LXXXII. THE METABOLISM OF DEVELOPING SALMON EGGS.

I. THE SIGNIFICANCE OF HATCHING AND THE RÔLE OF WATER IN DEVELOPMENT.

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MATERIAL.

Eggs of the Atlantic salmon (*Salmo salar*) were procured in the eyed stage and maintained in a small hatchery at a temperature of 8 to 10°. Younger stages were not reared in the laboratory, for although many could be kept alive for some weeks after the journey from the hatchery there was no further development. The series of analyses given is that of a group of 5000 eggs which came to the laboratory in the eyed stage and were from one female. Fifty specimens were taken for each test and the adherent moisture removed between sheets of filter-paper. They were then weighed in a dried extraction thimble supported on cotton wool in a tared weighing bottle. This was the wet weight. The eggs and larvae were dried to a constant weight (the dry weight) at 100°.

OBVIOUS CHANGES DURING DEVELOPMENT.

Experts in fish culture recognise several critical periods in the development of salmonid eggs. For a day or two after fertilisation they are hardy and can be moved about and arranged in the hatchery trays without injury; after this they become exceedingly delicate until the appearance of pigmented eyes within the shell. The first period is one of comparatively undifferentiated cell division, the second that of the organogenesis or morphological differentiation. In the eyed stage very few eggs die and much rough handling is not detrimental. About three weeks before hatching there ensues another period of great mortality, in the phase of histogenesis. Many of the embryos become opaque within the shell while the yolk remains transparent. A large number of such larvae have been hatched and examined. Necrosis usually begins as one or more ring-shaped areas in the tail region from which it spreads rapidly until the whole tail has become opaque. At this stage there is no tail circulation, poor vitelline circulation, feebly and irregularly beating heart and very little movement. No larva hatched in such a condition has recovered. A group of larvae in various stages of opacity was hatched and the "case histories" followed in

detail. Extracts from two typical examples are given. The embryos began normally to hatch about Jan. 15.

Case 1. Hatched Dec. 29. Physical conditions; dorsal fin sloughed into a large mass of necrosed tissue; adipose fin had dropped off leaving only a jagged edge; two ring-shaped opaque spots, one situated just posterior to the pectoral fin and the other under the adipose fin; heart rate 84 per minute (control heart rate 94); good circulation to all parts of the body. On Dec. 30 the dorsal fin sloughed off and from then on the fish began to recover, until on Jan. 9 there was no trace of the white patches, the heart rate was normal, and the fish very active.

Case 2. Hatched Dec. 30. Physical conditions; whole of tail half way to adipose fin enclosed in a white sheath of necrosed tissue with a large flap hanging loose; a second small patch under the adipose fin; heart rate 69 per minute (control heart rate 84); poor embryonic circulation and no blood supply over distal parts of yolk sac; very little activity. Hatching produced a slight temporary improvement, but after Jan. 2 the heart rate decreased and the general condition became worse until death on Jan. 6. The sudden temporary improvement after artificial hatching has been noted in most cases.

On Jan. 2, 130 typically affected eggs were selected and divided into two groups. Those of one group were hatched artificially by tearing the shell with fine forceps. Both groups were kept under similar conditions to determine the effect of hatching on the fatality of the condition. The final results were the same, 11 out of the 65 surviving in each case. Natural hatching had taken place before the conclusion of the experiment on Jan. 19. It was noticed that death occurred more quickly in the hatched group, due to the immediate attack of the necrosed tissue by the fungus *Saprolegnia ferax*, while the unhatched larvae might live for some days longer protected by the shell. It is believed that under different experimental conditions, using fungus-free water, the hatched group would have shown a larger proportion of survivors.

If bacteria or mycelia could go through the shell, infect the embryo within, and by destruction of the shell produce hatching, an interesting case of symbiosis would be suggested. To test this idea affected larvae and controls were hatched under aseptic conditions and reared in vials at a suitable temperature, protected from atmospheric contamination. Before hatching any possible infection on the outside of the shell was removed by immersion for one minute in 3 % formalin. Experiments determined that at this age an egg is capable of withstanding an immersion of 3 minutes in 3 % formalin without serious injury. It is believed that an exposure to formalin which would remove infection within the egg would also kill the larva. Controls and some of the experimental animals lived until the conclusion of the experiment some 10 weeks after hatching. Those which died were left in the vials and observed, but there was no decomposition except the usual autolytic one, and examinations of living and dead eggs showed no bacteria or fungus. The conclusion is that it is not from external infection that the larvae die within the shell.

