chloride (Doryl) orally resulted in a slight increase of tone with good contractions. Urethane of beta-methyl-choline chloride (Urecholine), injected subcutaneously, raised the tone baseline and produced excellent contractions lasting as long as 4 hours. Administered orally, Urecholine stimulated fair contractions. The effect appeared slowly, but was prolonged.

Conclusions. Death of rabbits one or 2 days after cervical vagotomy occurred as reported by Short[4] and others. During the first several hours after operation the animals seemed in good condition and stomach motility ceased. Levin[5] has described this as "shock." Motility recovered later and persisted as described by Opuls,[6] Auer[7] and others. Van Yzeren[8] noted chronic gastric ulcers in rabbits after vagotomy and this has been confirmed by others and by us.

In man gastric contractions were markedly depressed after vagotomy even though resting intragastric pressure increased slightly. These effects persisted. Ulcers healed or became quiescent. This difference in the effect of vagotomy, that is, healing of ulcer in man and development of ulcer in rabbits might be associated with decreased motility in man and increased or continued motility in rabbits.

Of the drugs studied (Table I) most restored motility or tone during the first hours after vagotomy in rabbits. In patients Urecholine was the most effective. Starr and Ferguson[9] first reported the clinical use of Urecholine. Machella *et al.*[10] described its effect in patients after vagotomy. Our observations confirm theirs but indicate also that some effect was obtained from Mecholyl and Doryl. Urecholine and Doryl have proved effective in overcoming gastric retention in patients treated by vagotomy providing scar tissue obstruction of the outlet of the stomach does not exist.

4 Short, R. H. D., *J. Path. and Bact.*, 1944, **56**, 355.

5 Levin, P. M., *J. Pharm. and Exp. Therap.*, 1938, **62**, 449.

6 Opuls, W., *J. Exp. Med.*, 1906, **8**, 181.

7 Auer, J., *Am. J. Physiol.*, 1909, **25**, 334.

8 Van Yzeren, *Z. f. klin. Med.*, 1901, **43**, 181.

9 Starr, I., and Ferguson, L. K., *Am. J. Med. Sc.*, 1940, **200**, 372.

10 Machella, T. E., Hodges, H. H., and Lorber, S. H., *Gastroenterology*, 1947, **8**, 36.

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Nutrition of Trout: Studies with Practical Diets.*

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Introduction. Both in anatomy and habit the trout appears to be a carnivore. In hatchery practice it was early established that these fish can live and grow to maturity on diets of animal origin.[1] Liver has been shown to be an adequate ration in itself and any modification of the ration has been related to attempts to replace liver with less expensive organs and animal products. Plant materials have been thought to have little promise. Embody[2] was unable to raise trout on a mixture of animal and plant materials. Subsequently he made proximate analyses of the stomach contents of fish raised under

* Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

1 McCay, C. M., Bing, F. C., and Dilley, W. S., *Trans. Am. Fish Soc.*, 1927, **57**, 240.

2 Embody, G. C., *Trans. Am. Fish Soc.*, 1918, **48**, 26.

3 Embody, G. C., and Gordon, M., *Trans. Am. Fish Soc.*, 1924, **54**, 185.

natural conditions,[3] and found carbohydrates to be low, protein, mineral and fat high, and the presence of considerable chitinaceous material.

McCay[1,4] utilizing purified rations of the casein-sucrose type, indicated that trout require fresh liver. Titcomb *et al.*[5] raised trout on mixtures of liver with fish and vegetable meals. More recently, Wilkinson,[6] in an extensive study on rainbow, brook, and brown trout found that mixtures similar to Titcomb's produced trout more economically than those completely of animal origin.

Field *et al.*[7] reported that vegetable meals supplemented with skim milk powder, gelatin, and concentrated liver fractions produced fair growth for a short period. Hatchery experiments[8] with one of these combinations showed that after 8 weeks growth ceased, but was resumed again when the ration was finely ground. Laboratory trials indicated that skim milk powder was an essential ingredient in this mixture, while liver fractions contributed little to the growth of the fish on these rations. The following laboratory and hatchery experiments were set up to improve Field's ration.

