Distribution of an Unidentified Antithyrotoxic Factor in Materials of Plant and Animal Origin.* (17918)

BENJAMIN H. ERSHOFF

From the Emory W. Thurston Laboratories, Los Angeles, and the Department of Biochemistry and Nutrition, University of Southern California, Los Angeles.

Available data indicate that requirements for a number of nutrients are markedly increased in the hyperthyroid animal(1). This is particularly true for some of the B vitamins. An increased requirement for thiamine (2,3), pyridoxine(4), pantothenic acid(4), folic acid(5) and, more recently, vitamin B_{12} has been demonstrated following the administration of large doses of thyroactive substances. In addition to the above, requirements are increased for at least one additional factor as well. This substance which has been termed the "antithyrotoxic factor of liver" will counteract completely the growth retardation of immature rats and mice fed massive doses of thyroid in conjunction with a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate(6,7). It is present in considerable concentration in the water-insoluble fraction of liver (liver residue) and is apparently distinct from any of the known nutrients(8-11). In the present communication data are presented on the comparative effects of liver residue and other materials of plant and animal origin on the gain in body weight of immature hyperthyroid rats.

Procedure and results. The basal ration employed in the present experiment consisted of sucrose 73.0%; casein,† 22.0%; salt mixture,‡ 4.5%; and U.S.P. desiccated thyroid,§ 0.5%. To each kg of the above were added the following synthetic vitamins: thiamine hydrochloride, 72 mg; riboflavin, 9 mg; pyridoxine hydrochloride, 15 mg; calcium pantothenate, 67.2 mg; nicotinic acid, 60 mg; 2-methyl-naphthoquinone, 5 mg and choline chloride, 1.2 g‖. Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson), 500 mg; alpha-tocopherol acetate, 1.5 mg and a vitamin A-D concentrate containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D¶. The various supplements to be tested were incorporated in the basal ration as per cent of the total diet, replacing an equal amount of sucrose. The supplements tested and the levels at which they were fed are listed in Table I. Male and female rats of the Long-Evans strain were selected at 22 to 25 days of age for the present study. Animals were kept in metal cages with raised screen bottoms to prevent access to feces, and were fed the various rations *ad lib.* Feeding was

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† Vitamin Test Casein, General Biochemicals, Inc.

‡ Hubbel, Mendel, and Wakeman Salt Mixture, General Biochemicals, Inc.

§ Thyroid Powder, U.S.P., Armour and Co.

‖ In view of the increased requirements for thiamine, pyridoxine, and pantothenic acid in the hyperthyroid rat(4), these vitamins were administered in excessive amounts in the present experiment in order to assure an adequacy of these factors in the diet.

¶ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vit. A and 80,000 U.S.P. units of vit. D per g.

TABLE I.

Effects of Liver Residue and Other Materials of Plant and Animal Origin on the Gain in Body Weight of Immature Hyperthyroid Rats. Eight animals were placed in each experimental group, 10 in control group on basal ration without thyroid, and 12 in control group on basal ration with thyroid.

