TABLE II. Fever Indices after Injection of .05 ml Typhoid Vaccine in Rabbits before and after HN_2. Peripheral leukocytes were reduced below 300/mm³ by HN_2.

	Before HN_2	After HN_2
	168	156
	192	176
	243	216
	186	220
	212	197
	161	191
	144	163
	212	176
	149	170
	198	162
Avg	186.5	182.7

to pyrogen injection with brisk fevers which could not be distinguished from those before HN_2 injection. Table II compares the febrile responses of 10 rabbits to typhoid vaccine before and after HN_2 treatment. Similar results were obtained in 6 animals given P-25 polysaccharide. These findings indicate that the production of fever by bacterial materials is not dependent upon the presence of a normal number of leukocytes in the peripheral circulation.

Discussion and summary. The finding of Stetson and Good that the period of inhibition of the Shwartzman phenomenon which follows injection of HN_2 in the rabbit coincides with the time of maximum depression of circulating leukocytes was confirmed. In addition, the prevention of severe leukopenia by cysteine resulted in retention of Shwartzman activity, further evidence for the role of the leukocyte in the Shwartzman reaction. Although injection of colloidal materials abolishes natural or acquired resistance to the Shwartzman phenomenon, thorotrast did not influence the Shwartzman non-reactivity produced by HN_2. The failure of HN_2-induced leukopenia to influence the febrile response of rabbits to pyrogenic bacterial materials indicates that pyrogen fever is not dependent upon the presence of normal numbers of circulating leukocytes.

Mrs. Frances Brown and Mrs. Anne McManus gave technical assistance in this work.

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Received September 9, 1952. P.S.E.B.M., 1952, v81.

Beneficial Effects of Vitamin B_{12} and Aureomycin in Rats Given Large Doses of Cortisone.*† (19860)

JOSEPH MEITES.‡§

From the Department of Physiology and Pharmacology, Michigan State College, East Lansing, Mich.

On corn-soybean meal or corn-powdered milk diets, vit. B_{12} or antibiotics have been shown to be partially or completely effective

* Published with the approval of the Director of the Michigan Agric. Exp. Station as Journal Article No. 1404.

† Part of these data were presented before the 36th Annual Meeting of the Federation of Am. Soc. for Exp. Biol., New York City, April 14-18, 1952.

‡ These studies have been aided in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

§ With technical assistance of Mrs. R. C. Ogle and Mrs. L. M. Miller.

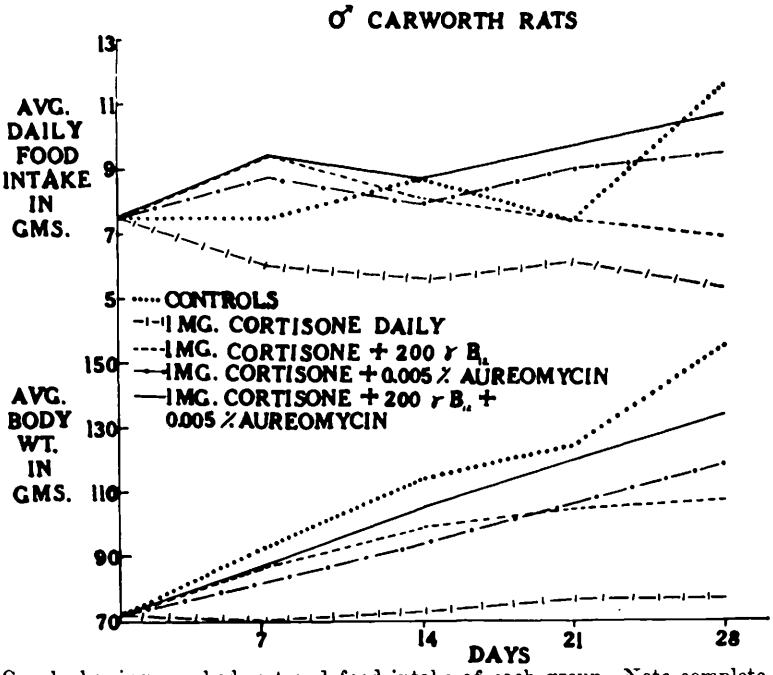

FIG. 1. Graph showing avg body wt and food intake of each group. Note complete inhibition of growth and reduction in appetite of the group which received cortisone alone, and improvement with vit. B_{12} and aureomycin.

in counteracting growth inhibition induced by administering large doses of iodinated casein, thiouracil, diethylstilbestrol or cortisone(1,2). These effects of the vitamin or antibiotics were usually accompanied by an increase in food intake and by greater efficiency in converting food into body-weight gains.

