LXIV. ON THE SIGNIFICANCE OF VITAMIN A IN THE NUTRITION OF FISH.

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It has recently been shown that the relatively large amounts of vitamin A found in the liver of the gadoid fishes is probably derived from their food and ultimately from the marine algae which synthesise this dietary factor. [Coward and Drummond, 1921; Hjort, 1922; Jameson, Drummond and Coward, 1922; Drummond and Zilva, 1922.] That the vitamin A probably fulfils some rôle in the development of the young fish is indicated by its presence in the gonads [McCollum and Davis, 1915; Hjort, 1922] and recently it has been shown that the amount stored in the reproductive cells of such a fish as the cod is extraordinarily large [Zilva and Drummond, 1922]. To study the fate of the vitamin in the fish ova it was decided to work with the brown trout which is easy to rear and maintain in the ordinary laboratory.

VITAMIN A IN TROUT EGGS.

Feeding tests on rats by the usual procedure demonstrated that trout eggs are very rich in vitamin A. A dose of two eggs per day per rat resulted in a resumption of normal growth, and it is to be regretted that tests were not made with smaller doses of the material, but none had been reserved (Fig. 2).

REARING OF YOUNG TROUT IN THE LABORATORY.

A thousand fertilised eggs were obtained from the Trout Fisheries, Stirling, and were allowed to hatch out in a single trough which we are describing because it enables trout to be reared with success for considerable periods of time in an ordinary laboratory, and we have encountered many cases where workers have failed to achieve this. All the eggs were placed in a flat boat-shaped receptacle (Fig. 1) 18" long and cross section 5" × 5". The sides are of wood but the ends and bottom are made of narrow-mesh zinc gauze. Such receptacles are placed in an ordinary porcelain sink of which the outlet has been adjusted so as to maintain a depth of half an inch of water whilst a constant stream of water is supplied by means of a pipe leading from the tap. The

incoming stream issues from a glass tube of half-inch diameter and is not directed centrally down the length of the "boat" but at an angle of about 20° to that axis. By this means the water stream does not pass directly out at the far end of the "boat" but tends to form a current round the sides. In this manner the unconsumed food, which must always be borne to the fish down stream, is carried round several times before being washed down the outflow pipe and wastage is thereby prevented.

The sinks and "boats" are kept scrupulously clean and the water current maintained without interruption. Partial shade for the trout is provided by placing a board crosswise over part of the sink. If these precautions are followed it is possible to rear the young trout almost without loss, at any rate for several months.

Fig. 1.

VITAMIN A IN LARVAL TROUT.

The ova hatched out with few exceptions and the larvae were allowed to develop without any extra food being given for four weeks. The depth of water was increased to about one inch. It is difficult to obtain information as to the feeding habits of young fresh-water fish, and as far as we are aware there are available no careful studies analogous to Dr Lebour's investigations on marine fish [1918; 1919, 1, 2]. It is commonly believed that larval trout take no food during that stage of their development and that they are purely carnivorous when they begin to feed as soon as the yolk sac is nearly absorbed. Some specimens of young brown trout about one month old from a trout hatchery were examined by us and were found to contain definite remains of partially digested green microscopic plants in their alimentary tracts. It is possible that these were taken in accidentally by the fish or that they were previously ingested by minute crustaceans which the fish had swallowed. The similar presence of marine plants in young fish has been described by Lebour.

About half-way through the larval period our experimental trout were tested again for vitamin A and it was found that although the contents of the yolk sac were about half absorbed there yet remained considerable amounts of this dietary factor. At this stage one young fish per day was sufficient

Fig. 2.

Fig. 3.

Periods on -A diet only in dotted lines.

Fig. 2. Resumption of growth of rats on feeding two trout eggs previous to basal diet each day.

Fig. 3. Resumption of growth of rats on feeding one larval trout with yolk sac half absorbed.

Fig. 4

Fig. 5.

Periods on -A diet in dotted lines.

Fig. 4. Very poor growth of rats on feeding one larval trout with yolk sac completely absorbed.

Fig. 5. Very poor growth of rats on feeding one trout that had been fed on cod muscle from the time of the absorption of yolk sac.

Periods on -A diet only in dotted lines.

Fig. 6. Resumption of growth of rats on feeding one trout that had been fed on liver and yolk of egg from the time of absorption of the yolk sac. Two short periods when the rats refused to eat the trout are seen in curves of rats 2566 and 2571. After a short time on -A only again the rats ate the trout and growth was rapid.

to cause a good resumption of growth in the test rats (Fig. 3). After about four weeks the yolk sacs were practically absorbed in every case and another test was made. From the results it was apparent that the vitamin *A* content of the fish had fallen very greatly and that actual utilisation of that originally present in the yolk sac had occurred (Fig. 4).

THE FEEDING OF POST-LARVAL TROUT.

Having found that the supplies of vitamin *A* present in the yolk sac of the newly hatched fish are apparently used up in the larval period, it was important to determine whether such fish are subsequently dependent on their food to maintain their supply of this factor.