It was found that immersion in formalin of an egg 3 weeks before its hatching time, caused it to hatch within a few hours. Scheminzky and Gauster [1924] showed that trout eggs which are continually exposed during development to the action of a weak electric current hatch a week earlier than the controls. The resulting larvae do not differ in any way from the normal. These authors attribute the result to the destruction of the egg capsule, but it may be due to nothing more obscure than a marked stimulation of embryonic activity.

Wintrebert [1912] has demonstrated the secretion of a protease by the trout embryonic ectoderm which dissolves the shell and permits hatching. It is one purpose of this paper to point out that the phenomena associated with the production of this enzyme have a far greater effect on embryonic metabolism than a plain statement of their final result would indicate. It is necessary for the larva to suspend body-building operations and attend to the problem of blazing a trail to the outside world. The effort profoundly modifies embryonic metabolism and it is not until some weeks after hatching that the uneventful course of development is resumed. The dissolving of a capsule so tough that it is broken down only with difficulty in caustic potash solution [Miescher, 1897] is accomplished by a concentrated protease solution in the perivitelline fluid. Variations in the water content and metabolism, and the frequent death of the embryos within the shell are explained by this developmental modification. The region of the embryo which first begins to die lies in the extremities (tip of tail, fins, etc.), and this further supports the idea that the enzyme directly attacks the embryonic tissue, for it is here that the embryo is most susceptible to the action of unfavourable environmental factors. (Hyman in 1921 exposed *Fundulus* eggs to lethal concentrations of cyanide solutions and found that the tail died first.)

THE EGG CAPSULE.

The chemistry of the salmonid egg shell is not known in detail. Steudel and Osato [1923] and Osato [1923] concluded from the nature of the amino-acids in herring egg shells that the fish egg is homologous only with the yolk of the bird's egg, while the shell of the latter is a new modification for terrestrial life. The albumin of the bird egg corresponds to the mucilaginous covering outside the shell of amphibian and some fish eggs, and the vitelline membrane is the homologue of the fish egg capsule. (The loose use of the word "chorion" for a fish egg shell suggests a homology which does not exist.) It follows that an aquatic egg shell may take a more active part in the scheme of development than a terrestrial one.

After fertilisation the capsule is pale in colour, of a dull sheen, and relatively thick and brittle. It can be stained intensely black with osmic acid [Scheminzky and Gauster, 1924]. Its strength increases for a time, but before hatching it becomes noticeably thinner, somewhat loose and folded, easily torn, and loses its sheen. Its reaction to osmic acid at this time indicates a

decrease in fat, or at least of the lipins containing unsaturated acids. Shortly before hatching the water increase in the egg causes the shell to become taut. Many eggs may be hatched at this stage by gently squeezing.

Nordgaard [1896] observed that if a dead opaque salmon egg were placed in a salt solution it became transparent. Gray [1920] found that any of the usual ways of killing a trout egg, such as heat, cold, electric shock, physical shock, etc., were followed by exosmosis of the electrolytes and this caused precipitation of the globulins with resultant opacity. The amount of exosmosis under various conditions could be quantitatively measured from the variations in the electrical resistance of the water in which the eggs were dying. The re-resolution of the globulins in normal saline demonstrated that under the unfavourable conditions the capsule had become permeable. The temporal relation between exosmosis and globulin precipitation was determined by Scheminzky and Gauster [1924], who found that the electrolytes leave the egg before the globulins are precipitated. Thus the primary injury is to the membrane rather than to the contents of the egg. Now Stockard [1921] and a number of other workers have demonstrated critical stages in embryonic development, at which it is particularly easy to subject an egg to a shock which will later produce an embryonic deformity. These conditions are generally attributed to changing properties of the protoplasm (varying oxidation rates of different parts at different times, etc.), but the final explanation may well be found in the egg membrane. It is worth noting that salmonids are the fish in which embryonic deformities have been most frequently observed in nature.