On a purified diet containing casein as the dietary protein and sucrose as the carbohydrate, Ershoff(3) reported that whole liver but none of the known B vitamins largely counteracted inhibition of body growth and alopecia in young rats fed massive doses of cortisone. Ershoff(3) suggested the presence of a separate protective factor in the liver different from all known B vitamins. This paper reports the results of an experiment in rats fed a purified diet containing casein as the dietary protein and glucose as the carbohydrate. On this diet it was found that vit. B_{12} and or aureomycin were effective in largely counteracting inhibition of body, hair and thymus growth induced by injecting large doses of cortisone.

Methods. Fifty young male Carworth rats were divided into 5 uniform groups of 10 each, and placed on the following purified diet: alcohol washed casein,‖ 25 g; glucose (Cerelose),¶ 62 g; corn oil (Mazola), 5 g; cod liver oil,** 5 g; salt mixture,†† 4 g. To each kilo of the above, the following vitamins were added: choline 1.0 g; thiamin, 2.0 mg; riboflavin, 5.0 mg; pyridoxine, 2.0 mg; niacin, 10.0 mg; Ca pantothenate, 28 mg; 2-Me-1,4 Naphthoquinone, 0.4 mg. After being on the above diet for a preliminary period of 2 weeks, each of the 5 groups was treated as follows for 28 days: 1, controls; 2, 1 mg cortisone acetate‡‡ daily by subcutaneous injection; 3, 1 mg cortisone acetate daily and 200 μg vit. B_{12} per kilo of

‖ "Vitamin Test" casein, Nutritional Biochemicals Corp., Cleveland, O.

¶ Corn Products Refining Co., Chicago, Ill.

** Contains 2000 USP units of vit. A and 250 units vit. D/g.

†† Salt mixture No. 2, Nutritional Biochemicals Corp., Cleveland, O.

‡‡ The B vitamins and cortisone acetate (Cortone) furnished through the kindnesss of Dr. E. Alpert of Merck and Co., Rahway, N. J.

diet; 4, 1 mg cortisone acetate daily and 0.005% aureomycin HCl§§ in the diet; 5, 1 mg cortisone acetate daily, 200 μg vit. B_{12} per kilo of diet and 0.005% aureomycin HCl. Individual body weights and food consumption were measured once weekly. Eosinophil counts were made from tail blood on the last day of the experiment. After killing the rats with ether, the adrenals and thymus glands were removed and weighed, and the latter were fixed for histological examination. All rats were maintained in metal cages with raised screen bottoms in an air-conditioned room at a temp. of 75 $\pm$ 2°F.

Results. Average body growth and food intake are shown in Fig. 1. All rats weighed approximately 71 g at the beginning of the experiment. The average final body weight and total food consumption per rat were as follows for each group: 1, controls, 155.4 $\pm$ 5.6 and 240.8 g; 2, cortisone acetate, 77.0 $\pm$ 4.2 and 162.4 g; 3, cortisone acetate and vit. B_{12} 107.5 $\pm$ 4.4 and 204 g; 4, cortisone acetate and aureomycin HCl, 117.2 $\pm$ 4.2 and 243.6 g; 5, cortisone acetate and vit. B_{12} and aureomycin HCl, 135.3 $\pm$ 6.8 and 251.2 g. It can thus be seen that cortisone completely suppressed body growth and reduced food intake, while supplementation of the diet with vit. B_{12} and/or aureomycin significantly counteracted these effects of the hormone.

Fig. 2 shows the typical appearance of 5 representative rats from each of the 4 cortisone-treated groups. It can be seen that the rats given cortisone alone are not only stunted in size but have developed a severe alopecia which was particularly marked over the head, neck and back areas. Either vit. B_{12} or aureomycin markedly reduced the alopecia, while the combination of the two appeared to completely eliminate it.

The effects of cortisone alone or in combination with vit. B_{12} or the antibiotic on the thymus gland are shown in Fig. 3. In the group treated with cortisone alone, there was marked atrophy of the thymus, disappearance of the cortical area and a great reduction in thymocytes. In the cortisone treated groups

§§ Kindly supplied by Dr. T. H. Jukes of Lederle Labs. Division, Pearl River, N. Y.

