animals developed bloody urine, some also bled from the nose and had diarrhea, and a large number showed tremor. At autopsy the first impression was always the more or less generalized congestion of all internal organs and the whiteness of the intestines caused by the diffuse filling with the thiouracil. This latter was occasionally obliterated in part by intestinal hemorrhage. Lung edema, as observed by Mackenzie and Mackenzie[4] after thiourea treatment occurred occasionally but was not always the first pathological change. When the lungs were congested, hemorrhage was usually absent. The most damaged organs were, first, the kidneys and, secondly, the liver; occasionally the spleen showed signs of damage, also.

Conclusion. Thiouracil is relatively nontoxic. Continued large doses cause cachexia, tremor, convulsions, bloody urine and feces. Most internal organs show hemorrhagic congestion; kidneys and liver seem to be damaged invariably; edema of the lungs is often, but not always, present.

4 Mackenzie, J. B., and Mackenzie, C. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 34.

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Increased Growth Produced by Gelatin in Trout Fed Meatless Diets.*

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For many years research on the nutrition of fish has indicated that trout require fresh meat in their diet for normal growth and maintenance.[1,2,3] The more recent work of McCay and his collaborators points to the existence of an indispensable labile factor present in fresh meat which has been termed "Factor H."[4] It has been demonstrated that the latter is required for growth and normal erythrocyte production in trout.

Restrictions in the use of meat as an animal feed during the present war food emergency threaten to restrict seriously the culture of trout in hatcheries throughout this country. This situation prompted a search for satisfactory substitutes for meat in fish diets.

Methods. All experiments were carried out in the Biological Laboratories of the James Nevin State Hatchery,‡ Madison. Healthy rainbow trout, *Salmo gairdnerii irideus,* were obtained directly from the stock raceways of the hatchery. The hatchery stock diet, consisting of ground fresh liver 47.5, ground carp 47.5, and dried brewer's yeast 5, was fed prior to the beginning of the experiment.

The procedure consisted of feeding the same experimental diet to 2 tanks of fish once daily, 6 days a week. An equal number of fish, from the same lot and chosen for their uniformity, were placed into one tank; 300 or more fish per tank were always used. Average weights were obtained once a week by transferring all the fish in a tank to a weighed quantity of water, and dividing the resultant fish weight by the total number of surviving fish.

Of an extensive series of diets which have

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† Biologist, Wisconsin Conservation Department.

1 Davis, H. S., and Lord, R. F., U. S. Dept. Comm., Bur. of Fish, Doc. No. 1079, 1930, 130.

2 McCay, C. H., *Ann. Rev. Biochem.*, 1937, **6**, 453.

3 Tunison, A. V., Brockway, D. R., Maxwell, J. M., Derr, A. L., and McCay, C. M., *Cortland Hatchery Report,* No. 11, N. Y. State Conserv. Dept., Albany, New York.

4 McCay, C. M., *Science,* 1928, **67**, 249.

‡ This project was made possible by the cooperation of the Biology Division of the Wisconsin Conservation Department and its Chief, Dr. Edward Schneberger.

been devised and tested, the following have been selected to illustrate the effect obtained with gelatin. The experimental basal ration consisted of skim milk powder 25, cottonseed oil meal 15, alfalfa leaf meal 10, cod liver oil 2, dried brewer's yeast 3, iodized salt 0.5, yellow corn meal 41.5, and *l*-ascorbic acid 4.54 g per 100 lbs. of ration. Various dried liver preparations§ were included at a level of 3%. The control meat diet was composed of the hatchery stock mixture of fresh liver, carp, and yeast.

Experimental. The basal ration supplemented with 1:20 liver powder, liver fraction "L" or fraction "M" were fed in unrestricted quantities for a period of 9 weeks. During this entire period the weight of the fish receiving all experimental diets was essentially unchanged from their initial weight. However, the fish receiving the control meat diet, alone, showed a gain of 67% above the starting weight during the same period.

Effect of force feeding basal rations in gelatin capsules. Since a large residue was always present after feeding the experimental rations in the usual way, it was suggested that a factor in their failure to induce growth might be one of palatability. Thus, to ensure a satisfactory test of the nutritional value of these diets, they were enclosed in gelatin capsules and hand-fed to the trout.‖ Sufficient diet was forced into the capsules so that each contained 1.5% (diet weighed in dry state) of the average body weight of the fish exclusive of the weight of the gelatin. The latter was estimated to be about 0.3% of the body weight. It was impossible to enclose the meat control diet in capsules. However, when this diet was fed to fish, it was consumed immediately and rarely was any residue seen. Therefore, 15 parts of melted gelatin was mixed with 85 parts of the meat diet and this was fed at an intake calculated at 1.8% (diet weighed in a wet state) of the total weight of the fish. daily.

