reported by Griffitts.[5] This worker found that *E. coli, Aerobacter aerogenes, Alcaligenes faecalis, Bacillus anthracis, Brucella abortus, Corynebacterium diphtheriae, Eberthella typhosa, Proteus vulgaris, Salmonella, Shigella, Staphylococcus aureus* and a few other bacterial species failed to agglutinate human erythrocytes. On the other hand, 23 strains of *Shigella alkalescens,* some strains of *Hemophilus pertussis, Vibrio comma,* and *Streptococcus pyogenes* showed hemagglutination of human erythrocytes. None of the species of bacteria which Griffitts reported to produce hemagglutination were isolated from stool suspensions cultured in the present study.

Summary. Saline suspensions of stools obtained from both normal and poliomyelitis patients showed hemagglutination of group "O" human, chick, rabbit, guinea pig and sheep erythrocytes. The rabbit cells were agglutinated in highest titers. No significant difference in hemagglutination titer was noted with the normal and poliomyelitis stools. Normal human, rabbit and monkey serum and 20% bovine albumin inhibited hemagglutination. The hemagglutinin was thermolabile and absorbed by group "O" human, chick and rabbit erythrocytes. Stool suspension Seitz filtrates failed to show hemagglutination, but the titer could be partially restored by methanol precipitation. Various species of bacteria isolated from stool suspensions failed to agglutinate chick, group "O" human and guinea pig erythrocytes but showed some agglutination of rabbit cells in low titer. Mouse brain and cord suspensions infected with the Lansing strain of poliomyelitis virus also failed to agglutinate chick, human group "O" and rabbit erythrocytes.

[5] Griffitts, J. J., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 358.

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17136. An Antithyrotoxic Factor for the Rat Not Identical with Vitamin B_{12}.*

BENJAMIN H. ERSHOFF.

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

It has been demonstrated that whole liver powder will prolong survival and counteract growth retardation of immature rats fed massive doses of desiccated thyroid.[1-3] Some confusion exists, however, regarding the nature of the factor or factors responsible for the above effects. On rations containing soybean meal the retardation in growth caused by massive doses of desiccated thyroid or iodinated casein has been counteracted both in the rat and chick by liver fractions with vitamin B_{12} activity.[4-6] In subsequent work similar findings were obtained with crystalline vitamin

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[1] Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 500.

[2] Ershoff, B. H., *Arch. Biochem.*, 1947, **15**, 365.

[3] Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition,* 1947, **34**, 431.

[4] Bosshardt, D. K., Paul, W. J., O'Doherty, K., Huff, J. W., and Barnes, R. H., *J. Nutrition,* 1949, **37**, 21.

[5] Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 129.

[6] Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 631.

B_{12}.[7,8] On rations containing casein as the dietary protein and sucrose as the dietary carbohydrate, however, the retardation in growth following administration of massive doses of thyroactive substances appears to be due to a deficiency of some other factor. This latter substance which has been termed the "antithyrotoxic factor of liver" is present in considerable concentration in the water-insoluble fraction of liver.[2,9] It is not present in significant amounts in liver fractions with vitamin B_{12} activity or other concentrates of vitamin B_{12}.[10] In the present communication further data are presented concerning the ineffectiveness of crystalline vitamin B_{12} as a source of antithyrotoxic factor for the immature hyperthyroid rat.

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.¶ In addition to the basal ration the following diets were also employed, consisting of basal ration plus each of the following supplements: (1) 30 γ of vitamin B_{12} per kg of diet,** (2) 10% whole liver powder,†† (3) 10% extracted liver residue,‡‡ and (4) 4% liver concentrate 1-20.§§ The liver fractions were added in place of an equal amount of sucrose. In addition to the above, two control diets were also tested. These consisted of (1) basal ration with thyroid omitted and (2) a similar ration supplemented with 30 γ of vitamin B_{12} per kg of diet. Sucrose replaced thyroid in both of the latter rations. Sixty-two female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average weight of 46.0 g for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib.* Feeding was continued for 28 days or until death, whichever occurred sooner. Results are summarized in Table I.

In agreement with earlier findings whole liver powder completely counteracted the growth retardation of immature rats fed massive doses of thyroid. The protective factor was retained in the water-insoluble extracted liver residue. Little if any activity was present in the water-soluble liver concentrate 1-20. Crystalline vitamin B_{12} was inactive. These findings indicate that extracted liver residue

[7] Nichol, C. A., Dietrich, L. S., Cravens, W. W., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 40.