Scheminzky and Gauster themselves do not believe that the varying susceptibility during development is due to the membrane, because the electric current required to kill an egg in the later stages increases, while the tensile strength of the shell decreases. (Hein [1907] determined the pressure necessary to crush an egg at various stages.) There is, however, no good reason to believe that permeability is determined by strength and thickness. Furthermore, during development a new membrane system comes to surround the yolk. In later stages two membrane systems, a band of muscle and a fluid-filled coelom, must be penetrated in addition to the shell before the yolk is reached. An inspection of Scheminzky and Gauster's fig. 19 suggests that the coelom surrounds the yolk at the 24th day, since the resistance of the egg rises suddenly at this time.

It has been agreed by most workers that the capsule of the living egg is normally permeated by water but not by electrolytic or colloidal substances. Svetlov [1929] has recently sharply criticised this conception. Using micro-methods he found a lower osmotic pressure in the perivitelline fluid of trout eggs than in the yolk, and concluded that the egg contents have the power of maintaining their osmotic pressure independently of the capsule. This does not deny the same function to the cell membrane. Kite [1913] found that most animal and plant cells regulate their permeability as much by the cytoplasm as by the cell wall.

Svetlov also found that salt ions passed through the capsule of a living egg and into the perivitelline fluid. Now Loeb [1912] has shown that the action of a solution of one salt on *Fundulus* eggs is to increase the permeability of the capsule. If a solution of two antagonistic salts such as NaCl and CaCl_2 were used there was no changed permeability. What Svetlov has really done therefore is to injure the membrane with an electrolytic solution, the ions of which afterwards passed through. Loeb points out that a dilute acid or alcohol solution has the same effect as a salt solution, and he says, "The increase in the permeability of the membrane for water and salts under the influence of acids is due to an alteration of the protein constituents of the membrane." Thorner [1929] immersed developing *Fundulus* eggs in KCl until the heart ceased to beat, and then removed them to fresh water or sea-water. In distilled water there was a slow extrusion of salt through the shell until after some days the heart recovered. The reaction in sea-water was quite different, the time required for recovery being short and directly proportional to the period of exposure to KCl. Since *Fundulus* eggs can develop in either fresh water or sea-water the customary reaction of the shell must be the same in both media. It follows therefore that after immersion in one salt the capsule behaves abnormally. Sumwalt [1929] found that the electrical potential of *Fundulus* eggs was changed to a marked degree if the eggs were immersed in solutions of various salts. In neutral solutions the capsule was more permeable for cations than for anions. Bivalent cations had about half the effect of univalent cations. As the acidity of the solutions was increased the cation and anion permeabilities approached one another until at p_H 3.7 they were equal. In more acid solutions reversal of permeability occurred. Sumwalt reviews the literature on this point which serves to show the pronounced effect of salt and acid on the capsule. Svetlov mentions a simple and convincing proof of the permeability of the capsule to salt under normal conditions. If a very young egg, which has become opaque while the protoplasm is still alive, is placed in normal saline the opacity disappears and normal development may continue; but as soon as it is replaced to tap water it dies. This really proves that it is possible to compensate for an injured capsule by rearing the egg in normal saline. The capsule, however, never returns to its normal condition as is shown when the egg is replaced to tap water.

Svetlov's vital staining experiments seem at first sight to prove his contentions. If eggs were immersed in neutral red or Nile blue sulphate the dye passed through the capsule and stained the contents intensely. Negative results followed similar experiments with trypan blue and Indian ink. Neutral red required 3 hours for the maximum effect, and Nile blue sulphate 12 hours. It is concluded that the egg capsule is permeable to some colloids under natural conditions. The first thing to notice is that neutral red and Nile blue sulphate belong to a toxic group of stains. Romeis [1928, p. 198] gives particular warning that even in highly dilute solutions neutral red is poisonous. Secondly, teleost embryos have been shown by Wislocki [1917] to be incapable of taking

up colloidal dyes in which they are immersed. Their impermeable integument distinguishes them from amphibia larvae [see McClure, 1918].

The writer has carried out vital staining experiments with the salmon (*Salmo salar*), the plaice (*Pleuronectes platessa*), and the goby (*Cyclogaster liparis*). Eggs were immersed in solutions of neutral red, methylene blue, ponceau S., and Janus green. Five hours' immersion in a 0.1 % solution of any of these stains coloured the capsule intensely, methylene blue being most readily adsorbed. When the shell was removed there was absolutely no trace of dye in the egg contents. Similar results followed three days' immersion in the dye. The perivitelline fluid of salmon eggs placed in a saturated solution of methylene blue for 15 hours was slightly stained, but the larvae within were either dead or dying. There was no case in which the stain passed through the shell and the embryo lived. The positive results only showed the staining reaction of dead tissue to dye-stuff.