Experimental. All the laboratory experiments were conducted in the biological laboratories of the James Nevin State Hatchery, Madison. Healthy yearling rainbow trout *(Salmo gairdnerii irideus)* were obtained directly from the stock raceways of the hatchery. Fish weighing 18-20 g were selected and divided into groups of 25. The average weight of fish was obtained weekly using the method outlined by Field *et al.*[7] Hemoglobin determinations were made at intervals on blood obtained by heart puncture. The hemoglobin was measured as acid hematin. At the termination of the experiment representative fish from each group were killed, weighed, and the livers removed and weighed. A control group of fish received a ration made up of liver, canned carp, and yeast, the stock diet in use at the Nevin State Hatchery. Other groups received Ration C and various modifications thereof. Ration C, Field's basal ration,[7] modified to increase the protein content and reduce the cost, consisted of: skim milk powder 25, alfalfa leaf meal 10, soybean oil meal 15, liver A† 10, brewer's yeast 5, iodized salt 0.5, red dog flour 22.5, cod liver oil 2, and gelatin 10 (either as capsules or finely ground). Vitamin C was added at the rate of 4.54 g/100 lbs of ration. Ration C (including the gelatin capsule, see below) is calculated to contain protein 40%, fat 5%, carbohydrate 45%, ash 5% and fiber 5%. The stock ration on the same basis contains protein 66%, fat 24%, carbohydrate 4%, ash 6%, and fiber 0%. The stock diet was fed by dropping an amount, approximately equal to 6% of the body weight, into the tank while Ration C and modifications were fed by gelatin capsule[9] at an isocaloric level (2.0% of the body weight). Results are recorded in Table I in terms of growth, hemoglobin, liver size and mortality. The liver to body weight ratio has been shown earlier[10] to be an important index of the health of the fish. Enlarged, pathological livers are associated with high mortality.

From the data presented in Table I it is evident that sprouted soybeans are inferior to the ordinary soybean oil meal (expeller process). After 7 weeks on Ration D, soybean sprouts, autoclaved at 10 lbs pressure for 10 minutes, were substituted and growth for the next 9 weeks was somewhat improved; but the enlarged livers produced by the raw-sprouted beans were not reduced in size. When soybean oil meal was replaced by increasing the red dog flour to 37.5%, no sig-

[4] McCay, C. M., and Dilley, W. E., *Trans. Am. Fish Soc.*, 1927, **57**, 250.

[5] Titcomb, J. W., Cobb, E. W., Crowell, M.F., and McCay, C. M., *Trans. Am. Fish Soc.*, 1928, **58**, 205.

[6] Wilkinson, J. T., *Trans. Am. Fish Soc.*, 1938, **68**, 96.

[7] Field, J. B., Herman, E. F., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 227.

[8] Field, J. B., unpublished data.

† Liver A is a product of Wilson and Company, Chicago, Ill.

[9] Field, J. B., Herman, E. F., and Elvehjem, C. A., *Copeia*, 1944, No. 3, 184.

[10] McLaren, B. A., Herman, E. F., and Elvehjem, C. A., *Arch. Biochem.*, 1946, **10**, 433.

TABLE I.
Results of Laboratory Feeding Trials with Different Vegetable Meals and Levels of Yeast.

Ration	Modification	Weekly gain (16 wks, g)	Hemoglobin g/100 ml blood	Liver wt/body wt × 100	Mortality, No. of fish
Series 1					
Stock	Liver—canned carp	2.4	8.6	1.1	1
C	Field[7] as modified in text	2.3	8.2	1.1	1
D	Sprouted soybeans replacing soybean oil meal	0.7 (7 wks)	7.1	3.1	1
	,, ,, autoclaved 10 lbs for 10 min	1.6 (9 wks)	7.0	3.0	1
E	Red Dog Flour replacing soybean	2.1	7.6	1.4	2
F	Ground oats replacing Red Dog Flour	2.5	8.4	1.0	1
G	8% wheat germ flour replacing 8% Red Dog Flour	2.2	6.9	1.2	1
H-1	Brewer's yeast omitted—poultry yeast 5%	1.7	4.1	4.0	2
2	,, ,, ,, ,, 10%	1.5	4.2	6.5	9
3	,, ,, ,, ,, 15%	1.6	3.5	7.2	12
Series II					
C		1.6	10.5	1.4	0
N	Glandular meal replacing liver A	1.7	12.5	1.5	0
M	Flavonne meal replacing skim milk powder	0.6	6.8	1.8	1

