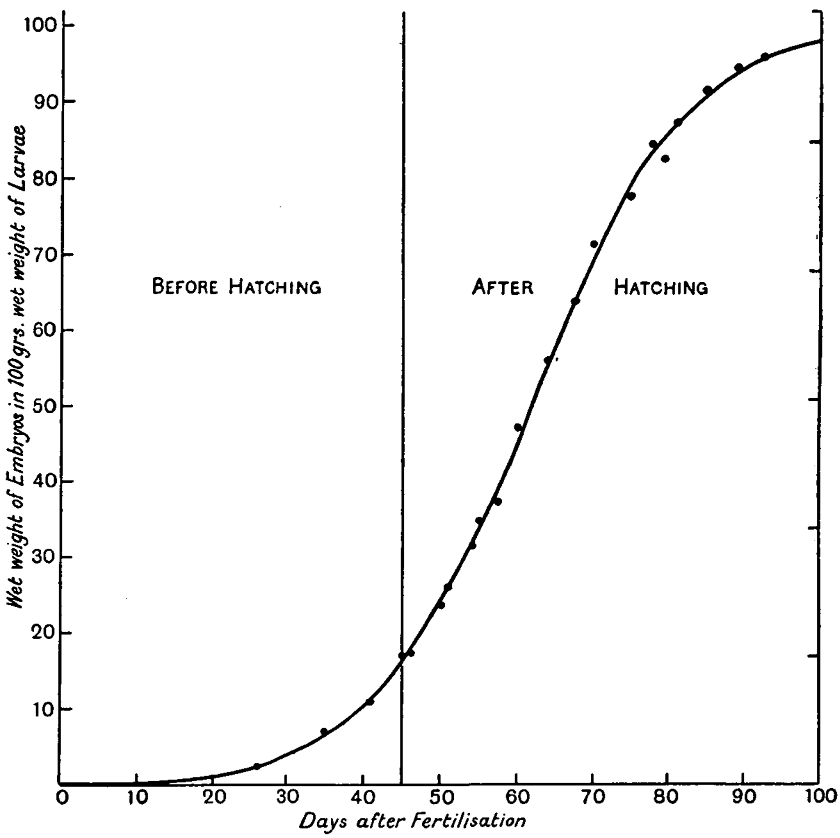

Fig. 3. Curve illustrating the relative amount of embryo and yolk at different ages of the larva.

It will be noticed that the fish embryo does not show an increasing percentage dry weight during the larval stage of development, and in this respect differs from that of the chick. The constant composition of the fish embryo is probably associated with the fact that during the larval period there is no bone formation or appreciable deposition of fat; the increase in mass is largely due to an increase in the size of the muscles, central nervous system and (to a minor extent) of the cartilage and skin. As the relative proportions of these organs remain practically constant, it is not surprising to find a constant percentage dry weight.

Now, if the initial weight of wet yolk in a newly fertilised trout egg is approximately 100 mg., and this is converted into embryo it is obvious that, since 41 mg. of dry yolk are present, 41 mg. dry weight of fish can be produced whose wet weight is $\frac{4100}{16} = 256$ mg. In other words, there is enough dry material in the original egg to produce a young fish two and a half times the original wet weight of the newly fertilised egg. On the other hand, there are only 59 mg. of water in the original yolk, and this is only sufficient for 70 mg. wet weight of fish. The observed weight of the fish at the end of the yolk conversion phase of development is actually about 150 mg. The explanation of this is as follows: the whole of the yolk present in the original egg is not converted into embryo; some of it is required for maintaining the larva in its normal active condition. We may define the ratio existing between the dry weight of embryo and the amount of dry yolk required to produce this quantity of embryo as the efficiency coefficient of development.

In actual practice the accuracy with which the efficiency of development can be determined depends on the degree of variation in the size of individual eggs. The size of eggs from different females varies considerably, but it is not difficult to obtain nearly all the eggs of a practically uniform size if a suitable female fish is used for each experiment. The following figures illustrate the variability of the eggs used (Table II):

Table II.

♀	No. of eggs weighed for sample	Weight in gm.	Average weight of one egg
A	10	1·163	·116
A	10	1·230	·123
A	10	1·120	·112
B	24	2·704	·113
B	30	3·465	·115
B	40	4·524	·113
B	20	2·300	·115
B	20	2·198	·110
B	20	2·217	·111

The efficiency of development at different periods between the 50th and 80th day is given in Table III.

Table III.