A number of artificially hatched salmon larvae were left for 24 hours in dilute solutions of neutral red, methylene blue, trypan blue, and nigrosine. On examination they were found to be unstained. Using a 1 % solution the results were the same as long as the larvae lived, but when they were about to die a diffuse staining occurred. With plaice and goby larvae after hatching the results were the same. Thus Wislocki's observations have been entirely confirmed.

It is concluded that in Svetlov's experiments the dye injured the capsule before passing through, and injured the living tissue before staining it. In the absence of further evidence we may continue to believe that under natural conditions there is no exchange of electrolytic and colloidal substances through the shell.

The recent work of Straub [1929] presents the egg capsule in a new rôle. He analysed the causes of observed differences between the osmotic pressures in the yolk and white of hens' eggs. The vitelline membrane is permeable to both water and dissolved substances, nevertheless in the living egg the yolk keeps a higher osmotic pressure than the white for weeks. The difference is lost very quickly in an egg killed by cold. It requires a measurable expenditure of work on the part of the egg to maintain this condition. In general it is in the form of electricity that the free energy of a chemical reaction can be made to do work. The vitelline membrane must be expected to have the properties of a galvanic element, that is, some of the intermediate stages of glucose oxidation must take place in such a way that their free energy maintains a constant-difference in potential across it. The membrane bears the same relation to the system as the copper wire between the poles bears to an electric battery. Neither is capable of acting alone as a producer of differences in potential. Straub predicts the histological structure that will be determined for the vitelline membrane of a hen's egg—a porous sheet in which the fluid-filled holes and solid parts provide the liquid and solid phases necessary for the passage of electric currents in both directions.

VARIATIONS IN WEIGHT AND THE RÔLE OF WATER IN DEVELOPMENT.

The data under discussion in this section are summarised in Tables I and II, and in Figs. 1 and 2. The curves are broken at hatching time owing to the loss of the shell and perivitelline fluid. Both egg and larval determinations have been made on the 65th and 70th days. The time over which hatching normally extends is shown on each graph by dotted ordinates, and broken lines give a similar indication of the approximate time during which the effects of hatching are felt. The special metabolic features associated with hatching show with particular clearness in the last eggs to hatch, and the changes in the curves caused by these eggs are of particular interest. As the larvae were selected from the main group at random the special features appear in varying degrees, so that before the effects of hatching are over some irregularity in the determinations occurs. In other words the internal factors—enzymes for example—which adversely affect the eggs during the last stages must injure the late-hatching larvae most.

Table I. *Variations in the dry material and water content during development.*

1	2	3	4	5	1	2	3	4	5
Days after fertilisa- tion	Ma- terial	Wet weight	Dry weight	Water (%)	Days after fertilisa- tion	Ma- terial	Wet weight	Dry weight	Water (%)
36	<i>e</i>	13.2784	4.3568	67.3	76	<i>l</i>	11.8804	3.9194	67.0
40	<i>e</i>	13.1162	4.2826	67.2	79	<i>l</i>	11.5903	3.7928	67.3
44	<i>e</i>	13.2764	4.2956	67.6	86	<i>l</i>	11.8374	3.7154	68.6
46	<i>s</i>	0.6750	0.2065	69.5	90	<i>l</i>	11.4210	3.4992	69.4
50	<i>e</i>	13.3762	4.3110	67.8	94	<i>l</i>	11.2256	3.4052	69.7
55	<i>e</i>	13.2046	4.3026	67.5	100	<i>l</i>	11.2000	3.2372	71.1
60	<i>e</i>	13.4595	4.3122	67.9	106	<i>l</i>	11.1556	3.0408	72.7
65	<i>e</i>	13.3143	4.2740	67.9	111	<i>l</i>	11.1808	2.9931	73.2
65	<i>l</i>	11.2842	4.0254	64.5	116	<i>l</i>	11.0876	2.8720	74.1
70	<i>e</i>	13.9796	4.2802	69.5	124	<i>l</i>	10.8034	2.5308	76.5
70	<i>l</i>	11.6766	3.8832	66.8					

Absolute figures represent the weights for 100 individuals in grams. In col. 2 *e* means eggs; *s*, shells; *l*, larvae.

