into the diet of a flock of laying hens resulted in progressively increasing resistance of the eggs to rickettsial growth, the resistance becoming almost complete at the end of 3 weeks. Similar dietary factors had no appreciable effect on the growth of Influenza A virus in eggs. These results, with other data submitted, indicate that fertile eggs obtained from commercial hatcheries may, under present conditions, be unsatisfactory for controlled microbiological studies.

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Counteraction of Cortisone Inhibition of Body, Hair and Thymus Growth by Vitamin B_{12} and Aureomycin.* (19184)

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Large doses of cortisone have been shown to inhibit both body(1,2) and hair growth (3,4) in rats. These effects can be at least partially attributed to the reduction in availability of protein to the organism(5,6). Large doses of cortisone have also been reported to reduce normal food intake in rats(7), which may further contribute to reduced body and hair growth. Vit. B_{12} partially or completely counteracted growth-inhibition in rats induced by administering large doses of thyroid-active substances(8-10), diethylstilbestrol (11), or 0.1% thiouracil(12). Several antibiotics have also been shown to be effective in prolonging survival time(13) and overcoming growth-inhibition(14) in hyperthyroid rats. In general, these studies are believed to indicate that marked changes in estrogen or thyroid levels within the body can increase the need for vit. B_{12} and perhaps other factors by the organism.

It was the purpose of the present study to determine whether vit. B_{12} and aureomycin could prevent large doses of cortisone from depressing body and hair growth in rats fed a ration deficient in vit. B_{12}. If large amounts of cortisone can aggravate an existent dietary deficiency, and thus account to some extent for inhibition of body and hair growth, then supplementing the diet with the missing nutrients should help to prevent these manifestations.

Methods. A total of 130 immature male albino rats of the Carworth strain were used in 3 separate experimental trials. All rats were divided into uniform groups by weight of 10 each, and were placed for 30 days on the following vit. B_{12}-deficient ration: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; soybean oil meal, 20%;‡ alfalfa leaf meal, 6%; brewers' yeast, 3%; and table salt, 1%. *Cortisone acetate* (*Cortone*)§ was injected subcutaneously, daily, in a 0.1 ml volume of saline containing 0.5 mg of the hormone. Crystalline vit. B_{12}§ was

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‡ Low fiber, solvent extracted, containing 50% protein. Manufactured by Archer-Daniels-Midland Co., Minneapolis, Minn.

§ Cortone and crystalline vit. B_{12} were furnished through the courtesy of Dr. D. F. Green, formerly of Merck and Co., Rahway, N. J.

TABLE I. Effects of Vitamin B_{12} and Aureomycin on Cortisone Action in Male Rats.

Group	Treatment	Avg body wt Orig., g	Avg body wt Final, g	Avg food intake Total, g	Avg food intake Per g gain body wt, g	Avg adrenal wt Actual, mg	Avg adrenal wt Per 100 g body wt, mg	Avg thymus wt Actual, mg	Avg thymus wt Per 100 g body wt, mg
1	Controls	54.5 (30)*	141.1 (28)*	293.1	3.4	22.3	16 ± .9†	277.8	198.5 ± 10.7†
2	200 μg B_{12}	57.8 (10)	158 (9)	299.7	2.9	25.7	16.3 ± 1	328.1	207.7 ± 13.6
3	.005% aureo.	58.2 (10)	171.8 (9)	315.3	2.7	28.5	15.6 ± .8	369.3	215 ± 10.7
4	200 μg B_{12} + .005% aureo.	57.9 (10)	178.3 (9)	351	2.9	27.9	15.6 ± .7	365.6	205 ± 9.6
5	.5 mg Cortone	54.8 (30)	121.3 (25)	260.4	3.9	13.2	10.2 ± .8	142.4	110.5 ± 8.3
6	.5 mg Cortone + 200 μg B_{12}	54.6 (30)	143.1 (27)	313.4	3.5	15	10.6 ± .4	223.1	160 ± 10.6
7	.5 mg Cortone + .005% aureo.	53.4 (20)	132.7 (19)	297.6	3.6	14.9	11.3 ± .8	195	144.8 ± 13.1
8	.5 mg Cortone + 200 μg B_{12} + .005% aureo.	53.4 (20)	151.8 (20)	325.4	3.3	17.3	11.6 ± .5	237	157.4 ± 11.8

* No. of rats per group. † Standard error of mean.

mixed into the ration in amounts of 200 μg per kilo, and aureomycin HCl¶ in amounts of 0.005%. Body weight and food consumption were measured on alternate days. At the end of the 30-day experimental period, the rats were killed and the adrenals, spleen, thymus and testes were removed and weighed. All rats were housed in an air-conditioned animal room at a temperature of 75 ± 1°F. The effects of cortisone and the dietary supplements on hair growth were determined in only 2 experiments. The hair was uniformly removed from the backs of the rats, from neck to tail, with a razor. In one experiment, the hair was not removed until the 18th day, leaving 12 days for regrowth of hair. In the other experiment, the hair was removed on the first day, leaving 30 days for regrowth of hair. At the end of the experiments, each group was laid side by side and each rat was rated individually for degree of hair regrowth. The ratings for each group were averaged. The following rating scale was used: 0, no regrowth; 1, slight regrowth; 2, moderate regrowth; 3, marked regrowth; 4, complete regrowth.

Results. The data from the 3 experiments were essentially similar and have been combined in Table I. It can be seen that when vit. B_{12} (Group 2) or aureomycin (Group 3) were fed individually or together (Group 4), they increased body weight gains above the controls (Group 1). The vitamin and antibiotic increased food intake more than is apparent here, since the controls of the individual experiment consumed only an average of 271 g of food per rat. Vit. B_{12} and aureomycin reduced the amount of food required for each g of gain in body weight. Adrenal and thymus weights were not affected by these dietary supplements.