At the end of 7 weeks of feeding, the average weight of the trout receiving the capsule-contained meatless ration had increased from 40.0 g to 50.2 g (trout larger than those used in *ad libitum* feeding experiments were required for the feeding of capsules), while the weight of the fish receiving the meat plus gelatin diet had increased to 43.8 g from a starting weight of 40.0 g. It would appear that the restricted meat intake of the latter group, which was calculated on a wet basis, furnishes an explanation for the sub-optimum rate of growth.

Effect of feeding of basal rations supplemented with gelatin and factors affecting palatability. The basal rations were supplemented in the following ways: plus 15% ground gelatin; plus 20% fresh ground spleen; plus 20% "stick";¶ plus anise oil (sufficient to give a distinct licorice flavor). For the first 4 weeks of the experiment, the rations were fed at an intake calculated at 5% of the total weight of fish in the group. Subsequent to the fourth week the level was increased to 10%. The group fed the original basal ration gained only slightly, anise oil did not increase the response, supplementing the ration with spleen or "stick" produced a slow uniform gain in weight throughout the course of the experiment. Although the fish fed the ration supplemented with gelatin lost some weight the first week, as they became accustomed to the new diet, they grew as rapidly as the group receiving the control meat diet (Fig. 1). It is noteworthy that the added gelatin serves only as a stimulus to initial growth with the meatless diets used for fish receiving these supplemented rations begin to lose weight rapidly after approximately 7 weeks (Fig. 1). It would appear that the failure to sustain growth rests in further deficiencies in the diets, one of which may be that of Factor H. In contradiction to an earlier report,[5] the Cornell workers re-

§ We are indebted to Dr. David Klein of the Wilson Laboratories, Chicago, for generous supplies of the liver fractions.

‖ The procedure of the hand-feeding technic as applied to controlled nutritional studies in fish will be more fully discussed elsewhere.

¶ "Stick" is a concentrated residue obtained from the aqueous liquor in which processed meats have been steeped or cooked. It was supplied by Oscar Mayer and Co., Madison.

5 Phillips, A. M., Tunison, A. V., Fenn, A. H., Mitchell, C. R., and McCay, C. M., *Cortland Hatchery Report*, No. 9, N. Y. State Conserv. Dept., Albany, New York.