♀	Age of larva in days	In 100 larvae			Efficiency	Average efficiency
		Dry wt. of embryo in gm.	Dry wt. of unused yolk in gm.	Dry wt. of yolk used in gm.		
A	50	0·42	3·07	0·78	·54	·58
B	50	0·53	2·83	0·86	·62	
A	60	0·80	2·56	1·29	·62	·63
B	60	1·07	2·05	1·64	·65	
A	66	1·28	1·88	1·97	·65	·65
A	70	1·55	1·52	2·33	·66	·65
B	70	1·73	1·02	2·67	·65	
A	80	2·24	0·57	3·28	·67	·64
B	80	1·92	0·48	3·21	·60	

According to Tangl (1903) the loss in the energy content of the egg between the time of fertilisation and of hatching is about 4 per cent., but it must be remembered that the larva hatches when the embryo is very small.

The above figures show that only something like two-thirds of the original yolk is converted into embryo while the remaining third is used up for other purposes. It is now possible to write a generalised equation for the conversion of yolk into

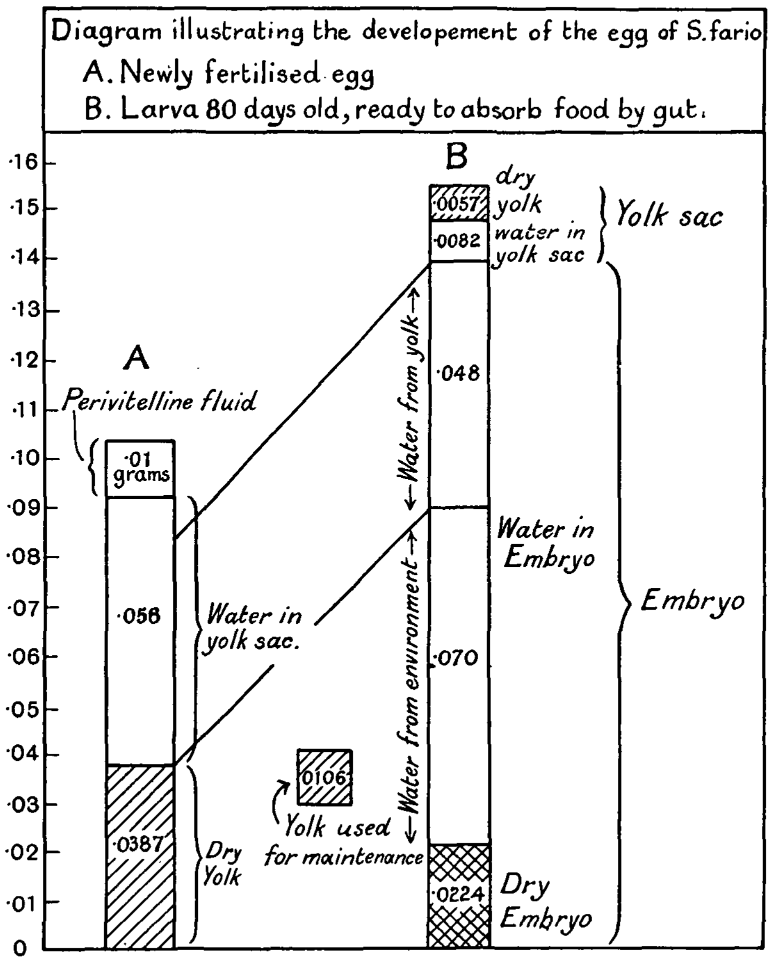

Fig. 4.

embryo which will at least hold good from the 50th–80th day of development at 10° C., during which period the size of the embryo has increased about 400 per cent.

WET YOLK	+	WATER	=	WET WT. FISH	+	DRY YOLK USED FOR OTHER PURPOSES
1·0 gm.	+	·70 gm.	=	1·56 gm.	+	·14 gm.

Fig. 4 illustrates the above changes for another experiment. The ordinates of the figure represent grams.

It is clear that the egg of the trout, and in all probability that of the frog, absorbs a considerable amount of water from its environment during the process of development. The significance of this fact will be discussed in a later paragraph (see p. 222). At the moment it is convenient to consider the fate of the 37 per cent. of yolk which is not converted into embryo. By estimating the oxygen consumption of the embryo and converting this into terms of yolk it was found that the whole of the loss in the dry weight of the egg could be accounted for. The apparatus used was a Barcroft manometer which was shaken in a water-bath at 10° C. at a rate of 72 oscillations per minute. Under these conditions the larvae rapidly attained a degree of activity comparable to that exhibited in the hatchery.

Until the embryos were about 72 days old, their rate of respiration in the manometer was extremely regular over a period of at least 1 hour, and from this it is assumed that the observed values are at least of the same order as that under hatchery conditions. As the larvae increased in age, however, it was found that the respiration of individual specimens varied somewhat, and was often rather unsteady. It would therefore be desirable to check these values by some alternative method.

From the percentage composition of the yolk given by Fauré-Fremiet (1925) 1 gm. of oxygen is equivalent to 0·33 gm. of nitrogenous yolk, and to approximately the same amount of fat. The observed amount of yolk in the newly fertilised eggs from which the material in Table IV was derived was 0·03772 gm. per egg.