Table II. *Growth of embryos and plastic efficiency coefficients.*

1	2	3	4	5	6	
Days after fertilisation	Embryo wet weight	Yolk wet weight	Embryo dry weight	Yolk dry weight	Plastic efficiency coefficient	
					days	coeff.
36	0.223	12.450	0.036	4.115	—	—
86	1.113	10.724	0.175	3.543	—	—
90	1.612	9.809	0.261	3.235	86-90	0.413
94	1.784	9.442	0.288	3.116	90-94	0.094
100	2.698	8.502	0.432	2.804	94-100	0.462
106	3.744	7.414	0.598	2.445	100-106	0.462
111	4.076	7.105	0.652	2.342	106-111	0.524
116	4.630	6.458	0.741	2.132	111-116	0.424
124	6.035	4.768	0.962	1.573	116-124	0.396
—	—	—	—	—	36-124	0.364

Fig. 1 shows that there is no measured decrease in the dry weight until a few days before hatching. This is in line with the idea that there is no loss of substance through the shell. Just before hatching the membrane becomes thin

and loose, permitting an increased water content. The previously noted loss of fat in the egg capsule during development may partly account for the decrease in dry weight. In the last stages the capsule has become permeable and substance has passed through. Very little movement is possible for the larva within the egg, but after hatching it can swim about freely. The increased activity causes a more rapid loss in dry weight until an unexpected plateau appears at the 106th day, the former slope being resumed again 10 days later. May it not be that this period is one in which there is a change in the nature of the sources of energy with which the yolk provides the embryo for its building operations?

Fig. 1.

Fig. 2.

Studies on the mechanics of development of trout eggs and larvae have recently come from two sources. Since they differ somewhat with respect to observations as well as to theoretical conceptions it is necessary to examine them here with a view to determining which agrees more closely with related facts.

Kronfeld and Scheminzky [1926] measured the embryonic length and the wet and dry weights of the embryo, yolk sac, and egg capsule during development. The species used were *Trutta fario* and *Salmo lacustris* as well as hybrid eggs. In each experimental series they used eggs of one group with unfertilised

eggs as controls. The method involved preliminary hardening in 2 to 4 % formalin, but they produce evidence to show that this caused no significant changes. The water content of the egg as a whole did not appreciably vary up to the time of hatching. They have not distinguished between early and late hatching eggs and have therefore missed the sudden rise in the percentage of water which occurs just before the hatching of the latter. There was a loss of water at hatching, owing to the loss of the capsule and perivitelline fluid, followed by a rapid rise which continued until the end of development. They found that the water content of the embryo alone dropped until hatching and then rose suddenly, to reach its final level of 86 % three weeks later. The yolk sac alone contained 66 % of water in the early stages. From this tension the water dropped to as low as 50 % a week after hatching, followed by a rise so that the normal figure was reached again two weeks later. The developing embryo faced the problem of building living tissue of high water content from yolk of low water content. Presumably the living cells could only absorb water through the yolk sac, and for some days after hatching the water taken was not replaced. When the living tissue was almost in equilibrium again the water in the yolk sac commenced to rise. There was evidently some delicate adjustment of organism to environment, on the nature of which we may be able to speculate after considering the chemical changes [Hayes, 1930].

An investigation was also made of the specific growth rate during development, that is how much new tissue can be built per unit embryo per unit time at different ages. According to Kronfeld and Scheminzky's fig. 4 the curve of building power rose to two peaks, one of them 16 days before hatching, and the other 23 days after hatching. Anatomically the first maximum corresponded to the formation and growth of the chief organs of the body, the ensuing drop being due to a form of starvation owing to lack of water. The drop after the second maximum they attributed to the lack of some necessary material in the yolk, and they point out that this is the time at which hatchery experience has shown it is best to begin feeding the larvae.

Similar investigations to those just described have been carried out on the same species of trout (*Salmo fario* in British terminology) by Gray [1926, 1928]. The variations of the wet and dry weight of the egg or larva, embryo, and yolk (by difference) were found. The figures presented show that the embryonic water content remains constant at approximately 84 %, and that of the yolk at 59 %. These results indicate that the hatching process does not affect the water content of the yolk or embryonic body. The dry weights given in the second paper are calculated on this assumption.