FIGURE 1. THE INCREASED GROWTH INDUCED IN TROUT FED A MEATLESS DIET SUPPLEMENTED WITH GELATIN

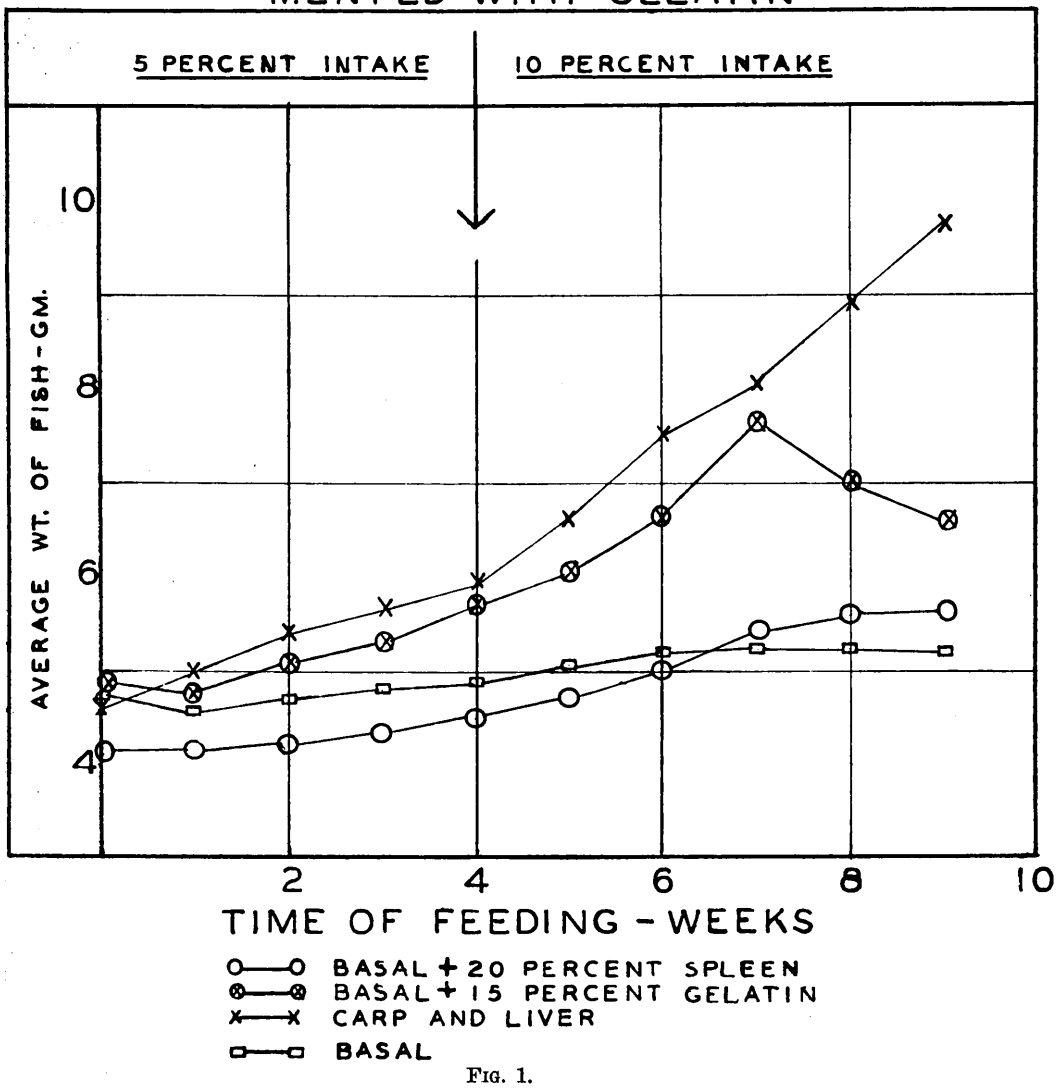

FIG. 1.

cently stated that dried liver preparations are devoid of anti-anemic properties.[3]

Discussion. The increased requirement of the chick for arginine and glycine for the production of muscle creatine and feathers[6] suggests that an analogous situation may exist in the trout. It has been found that gelatin satisfactorily supplies these necessary amino acids in the nutrition of the chick.[7] Walters[8] states that fish scales and bird feathers may have a homologous origin. Thus, it can be suggested that formation of the epithelial surface and dermal scales of the trout provides a nutritional demand similar to that in the

[6] Hegsted, D. M., Briggs, G. M., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **140**, 191.

[7] Briggs, G. M., Jr., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1942, **144**, 47.

[8] Walter, H. E., *Biology of the Vertebrates*, New York, 1939, p. 247.

chick.**

Summary. It has been shown that growing trout require a diet containing fresh meat. Mixed diets devoid of fresh meat did not produce any increase in the growth of trout. Varying the nature of the dried liver fractions used did not stimulate growth. When these diets were enclosed in gelatin capsules and force fed to the fish, a marked gain in weight was obtained. Supplementing the meatless diets with gelatin resulted in satisfactory initial growth. The supplemented diets did not sustain continuing growth beyond 7 weeks suggesting that further deficiencies exist in these rations.

** The growth promoting property of the gelatin-supplemented diets has been substantiated in practical feeding tests carried out at the Westfield (Wis.) State Hatchery. Brook trout (*Salvelinus fontinalis*) fingerlings were fed either the experimental diet or the hatchery stock diet of pork spleen mixed occasionally with brewer's yeast at 10% of the body weight. After 8 weeks the fish receiving the meat diet had gained 52% over the initial weight while those receiving the experimental diet had gained 40%.

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Studies on Certain Filtrable Viruses. VI. Antigenic Properties of Entire Embryo Fowl Pox Vaccine.

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In a previous report,[1] the results of immunizing farm flocks with fowl pox vaccine prepared from entire chick embryo were described. The vaccine consisted of a composite of the chorio-allantoic membrane, yolk, yolk sac, albumen, embryo proper, and fluids from previously inoculated embryos that showed gross lesions on the chorio-allantoic membrane. While a high concentration of virus was noted in the chorio-allantoic membrane, the presence of virus in other parts of the embryo was also demonstrated.[2] The dehydrated vaccine was suspended 1-100 in tryptone broth and applied by the 3-stick method in 35 healthy farm flocks, comprising 25,164 chickens. Nine days after inoculation, more than 6000 birds, approximately one-fourth of the vaccinated birds, were observed with 95.85% showing takes. Forty-three days after inoculation, 71 culled chickens from 3 of the flocks were brought to the Laboratory and artificially exposed to fowl pox virus by the feather follicle method. Eighty-eight and seven-tenths per cent proved refractory.

The present note concerns an observation on the duration of the immunity produced by entire embryo vaccine. Seventy-one birds selected from 4 of the flocks were exposed, one year subsequent to vaccination, on the patagium by the 3-stick method to fowl pox virus in a 1-100 suspension in tryptone broth. The

TABLE I.
Artificial Exposure of Chickens Vaccinated One Year Previously with Entire Embryo Fowl Pox Vaccine.

Flock	Birds exposed* 8/18/43	Reaction to exposure 8/25/43	9/1/43	9/9/43
1	35	—	—	—
2	12	—	—	—
3	15	+ (3)†	+ (3)†	+ (1)†
4	9	—	—	—
	—	—	—	—
Total	71	+ (3)	+ (3)	+ (1)
Nonvaccinated controls	10	+ (10)	+ (10)	+ (5)

* Fowl pox virus 3-stick method.
— = No lesion.
\+ = Fowl pox lesion
† No. of birds.

1 Thorning, W. M., Graham, Robert, and Levine, N. D., *Am. J. Vet. Res.*, 1943, **4**, 250.

2 Thorning, W. M., Thesis in partial fulfillment of Master of Science degree, University of Illinois Graduate School, 1942.