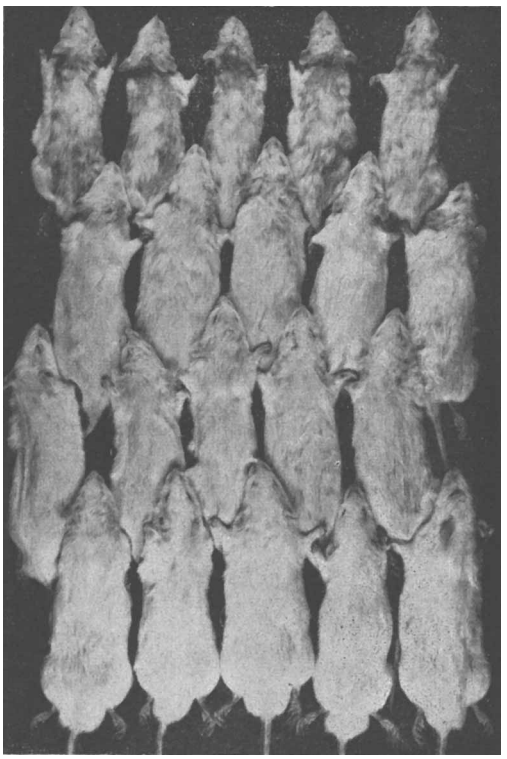

FIG. 2. Appearance of representative rats in the exp. From top to bottom, each group was treated as follows: Cortisone only; cortisone and vit. B_{12}; cortisone and aureomycin; cortisone, vit. B_{12} and aureomycin. Note the significant improvement in hair and body size of the rats given vit. B_{12} and/or aureomycin.

whose diets were supplemented with the vitamin and/or antibiotic, the thymus glands were more normal in size and appearance. Distinct cortical and medullary areas were present and the number of thymocytes was increased. The average thymus weight per 100 g of rat for each of the 5 groups was as follows: 1, 216.3 mg; 2, 52.0 mg; 3, 111.6 mg; 4, 115.6 mg; 5, 142.8 mg.

Eosinophil counts were reduced from an average of 330 per cu ml in the control rats to 28 in the rats given cortisone alone. Neither vit. B_{12} nor aureomycin significantly altered this action of cortisone on the eosinophils. The average weight of the adrenals of the control rats was 32.9 mg as compared to 18.8-23.4 mg in the rats given cortisone with or without vit. B_{12} and aureomycin.

Discussion. The results of this experiment, in which a purified casein-glucose diet was

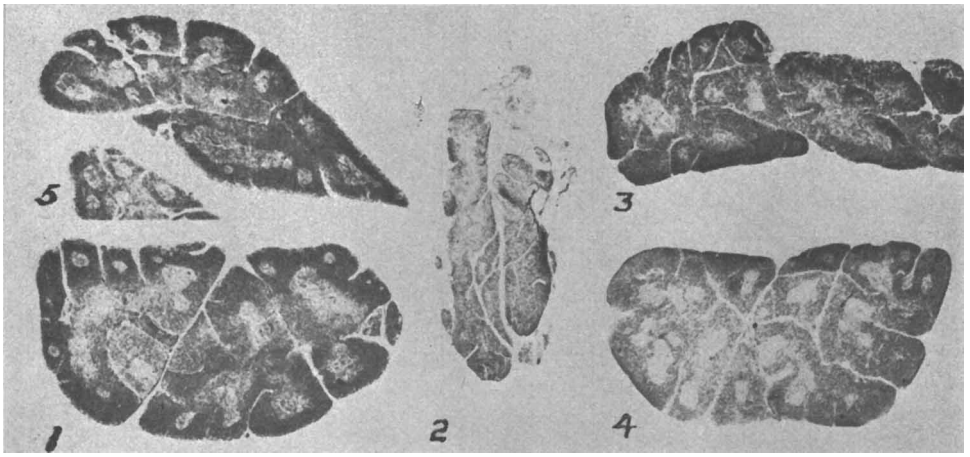

FIG. 3. Histological sections of representative thymus glands from each of the 5 groups. Treatment was as follows: 1, control; 2, cortisone only; 3, cortisone and vit. B_{12}; 4, cortisone and aureomycin; 5, cortisone, vit. B_{12} and aureomycin.