Supplements fed with basal ration*	Initial body wt, g	Avg gain in body wt in 28 days, g†
♂		
(Basal ration without thyroid)	45.9	105.3 ± 5.2 (10)
0	47.1	51.0 ± 3.4 (9)
Vit. B_{12}	46.4	53.4 ± 3.6 (6)
Synthetic vit. mixture‡	46.3	81.5 ± 2.4 (5)
Heart	46.1	60.7 ± 2.1 (5)
Whole egg	46.4	63.3 ± 3.4 (5)
Muscle (beef flank)	46.3	63.5 ± 3.7 (6)
Thymus	46.0	67.0 ± 3.5 (6)
Streptomycin mash	46.2	67.3 ± 3.0 (7)
Placenta	46.5	72.3 ± 5.8 (6)
Brain	46.4	73.8 ± 4.4 (7)
Duodenum	46.3	75.5 ± 4.5 (6)
Sardine meal	46.3	76.9 ± 2.1 (8)
Soybean flour§	46.1	91.4 ± 3.4 (8)
Kidney	45.9	110.2 ± 4.1 (7)
Liver residue‖	45.8	112.9 ± 4.5 (7)
Aureomycin mash	46.0	118.3 ± 4.9 (8)
♀		
(Basal ration without thyroid)	44.0	91.9 ± 3.6 (10)
0	47.8	44.3 ± 3.6 (10)
Vit. B_{12}	46.2	46.0 ± 3.1 (6)
Synthetic vit. mixture‡	46.9	75.7 ± 1.9 (5)
Aureomycin HCl (500 mg per kg/diet)	46.4	46.7 ± 3.2 (4)
Aureomycin HCl (100 mg per kg/diet)	46.4	49.3 ± 3.3 (5)
Whey	45.9	52.9 ± 3.6 (5)
Alfalfa	46.2	60.6 ± 3.7 (8)
Yeast	46.5	62.4 ± 3.0 (8)
Penicillin mash	47.0	74.6 ± 3.2 (8)
Soybean flour§	46.4	84.3 ± 4.0 (5)
Liver residue‖	45.9	95.4 ± 3.9 (7)
Aureomycin mash	45.8	103.0 ± 4.1 (8)

The values in parentheses indicate the number of animals which survived and on which averages are based.

* The indicated factor constituted 10% of the diet except in the case of vit. B_{12} fed at a level of 30 γ per kg of diet, and aureomycin HCl fed at levels of 100 and 500 mg per kg of diet. Supplements were obtained from the following sources: Cobione (Crystalline Vit. B_{12} Merck); extracted liver residue, Wilson Laboratories; aureomycin mash, APF-5, Lederle Laboratories; kidney, Viobin Corporation; heart substance powder, Armour and Co.; powdered whole egg, Washington Cooperative Farmers Assn.; beef flank, Southern Calif. Gland Co.; thymus powder, Armour and Co.; streptomycin mash, Bi-Con APF 3+, Chas. Pfizer and Co.; placenta powder, Armour and Co.; brain powder, Armour and Co.; duodenum, Viobin Corporation; sardine meal, Van Camp Corporation; soya powder, El Molino Mills; dry cheese whey solids, Western Condensing Co.; alfalfa concentrate, Test Laboratories; brewers' type yeast No. 200, Anheuser-Busch; and penicillin mash, dried penicillin mycelia, Eli Lilly and Co. We are indebted to Dr. E. L. R. Stokstad of Lederle Laboratories for the crystalline aureomycin HCl employed in the present experiment.

† Including stand. error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

‡ The following vitamin supplements were added per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; biotin, 4 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-naphthoquinone, 10 mg; and vit. B_{12} (Cobione), 30 γ.

§ Full fat soybean flour containing approximately 22% fat.

‖ Extracted liver residue consists of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances.

continued for 28 days or until death, whichever occurred sooner. Results are summarized in Table I.

In agreement with earlier findings massive doses of thyroid retarded significantly the gain in body weight of immature rats fed a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate. No increase in growth resulted from the administration of crystalline vitamin B_{12}; supplements of all the known B vitamins, however, promoted a significant increase in body weight although the growth increment was less than that obtained with rats fed the basal ration with thyroid omitted. Liver residue, desiccated and defatted kidney and a fermentation product derived from cultures of *Streptomyces aureofaciens* (APF-5)** completely counteracted the growth retardation of immature rats fed massive doses of thyroid under conditions of the present experiment. Some increase in body weight over that obtained on the basal ration was observed following the administration of desiccated and defatted heart, beef flank, thymus, placenta, duodenum and brain as well as powdered whole egg, fish meal, whey, alfalfa, yeast, streptomycin residue (Bi-Con APF-3+) and a sample of penicillin mash. Gain in body weight on these supplements, however, was significantly less than that obtained on the basal ration with thyroid omitted. Soybean flour as previously reported(12) showed considerable activity as a source of growth-promoting factor for the hyperthyroid rat. Crystalline aureomycin HCl at levels of 100 or 500 mg per kg of diet, however, was without significant effect.