Table IV.

Day	O_2 consumed per gm. embryo per hour c.c.	Yolk equivalent to O_2 consumption gm.	Yolk equivalent of embryo gm.	Unused yolk gm.	Total yolk gm.
46	·55	·00138	·00304	·03321	·03763
50	·55	·00188	·00378	·03157	·03723
53	·55	·00234	·00453	·03075	·03762
57	·55	·00311	·00688	·02768	·03767
60	·55	·00384	·00848	·02501	·03733
68	·55	·00645	·01300	·01853	·03798
71	·48	·00777	·01550	·01763	·04090
75	·44	·00956	·01808	·01476	·04240
78	·33	·01083	·02016	·00779	·03878
80	·30	·01140	·02304	·00738	·04182

The calculated average yolk value of an egg as shown by these figures is 0·03872 gm. per egg, which agrees well with the observed value. From this it may be concluded that the actual synthesis of living material from yolk is either an exceedingly efficient process or that the by-products of growth are oxidised. The first conception is perhaps more in keeping with the facts. Until the 80th day it is difficult to detect any reduction in the specific growth-rate of the embryo, and an examination of Table IV shows that the respiratory level per gram of embryo begins to fall about 10 days prior to this. Again, after the 80th day the growth-rate drops rapidly and although there is a drop in the respiratory level it is not of the order one would expect if the material oxidised were derived in any marked amount from the by-products of growth (Table V).

Table V.

c.c. O_2 used per gm. per hour at 10° C. during	
Closing phases of embryonic growth	Subsequent period of inhibited growth
·48	·42
·44	·34
·34	·44
·30	·30

Again, during the opening phases of post-embryonic growth when the growth-rate is again considerable there is undoubtedly a fall in the respiratory level. Assuming that the figures given by Gortner (1913) are applicable to *Salmo fario* it looks as though there were a considerable oxidation of protein during development, which is in keeping with the fact that fish do not store large quantities of fat, but that muscle proteins are available for maintenance during starvation or for the formation of the eggs themselves. This evidence suggests that the nitrogenous and fatty yolk of the fish has a dual function to perform, (*a*) to form the embryo, (*b*) to provide the embryo with an adequate supply of energy by oxidation. This view, which is in harmony with the fact that the respiratory level of the mammalian embryo is roughly the same as that of the mother (Bohr, 1900), points to the conclusion that the conversion of yolk into embryo goes on with little or no loss of energy; the loss in the energy content of the egg is due to the fact that the tissues when formed expend energy which is derived from the yolk.

DISCUSSION.

It has been shown that the eggs of the trout, and probably also those of the frog, do not contain sufficient water to suffice for complete development, and that the deficiency is made up by an absorption from the external medium. As far as can be determined from the literature the percentage dry weight of the eggs of all aquatic organisms is higher than that of the fully-formed tissues of the organism. If, therefore, it may be assumed that most, if not all, aquatic organisms during development are dependent on the environment for a supply of water, an interesting problem of phylogeny is opened up. The primitive vertebrate is, with good reason, regarded as the offspring of an aquatic type, but at some stage in the history of the truly terrestrial animals there must have come a time when oviposition occurred on land and not in water. During the earlier stages of their evolution terrestrial vertebrates no doubt laid their eggs in water, where possibly the factor of greatest survival value consisted in the newly hatched individual having reached a stage in the development at which it might fend for itself and be of somewhat active habits. Now an animal such as the trout necessarily hatches at a very early stage since the increasing volume of the larva soon reaches the volume of the original egg shell: further development without hatching would crush the embryo against the egg membranes. In aquatic organisms a postponement in the date of hatching is only possible when either the original egg membrane is highly elastic or when it is separated from the yolk by a wide perivitelline space. Now an aquatic egg containing sufficient perivitelline space to allow the act of hatching to be postponed until the young organism is fully formed (and comparatively unhampered by remaining yolk), would form just the system most easily adaptable to terrestrial habits. Such an egg laid on land would undergo its normal development as long as it is protected from undue evaporation during the incubation period. Thus the experimental data point strongly to the suggestion that the Reptilia and their derivatives arose from an aquatic type of organism whose eggs were laid in water but which did not hatch until the yolk sac period of development had reached an advanced stage. The wide perivitelline space which was then a necessary feature for the accommodation of the enlarging embryo became on land the means whereby water was supplied for development. It is curious to note that wide perivitelline spaces are characteristic of Elasmobranch eggs whereas the eggs of most living Crossopterygians and Dipnoans resemble those of the trout. On the other hand, the eggs of *Ceratodus* or of many Amphibia could conceivably develop on land if the gelantinous coats were available as a source of water supply.