Gray has also given figures for the specific growth rate during development, which are in agreement with those of Kronfeld and Scheminzky. His observations began at the 44th day, or after the time of the first maximum. The two curves inflect when the larva has completed about 70 % of its development. The agreement on this point may be accepted as proof that by this stage both the embryo and yolk have an unchanging water content. It is possible to

calculate the weight of the salmon embryo and yolk at this stage by weighing the whole larva wet and dry (see below). According to all workers the larval wet weight increases for some time after hatching, and then decreases until the embryo begins to take outside food. The water content however (Fig. 2), after the fluctuations subsequent to hatching, rises steadily until the embryo begins to feed. Therefore in the later stages the loss in dry material is greater than the gain in water. Increases in water are associated with the transformation of yolk into living tissue, and losses in dry substance are due to the catabolism of the embryo.

In Table II the plastic efficiency coefficients represent the ratio

$$\frac{\text{gain in embryonic dry material}}{\text{loss in yolk dry material}}.$$

This coefficient has been determined for a number of forms, and in a given species it may be assumed to be of the same order throughout larval development. It is given here for the salmon with the object of showing that by determinations of the wet and dry weight of the larva it can be decided independently whether the embryo and yolk have a constant water content. Data from the egg are invalid since two unknown variables are included, the shell and the perivitelline fluid. Assume, as Gray did, that both embryo and yolk actually maintain a stabilised water content which may be found experimentally and applied to larvae of other ages. The dry weight of the embryo, yolk or larva is equal to the wet weight multiplied by the percentage of dry substance. The total larva is equal to the sum of the embryo and yolk. Instead of "yolk wet weight" can be written "larva wet weight minus embryo wet weight." Hence the following equation is true:

$$\begin{aligned} \% \text{ dry wt. larva} \times \text{wet wt. larva} &= \% \text{ dry wt. yolk (wet wt. lar.} - \text{wet wt. emb.)} \\ &+ \% \text{ dry wt. emb.} \times \text{wet wt. emb.} \end{aligned}$$

The only unknown is the embryonic wet weight which is found by solving the equation. The equation can be conveniently expressed by a formula. Let T = total wet weight of larva given in col. 3, Table I; let x = total wet weight of embryo; let a = % dry weight of larva found by subtracting the value in col. 5, Table I from 100; let b = % dry weight of yolk experimentally found to be 33 in later larval stages; let c = % dry weight of embryo experimentally found to be 16 in later larval stages. The value 16 for c is among the highest found, but the position of any possible inaccuracy will be obvious when one remembers that it is dependent on the removal of all moisture from the outside of very small embryos dissected away from their yolk sacs. The fresh embryos used were fragile and it was difficult to remove all the water. The use of the figure 16 brings the theoretical results into harmony with direct observations. The following is the equation:

$$aT = b(T - x) + cx,$$

or it may be written

$$x = \frac{(b-a)T}{b-c}.$$

An attempt was made to apply this formula to each of the larval weights determined. The 65th and 70th day calculations gave the embryonic weight as a minus quantity; the 76th day as exactly zero; and on the 79th day 1.715 g. or double the experimentally observed value. The figures from the 86th day forward are given in col. 2, Table II, and in col. 6 the plastic efficiency coefficient (P.E.C.) is recorded. The latter figures (with one exception due to experimental error) agree quite well and show that after the 86th day the embryo and yolk have a constant proportion of water. The lack of uniformity of the calculations made before the 8th day provides independent evidence in favour of Kronfeld and Scheminzky's statement that the water content does not become stabilised for some time after hatching.

Col. 6, Table II also compares the P.E.C. over a long period (36 to 124 days) with that of the later larval stages. From this it may be seen that the coefficient increases somewhat during development. The 36-day figures quoted were obtained in the usual way and a correction applied for the shell. Their accuracy is probably not of a very high order, but this is offset by the small size of the embryo.