nificant changes were noted. When Ration C was modified by replacing the red dog flour by finely ground rolled oats, growth, hemoglobin and liver to body weight ratio were the best of any group in the series. Substitution of 8% of the red dog flour in Ration C by wheat germ flour, a good source of vitamins, and minerals, lowered the hemoglobin from 8.2 to 6.9 and did not improve growth. When the brewer's yeast (Anheuser Busch) was omitted and 5% of livestock and poultry grade yeast substituted (Ration H-1) growth was poor, hemoglobin low, and the livers enlarged, lobulated and mustard yellow in color. Higher levels of this yeast augmented these deleterious effects and increased mortality.

As the foregoing experiments were being concluded 2 commercial products were brought to our attention, glandular meal and flavonne meal. The producers suggested that they could be substituted for liver A and skim milk powder respectively. The effect of these substitutions in Ration C is presented in the second series in Table I. In comparison with the controls it is apparent that glandular meal is equal or superior to liver A and at present is considerably cheaper. Flavonne meal cannot be considered as a substitute for skim milk powder.

In the second series the control group receiving Ration C did not grow as well as in the preceding experiment. The selection of fish of standard size (18-20 g) from the same hatch, now 4 months older, probably allowed the use of the stunted, slower growing fish.

It seems apparent from these experiments that under laboratory conditions it is possible to force feed yearling trout a practical ration and produce good growth, satisfactory hemoglobin values, and livers of normal size, shape and color. Using these factors as criteria of health, then the rations C, F, and G compare favorably with the fresh liver-canned carp control diet. These rations contain no fresh meat and are composed of 55% plant materials.

The question arises whether fish would eat such a ration under hatchery conditions. To test this, field experiments were devised and

TABLE II.
Results of Large Scale Feeding Trials at Westfield Hatchery by Mr. E. W. Hackman.

	No. of fish	Weekly gain 11 wks	Mortality No. of fish	
			6 wks	11 wks
Ration C	1400	0.86	19	38
Pork spleen	700	0.91*	313	—
Pork spleen plus yeast	700	0.77	5	99

* Weekly gain for 5 weeks. The figure reflects the loss of all the weaker more slowly growing fish from furunculosis.

carried out in the Westfield State Hatchery under the direction of Mr. E. W. Hackman. Ration C was selected for the field trial since it gave almost as good growth as Ration F, and was more economical.

Four groups of 700 each of 10 g brook trout *(Salvelinus fontinalis fontinalis)* were weighed and placed in separate tanks. The fish were hand-counted and hand-sorted to avoid injury. Two control groups received spleen, one with and one without poultry yeast. The remaining 2 groups were fed the experimental diets (Table II).

The ration was prepared in the dry form at the Department of Biochemistry and sent to the hatchery where it was mixed with 1½ times its weight of water and allowed to set (about 40 minutes) until it had a firm gelatinous consistency, not unlike liver. It was fed daily by dropping chunks into the tank.

Unfortunately 3 weeks after this experiment was set up, furunculosis broke out and spread throughout the hatchery. At the end of 6 weeks, 313 fish receiving spleen alone had died, and this group was discontinued. There was negligible mortality in the other 3 groups and these were maintained on their respective rations for an additional 5 weeks, until the disease had reached epidemic proportions. During this period 94 of the fish receiving spleen and yeast died and the rate of mortality increased rapidly during the last 2 weeks. Of the 1400 fish fed meatless rations only 38 died during the entire experiment.

Growth on Ration C was definitely better than on pork spleen even when supplemented with yeast. The 2 groups received slightly different liver preparations.

The weekly gain of these fish, while superior to that of the controls, was definitely lower than that of rainbow trout receiving Ration C in capsule (2.1 g/wk at 11 weeks). These fish were only half the weight of those fed in the laboratory experiments and would not be expected to make as great an absolute increment in weight, in a given period, as the larger fish. When the gain in weight is expressed in per cent of the initial weight it is seen that these fish grew at an even faster rate (98% in self-fed as against 67% in capsule-fed at 11 weeks).