Discussion. Previous findings indicate that whole liver contains one or more factors apparently distinct from any of the known nutrients whose requirement is increased in hyperthyroid rats fed a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate. A deficiency of this factor results in a marked retardation of growth in the immature thyroid-fed rat which may be completely counteracted

** The material employed, which was kindly provided by Dr. E. L. R. Stokstad, contained approximately 5 mg of aureomycin per g. Tests were subsequently conducted to determine the growth-promoting effects of smaller doses of this material. APF-5 when fed at a level of 2% of the diet had no significant effect on rate of growth. APF-5 at a 5% level markedly increased the gain in body weight but the weight increment was less than that obtained with the 10% supplement.

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by the administration of desiccated whole liver or its water-insoluble fraction but not by supplements of the various known nutrients (8-11). Results of the present experiment indicate that desiccated and defatted kidney and a fermentation product derived from cultures of *Streptomyces aureofaciens* (APF - 5) were as active as liver residue in counteracting the growth retardation of immature rats fed massive doses of desiccated thyroid. Full fat soybean flour also showed considerable activity in this regard. Stokstad *et al.* (13) have recently observed that aureomycin mash contains a factor apparently distinct from any of the known nutrients which is essential for the optimal growth of chicks. The activity of aureomycin mash under conditions of the present experiment suggests the possibility that the "antithyrotoxic factor of liver" and the chick growth factor of Stokstad *et al.* may be one and the same factor. Crystalline aureomycin HCl, however, which exerts growth-promoting activity in the chick(14). turkey and pig(15) is inactive as a source of growth-promoting factor for the hyperthyroid rat. The ineffectiveness of whey (Table I) appears to distinguish the active principle in the present experiment from the "whey factor" described by Hill(16). Some increase in body weight over that obtained on the basal ration was observed in thyroid-fed rats under conditions of the present experiment following the administration of desiccated and defatted heart, beef flank, thymus, placenta, duodenum or brain as well as powdered whole egg, fish meal, whey, alfalfa, yeast or streptomycin and penicillin mash. The gain in body weight of rats fed these supplements, however, was less than that of animals receiving an additional supply of the known B vitamins suggesting that the growth-promoting effect of these supplements may have been due, at least in part, to their content of known B vitamins.

Summary. Growth was markedly reduced in hyperthyroid rats fed a purified ration containing casein as the dietary protein and sucrose as the dietary carbohydrate. The retardation in growth was completely counteracted by the administration of a water-insoluble fraction of liver, desiccated and defatted kidney and a fermentation product derived from cultures of *Streptomyces aureofaciens*. Full fat soybean flour also showed considerable activity. Crystalline aureomycin HCl was inactive. Other materials of plant and animal origin showed little if any activity. The protective factor was apparently distinct from any of the known nutrients.

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Absorption, Distribution and Excretion of Phenindamine (Thephorin). (17919)

ROBERT E. DAILEY,* E. LEONG WAY,† J. R. PARIKH,† CHEN-YU SUNG,† AND D. L. HOWIE

From George Washington University, School of Medicine, Washington, D.C., and Division of Pharmacology, School of Medicine, and the College of Pharmacy, University of California.

The compound, phenindamine (2-methyl-9-phenyl 2,3,4,9 tetrahydro-1-pyridindene), is available under the trade name Thephorin for the treatment of various allergic conditions. Its antihistaminic and other pharmacologic properties have been described by

* Present address: Smith, Kline & French Laboratories, Philadelphia, Pa.

† Present address: University of California, Medical Center, San Francisco, Calif.