The form of the growth curves given by Gray, and Kronfeld and Scheminzky shows that after the second maximum an ever-increasing proportion of the yolk supplied is burned to provide energy of maintenance, while a decreasing share is built into new tissue. In other words basal metabolism steadily replaces growth. If no outside food were provided the embryo would finally cease to grow, become emaciated, and die. Thus if the P.E.C. given above had extended until the disappearance of the yolk sac it would necessarily have declined, indicating that the larva was wasting more food at the end of development. The conceptions of growth recently developed by Terroine and his collaborators show that this is not really the case. [Complete references may be found in Terroine and Wurmser, 1922, and in de Caro, 1928.] We are dealing here only with matter, and leaving out of consideration the energy of maintenance of the cells already formed. Any cell must carry on metabolism if it is to remain alive and this basal metabolism is not waste. The real energetic efficiency must be calculated according to the formula

$$\frac{\text{energy gained by embryo}}{\text{energy lost by yolk} - \text{energy of maintenance}}.$$

Some observers have attempted to distinguish between non-basal metabolism (wasteful burning of food) and basal metabolism (energy of maintenance) by measuring the surface area and assuming that the latter bears a constant relation to basal metabolism. Not wishing to rely on surface laws Terroine and Wurmser made their experiments directly, by finding the way in which the growth of slime moulds was hastened or retarded by adjustments of the p_H of the culture medium. The real developmental efficiency of different embryos, such as the frog, silkworm and chick, is of the same order. They waste from 20 to 30 % of the energy provided for their development. Accurate figures for the real energetic efficiency of a fish are not yet available.

SUMMARY.

1. The wet weight of a salmon egg increases slowly until a short time before hatching, then rapidly. In the larva the wet weight first increases and then decreases. The dry weight drops rapidly after hatching.

2. Observations on hatching show that the enzyme which dissolves the shell has a very unfavourable effect on the embryo.

REFERENCES.

- de Caro (1928). *Bull. Soc. Chim. Biol.* **10**, 456.
 Gray (1920). *J. Physiol.* **53**, 308.
 — (1926). *Brit. J. Exp. Biol.* **4**, 215.
 — (1928). *Brit. J. Exp. Biol.* **6**, 110.
 Hayes (1930). *Biochem. J.* **24**, 735.
 Hein (1907). *Allgem. Fischereizeitung* (München), No. 16.
 Kite (1913). *Biol. Bull.* **25**, No. 1.
 Kronfeld and Scheminzky (1926). *Roux's Arch. Entwksmechan.* **107**, 129.
 Loeb (1912). *Science*, N.S. **36**, 637.
 McClure (1918). *Amer. Anat. Memoirs*, No. 8.
 Miescher (1897). *Histochemische und physiologische Arbeiten*, **2**, 359. (Leipzig.)
 Nordgaard (1896). *Bergens Mus. Aarbog*, **4**, 1.
 Osato (1923). *Z. physiol. Chem.* **131**, 151.
 Romeis (1928). *Taschenbuch der mikroskopischen Technik*. (12 Auflage. Oldenburg, München und Berlin.)
 Scheminzky and Gauster (1924). *Roux's Arch. Entwksmechan.* **101**, 1.
 Steudel and Osato (1923). *Z. physiol. Chem.* **127**, 220.
 Stockard (1921). *Amer. J. Anat.* **28**, 115.
 Straub (1929). *Rec. Trav. Chim.* **48**, 49.
 Sumwalt (1929). *Biol. Bull.* **56**, 193.
 Svetlov (1929). *Roux's Arch. Entwksmechan.* **114**, 771.
 Terroine and Wurmser (1922). *Bull. Soc. Chim. Biol.* **4**, 519.
 Thorner (1929). *Biol. Bull.* **56**, 157.
 Wintrebert (1912). *Compt. Rend. Soc. Biol.* **72**, 724.
 Wislocki (1917). *Anat. Rec.* **12**, No. 4.

(Note added to proof.) In a further contribution (*Pflüger's Arch.* 1929, **223**, 645), Scheminzky discusses at length the discrepancies between his results and those of Gray. If Scheminzky discards his own experimentally obtained figures for the variations in embryonic water content during development, and assumes as Gray does that this is constant, he is able to apply Gray's equations to his other results and obtain a similar curve. He finds an agreement in experimental observation, the disharmony of the conclusions being due, first to the above mentioned assumption, and second to a difference in mathematical treatment. "Die Wachstumskurve nach Gray stellt somit einfach die in einem bestimmten Zeitpunkt vorhandene Menge von embryonaler Substanz dar, ohne über die Intensität des Wachstums eine Aussage zu machen."