During the spring of 1945, the supply of fresh meat suitable for fish feeding greatly decreased. The Wisconsin Conservation Department had Garver's Supply Company, Madison, prepare 3 tons of Ration H-1. It was well mixed and finely ground. This feed was distributed among several trout hatcheries and a controlled experiment was set up in which 7000 yearling rainbow trout were fed this ration. 3500 of the same age were fed the spleen-carp mixture. Both groups received the same amount of feed on the wet basis. The fish were weighed at the beginning and end of the experiment. Over a 6-month period both groups grew at approximately the same rate,—dry ration 412% gain—meat ration 400%. The losses were negligible, 0.3% (dry ration) and 0.6% (meat mixture).

Discussion. The demonstration in the laboratory experiments that rations containing over 40% carbohydrate could successfully support essentially normal growth and health in yearling rainbow trout provides an interesting contrast to the earlier work with purified rations[10] where carbohydrates in excess of 20% (glucose, sucrose, lactose or raw

starch) produced marked liver pathology. Tunison *et al.*,[11] who fed these carbohydrates by capsule to fish receiving meat diets, also found that enlarged livers, rich in glycogen, resulted. The carbohydrate in rations C, E, F and G is largely in the form of starch and since the rations are not subjected to heat must be considered as raw. Two possibilities suggest themselves as explanations: (1) That a diet of natural foods contains some substance or substances which protect the fish against the deleterious effect of high carbohydrate. If this is the case these protective substances are apparently not, according to the work of Tunison,[11] present in animal organs. (2) That the physical nature of these natural materials with their intimate mixture of all the proximate principles exerts the protective effect observed.

Not all of the rations of natural materials prevented liver enlargement. Ration D, containing sprouted soybeans, and the H rations, in which poultry yeast replaced brewer's yeast, produced moderately to greatly enlarged livers. In the case of the H rations it cannot be easily argued that the substitution of one type of yeast for another resulted in the omission of a protective factor since enlarged livers were produced with Ration D (sprouted soybeans) in which there was no change in the yeast. Furthermore brewer's yeast does not give protection against excessive carbohydrate when included in purified rations.[10] It is possible in both Rations D and H that a toxic factor is involved which operates to produce liver enlargement independent of the carbohydrate level. At least in Ration D the factor is not destroyed by autoclaving. This would seem to indicate that it is not the trypsin inhibitor since the latter is heat labile.[12] At this stage of the investigation and without further work it is impossible to determine why these ingredients lead to liver enlargement.

The field trials at the Westfield Hatchery with Ration C and at Nevin Hatchery with Ration H indicate that essentially similar growth can be obtained when these rations are self-fed as when force-fed.

Summary. A practical meatless ration for trout containing 55% plant material is described which is essentially equivalent to a standard liver, canned carp diet so far as growth and hemoglobin levels are concerned.

The effect of various substitutes in the ration is described.

Although this ration contains 45% carbohydrate, liver enlargement was not noted except when sprouted soybeans or poor quality yeast were included. Large scale field trials indicated that Ration C was superior to pork spleen, and equivalent to pork spleen supplemented with canned carp, as a diet for yearling brook trout on the basis of both growth and mortality.

We wish to thank Wilson Laboratories, Chicago, for the liver fractions; Armour and Company, Chicago, for the glandular meal; Dawes Vitamilk Company, Chicago, for the Flavonne.

Grateful acknowledgment is due D. Mott Cannon, M.D., for his helpful suggestions and interest and Mr. Wendell Anderson, foreman, Nevin State Fish Hatchery, for his wholehearted cooperation in supplying suitable fish for the laboratory experiments.

[11] Tunison, A. V., Brockway, D. R., Maxwell, J. M., Dorr, A. L., and McCay, C. M., N. Y. Conservation Dept., Cortland Rept. No. 11, 1942.

[12] Hamm, W. E., and Sandstedt, R. M., *J. Biol. Chem.*, 1945, **161**, 635.