The significance of the so-called "white" of the hen's egg can be realised by the fact that after nineteen days of incubation the embryo has absorbed about 10 c.c. of water from the albumen. The eggs of all birds and of many reptiles are of the same type, but in some lizards the necessary water is partially supplied from external sources and the eggs swell appreciably after being laid. Apart from other advantages the value of the viviparous habit is obvious; it entirely removes the necessity for the parent organism to provide in the newly fertilised egg enough water to last the embryo through the whole period of incubation.

It is also probable that the presence of albumen in the "white" of the eggs of birds and reptiles is itself an adaptation for the provision of water to the embryo. It is known that the membrane surrounding the yolk of the egg is permeable to water but impermeable to salts and other osmotically active substances. Were the yolk surrounded by pure water, the latter would rapidly pass into the yolk and thereby produce a large and mechanically weak ovum. If the aqueous surroundings, on the other hand, consisted of crystalloid substances the ease with which the embryo could obtain water would become increasingly less with increasing age owing to the rising osmotic value of the external salts. Since, however, the initial osmotic equilibrium between embryo and yolk, on the one hand, and the aqueous surroundings on the other, is effected by means of a colloid, then although water is withdrawn from the latter its osmotic pressure does not rise as much as would that of a solution of a crystalloid. The existence of an albuminous solution around the yolk of terrestrially developed eggs appears to be an admirably adapted mechanism for providing the growing embryo with water.

A second point of some general interest lies in the value put forward as the efficiency coefficient of development, viz. ·63. A study of the literature reveals the fact that this value applies within reasonable limits to a wide variety of organisms. Table VI (which is derived from Murray's data) gives the efficiency of development of the chick. For the period of 7th–12th day during which the specific growth-rate of dry tissue is more or less constant the efficiency is practically the same as for the trout—at a later stage it appears to rise to about ·75.

Table VI.

Efficiency of Development in the Chick (data from Murray).

Day	Dry wt. of embryo	Dry wt. of yolk used	Efficiency
7	0·0430	0·0640	·67
8	0·0738	0·1148	·64
9	0·1181	0·1911	·62
10	0·1863	0·3043	·61
11	0·2888	0·4668	·60
12	0·4495	0·7075	·63
14	1·099	1·594	·69
16	2·360	3·217	·73
18	3·887	6·233	·74

Table VII shows the efficiency coefficient of various organisms.

Table VII.

Organism	Efficiency coefficient	Source of data
Aspergillus	·59	Terroine and Wurmser
Silkworm	·59	Farkas
Trout	·63	Gray
Frog	·58	Fauré-Fremiet
Chick	·63	Murray

In the case of germinating seeds the recent work of Terroine, Trautmann and Bonnet (1926) shows that the efficiency coefficient of development varies considerably (from ·72 to ·51) with the nature of the reserve material in the seed. These authors regard the process of germination as essentially a conversion of the reserve material in the seed into a seedling with a very high carbohydrate content. There is reasonable grounds for believing that the conversion of fat into carbohydrate involves an energy loss of 23 per cent., whilst the conversion of protein into carbohydrate involves a loss of 35 per cent. By means of these two figures the calculated efficiency of the different seeds agrees closely with the observed results. In the case of animals, however, there seems no reason why the process of yolk conversion should pass through a carbohydrate phase, and, as already pointed out, the loss of energy during development is more probably due to expenditure of energy by the tissues when formed and not lost in the actual process of tissue formation.

One final point may, perhaps, be mentioned. Needham (1926) has recently suggested that the changing cycles of energy reserves in the chick (viz. first carbohydrate, then protein, and finally fat), may have some phylogenetic significance.

If, however, we are right in believing that the trout embryo derives its energy in no small degree from protein, then the period of the chick's development during which it possesses the morphological features of its piscine ancestor (2nd–5th day) is passed in the carbohydrate phase, whereas teleostean fishes have apparently progressed to a stage beyond that of the ancestral Crossopterygian (or Dipnoan!) and are using protein as a supply of energy.

SUMMARY.

1. During the yolk sac phase of development the larval trout absorbs water from the environment. The percentage dry weight of the embryo itself remains constant at about 16 per cent. and that of the yolk at about 41 per cent.

2. The efficiency of development between 50th and 80th day of incubation is constant about ·63; in other words in order to produce 63 gm. dry weight of embryo, 100 gm. dry weight of yolk are consumed. The loss of 37 per cent. dry weight of yolk is accounted for by the oxidative processes going on in the embryo.

3. Vertebrate animals which lay eggs on land must provide an additional water supply to that present in the yolk. The type of egg laid by reptiles or birds is most simply derived from some aquatic type which possessed a perivitelline space wide enough to allow development to proceed to an advanced stage before the act of hatching occurred.

4. Attention is drawn to the fact that the efficiency of development of various types of animal organisms is of the same order of magnitude.

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