Accordingly the remaining fish were divided up into three groups of about 250 each which were treated as follows:

Group I. Diet rich in vitamin *A*; fresh minced rats' liver and ground up yolk of hard boiled egg.

Group II. Diet deficient in vitamin *A*, freshly ground up cod muscle.

Group III. No extra food.

It was found necessary to feed Groups I and II several times a day by slowly dropping the finely minced food pulps into the stream of water in the "boats" so that the small particles were borne down to the fish on the current when the trout would immediately arrange themselves upstream and feed voraciously.

The influence of the feeding on the trout themselves was marked. Those in Group I grew rapidly and were very active, plump and well. Except by an accident late in the experiment when a large number of deaths followed the stoppage of the current of water, there was hardly a death in this group.

The fish on the vitamin *A*-deficient diet of cod muscle lived fairly well but did not grow much or show the vigour of those in Group I. Their weaker constitution was demonstrated by the increasing frequency of deaths as the experiment progressed. The group kept without food exhibited little or no growth but few died until about three weeks after the experiment had been in progress when deaths were suddenly very frequent.

INFLUENCE OF FOOD ON VITAMIN *A* RESERVES OF POST-LARVAL TROUT.

The feeding tests on rats demonstrated in a very striking manner the importance of the qualitative composition of the food supply for maintaining the vitamin *A* in the tissues of these fish. Very slight or no growth (Figs. 4 and 5) was given by supplements of the fish of Group III or those fed on the white fish muscle known to be deficient in vitamin *A* [Drummond, 1918].

The fish from Group I, however, served as an excellent source of the vitamin for rats and led to a prompt resumption of growth (Fig. 6).

Confirmation of this significance of the food supply was given by the survivors of Group II whose diet was later supplemented by an addition of

egg-yolk pulp. They themselves immediately began to grow again and, when used as test material for the one rat surviving this test, showed that their tissues had once again become stored with the vitamin A. Similarly, those trout which had been previously starved were fed for ten days with egg; and the rats which had been used to test the starved trout were in the meantime fed only on the diet deficient in vitamin A. But when the trout now being fed were added to their diet, the rats immediately resumed growth.

The demonstration of the importance of this qualitative aspect of the diet of young fish may be of some interest in connection with the well-known critical period which coincides with the absorption of the yolk sac. Trout hatchers have assured us that this period is one of great uncertainty in the rearing of young trout, and it is possible that our experiments may suggest to them some modification of the usual existing methods of feeding which might lessen the loss which frequently occurs at that time. Some of our results, which are not fully recorded in this paper, tend to show us that mashed liver, the usual dietary of the fish in hatcheries, may not always supply sufficient of the vitamin A for their optimum development and well-being. The full absorption of the contents of the yolk sac is an equally critical stage in the development of marine fish. Fabre and Domergue [1900, 1905] found that certain young fish unable to obtain external food at this stage become anaemic and die, but that an addition of microscopical plant organisms to the water enables them to be reared successfully. The application of such observations to the practical question of sea fishery is well pointed out by Hjort [1914] who emphasises the importance of the immediate availability of suitable plankton food for the survival of larval marine fish.

SUMMARY.

1. The ova of the brown trout normally contain relatively large amounts of the vitamin A.
2. During the subsequent development of the young larval fish this substance is in some way utilised so that at or shortly after the stage at which the contents of the yolk sac are absorbed the supplies are almost exhausted, so far as our technique enables us to ascertain.
3. It is probable that this fact is one of the reasons why this stage is so critical a period in the development of the young fish.
4. If, in the post-larval period or even before the yolk sac is completely absorbed, the fish are given food rich in vitamin A their growth and development are satisfactory and they appear able to store that factor in their tissues.
5. On a diet containing adequate protein but deficient in the factor A no such storage occurs and growth is subnormal.
6. These experiments confirm the previous findings, referred to in the text, which show that the stores of vitamin A in the tissues of fish can be derived from the food.

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REFERENCES.

- Coward and Drummond (1921). *Biochem. J.* **15**, 530.
Drummond (1918). *J. Physiol.* **52**, 95.
Drummond and Zilva (1922). *Biochem. J.* **16**, 518.
Fabre and Domergue (1900). *Bull. de la marine marchande*, Paris.
—— (1905). *Développement de la Sole*, Paris.
Hjort (1914). *Rapports, Conseil International pour l'exploration de la Mer*, **20**.
—— (1922). *Proc. Roy. Soc. B.* **93**, 440.
Jameson, Drummond and Coward (1922). *Biochem. J.* **16**, 482.
Lebour (1918). *J. Marine Biol. Ass.* **11**, 434.
—— (1919, 1). *J. Marine Biol. Ass.* **12**, 9.
—— (1919, 2). *J. Marine Biol. Ass.* **12**, 22.
McCollum and Davis (1915). *J. Biol. Chem.* **20**, 641.
Zilva and Drummond (1922). *Lancet*, i. 1243.