[8] Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 392.

[9] Ershoff, B. H., and McWilliams, H. B., *Science*, 1948, **108**, 632.

[10] Ershoff, B. H., *Exp. Med. and Surg.*, 1948, **6**, 438.

† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Sure's Salt Mixture No. 1.[11]

[11] Sure, B. J., *J. Nutrition*, 1941, **22**, 499.

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

‖ In view of the increased requirements for thiamine, pyridoxine and pantothenic acid in the hyperthyroid rat,[12] the B vitamins in the present experiment were administered in excessive amounts in order to assure an adequacy of these factors in the diet.

[12] Drill, V. A., and Overman, R., *Am. J. Physiol.*, 1942, **135**, 474.

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

** The vitamin B_{12} employed in the present experiment was obtained from Merck and Co., Rahway, N. J.

†† Whole Dried Liver Powder, Armour and Co., Chicago, Ill.

‡‡ Extracted Liver Residue, Wilson Laboratories, Chicago, Ill. This material consists of the coagulated, water-insoluble material remaining after the removal of the extractable water-soluble substances.

§§ Liver Concentrate Powder 1-20, Wilson Laboratories, Chicago, Ill. This material consists of the water-extractable material of raw liver.

TABLE I.
Comparative Effects of Vitamin B_{12} and Various Liver Fractions on the Growth of Immature Rats Fed Massive Doses of Desiccated Thyroid.

Supplements fed with basal ration	% thyroid	No. of animals	Initial body wt, g	Avg gain in body wt after 28 days of feeding,* g
None	.5	10	46.1	47.8 ± 4.0 (6)
Whole liver powder	.5	10	46.0	109.7 ± 2.1 (8)
Extracted liver residue	.5	10	46.0	103.4 ± 4.4 (9)
Liver concentrate 1-20	.5	10	46.1	63.3 ± 3.2 (9)
Vit. B_{12}	.5	10	46.1	48.3 ± 3.5 (7)
None	.0	6	45.7	98.8 ± 5.8 (6)
Vit. B_{12}	.0	6	45.8	97.6 ± 3.9 (6)

The values in parentheses indicate the number of animals that survived of which this is an average.

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

contains one or more factors other than vitamin B_{12} which were responsible for its antithyrotoxic effects under conditions of the present experiment.

Available data indicate that requirements for a number of nutrients are markedly increased in the hyperthyroid animal.[13] This is particularly true for some of the B vitamins. An increased requirement for thiamine,[12,13] pyridoxine,[12,13] pantothenic acid,[12,13] folic acid[14] and more recently vitamin B_{12}[7,8] has been demonstrated following administration of large doses of thyroactive substances. In addition to the above, requirements are increased for at least one additional factor in the hyperthyroid animal. This substance, which has been termed the "antithyrotoxic factor of liver", is retained in the water-insoluble fraction of whole liver powder. Recent findings[2,3] with those of the present experiment indicate that this factor is distinct from any of the known nutrients including vitamin B_{12}.‖‖

Summary. Growth was markedly reduced in hyperthyroid rats fed purified rations 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. Crystalline vitamin B_{12} was ineffective. The protective factor in liver is distinct from any of the known nutrients including vitamin B_{12}.

13 Drill, V. A., *Physiol. Rev.*, 1943, **23**, 355.

14 Martin, G. J., *Amer. J. Dig. Dis.*, 1947, **14**, 341.

‖‖ While this paper was in press Betheil and Lardy[15] reported that crystalline vitamin B_{12} *partially* counteracted the growth retardation of immature male rats fed massive doses of thyroid in a ration similar to that employed in the present experiment. Growth was significantly less, however, than that occurring in animals fed a similar diet containing 10% whole liver powder. It would appear that Betheil and Lardy were dealing with a multiple deficiency induced by thyroid feeding. Vitamin B_{12} was one of the deficient factors, but optimum growth was not obtained unless animals were supplied an additional factor or factors present in whole liver. Emerson[16] has recently found that Vitamin B_{12} may be stored in considerable amounts in the tissues of the young rat during the period of pregnancy and lactation. It is not unlikely that differences in the growth of immature hyperthyroid rats fed vitamin B_{12} may be due, at least in part, to the vitamin B_{12} content of the pre-test dietary regime.

15 Betheil, J. J., and Lardy, H. A., *J. Nutrition*, 1949, **37**, 495.

16 Emerson, G. A., *Fed. Proc.*, 1949, **8**, 381.

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