fed, are in general agreement with the data previously reported in rats fed a corn-soybean meal diet(1,2). In both experiments, vit. B_{12} and or aureomycin largely protected young male rats against the inhibitory effects of large doses of cortisone on body, hair and thymus growth. It is of interest that on the corn-soybean meal diet, the dose of cortisone used (0.5 mg daily) did not induce alopecia, although it decreased the rate of hair regrowth on the backs of rats from which it had initially been removed. Even 1 mg of cortisone daily did not induce alopecia in rats fed this diet, although it completely inhibited body growth (unpublished observations). On both diets, the combination of vit. B_{12} and aureomycin was more effective than either alone in overcoming cortisone "toxicity."

Ershoff's(3) inability to counteract to any degree the inhibitory effects of huge doses of cortisone with combinations of the known B vitamins, including vit. B_{12}, is at variance with the results reported here. However, the diets and strain of rats employed in the two laboratories were different. Previously, Ershoff(4) had reported that when his same casein-sucrose diet was used, thyrotoxicosis in young rats could be overcome by whole liver but not by Vit. B_{12}. Other workers, however, could at least partially counteract thyrotoxicosis with vit. B_{12} on casein-sucrose (5,6), casein-glucose(7), soybean meal(6,8,9) or corn-whole milk(10) diets. In commenting on the differences between their results and those of Ershoff(4), Lewis *et al.*(6) suggested that altering the carbohydrate in the diet may change the intestinal synthesis of other factors. Furthermore, the favorable results obtained with whole liver might possibly be due to its glycogen content, since activity was reported only when liver was fed at a 10% level. It would be of interest to determine whether liver has any advantage over vit. B_{12} and aureomycin in counteracting large doses of cortisone in rats fed the casein-glucose diet of the present experiment.¶¶

It is believed that the large doses of cortisone given the rats in this experiment increased their requirements for vit. B_{12} and other factors. A similar opinion has been expressed regarding the effects of marked changes in levels of other hormones on nutri-

Ershoff's own data indicate that supplementation with the known B vitamins enabled his female rats to at least partially overcome inhibition of body growth induced by feeding 100 mg of cortisone acetate per kg of diet.

¶¶ In a similar experiment just completed, 10% whole liver powder was found to be more effective than either vit. B_{12} or aureomycin in counteracting cortisone in rats. Best results were obtained by combining all 3 factors in the diet. The question as to whether liver contains a factor different from all known B vitamins or whether its carbohydrate, fat or proteins account for its effectiveness remains unanswered.

tional requirements(2). Wahlstrom and Johnson(11) had previously observed that large doses of cortisone injected into baby pigs on a vit. B_{12}-deficient diet increased the urinary excretion of vit. B_{12} and reduced body growth and survival rate. Since it is well established that cortisone can increase the loss of body protein, as indicated in this experiment by retardation of body, hair and thymus growth, the favorable effects of vit. B_{12} can perhaps be attributed to an essential role in protein(12,13) and nucleic acid(14, 15) synthesis. Aureomycin and other antibiotics have been shown to exert a sparing action on several B vitamins, including vit. B_{12}(16-18). It is of interest that increased food intake(19), testosterone(19,20) and growth hormone(21,22) have all been observed to protect the body against the catabolic actions of ACTH and cortisone in a manner similar to that reported here for vit. B_{12} and aureomycin. It seems possible that the factor which all of these have in common is their ability to enhance protein retention within the body.

Summary. 1. Fifty young male albino rats were uniformly divided into 5 equal groups and placed on a purified casein-glucose diet. After a preliminary period of 2 weeks, all except a control group were injected for 28 days with 1 mg of cortisone acetate daily with or without vit. B_{12} and/or aureomycin. 2. Cortisone acetate alone induced complete inhibition of body growth, severe alopecia and marked atrophy of the thymus gland. Vit. B_{12} (200 μg. kilo diet) or aureomycin (0.005%) largely counteracted these inhibitory effects of cortisone acetate, and the combination of the two was more effective than either alone(3). It was concluded that large doses of cortisone acetate given to young rats on a casein-glucose diet, increased their requirements for vit. B_{12} and other factors. These data corroborate results previously obtained in a similar experiment on rats fed a corn-soybean meal diet.

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