Cortisone (Group 5) reduced body growth and food intake, and decreased the efficiency of transforming food into body weight gains. Adrenal and thymus weights were significantly reduced, the latter weighing only about half as much as in the controls. Vit. B_{12} (Group 6) completely and aureomycin (Group 7) partially counteracted the cortisone-induced inhibition of body growth and food intake, and both increased growth efficiency. The combination of the vitamin and antibiotic (Group 8) was more effective in all these respects than either substance alone. Although neither substance prevented the decrease in adrenal weight, they were both partially but significantly effective in counteracting the cortisone-induced decrease in thymus weight. This was found to be true in all 3 individual experi-

¶ Crystalline aureomycin HCl was kindly supplied by Dr. T. H. Jukes of Lederle Labs. Division, Pearl River, N. Y.

ments. The data pertaining to spleen and testes have not been presented in tabular form since neither cortisone (in the dose given) nor the dietary supplements significantly altered the weights of these organs.

The average rating for regrowth of hair in the rats from whom hair had been removed 12 days previously was as follows for each group: controls, 3.0; .5 mg cortisone, 1.1; .5 mg cortisone and 200 μg vit. B_{12}, 2.1; .5 mg cortisone and .005% aureomycin, 2.2; .5 mg cortisone, 200 μg B_{12} and .005% aureomycin, 2.7. The ratings for the rats from whom hair had been removed 30 days previously are given in the legend under Fig. 1. In general, the results of the two experiments are in agreement.

Discussion. In 3 similar experiments it was found that a relatively large dose of cortisone injected into immature rats on a ration deficient in vit. B_{12} aggravated the existent dietary imbalance. This was indicated by the partial retardation of body growth and appetite and in reduced ability to transform food into body weight gains. The incorporation of vit. B_{12} into the ration completely counteracted the cortisone-induced inhibition of body growth, although growth did not reach the level of the non-injected rats given vit. B_{12}. When vit. B_{12} and aureomycin were given together to the cortisone-injected rats, growth proceeded beyond the level of the non-injected control rats.

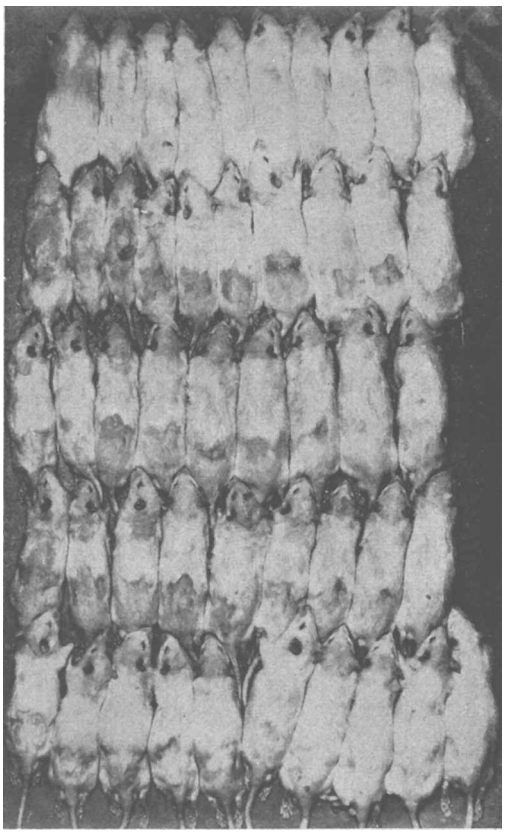

FIG. 1. From top to bottom, average rating for regrowth of hair for each group was as follows: controls, 4; .5 mg cortisone, 1.7; .5 mg cortisone and 200 μg B_{12}, 2.7; .5 mg cortisone and .005% aureomycin, 2.3; .5 mg cortisone, 200 μg B_{12} and .005% aureomycin, 3.2.

The ability of vit. B_{12} and aureomycin to partially counteract suppression of hair growth in these experiments was associated with their ability to simultaneously overcome inhibition of body growth. Hair and body growth were not suppressed to the same degree by cortisone, nor were these inhibitory effects counteracted to the same extent by vit. B_{12} and aureomycin. However, it was notable that on an individual as well as on a group basis, the heavier rats invariably showed more regrowth of hair than the lighter rats, and vice versa. The parallelism of these actions by cortisone and the two dietary supplements on hair and body growth suggests that similar mechanisms were in operation.

It is of considerable interest that vit. B_{12} and aureomycin partially protected the thymus against atrophy by cortisone. We have previously observed (unpublished data) that the decrease in spleen weighed induced by injecting 1 mg daily of cortisone into young rats can also be partially prevented by feeding extra quantities of vit. B_{12}. Pentz *et al.*(15) reported that liver and vit. B_{12} partially counteracted the loss in thymus weight of thyroid-fed rats having greatly hypertrophied adrenals. Similarly, several antibiotics were shown to partially or completely prevent loss of thymus weight in hyperthyroid rats(16). Inasmuch as the adrenal atrophy induced by cortisone in these experiments was not at all prevented by vit. B_{12} or aureomycin, it appears that the protective action on the thymus gland was a direct one. It would be interesting to determine whether vit. B_{12} or anti-

biotics would protect other lymphoid tissues against cortisone action.

Experiments now underway in this laboratory indicate that large doses of cortisone (1 mg daily) may induce deficiencies of vit. B_{12} even when a diet normally adequate in this factor is fed. Extra amounts of vit. B_{12} in an otherwise adequate diet have also enabled rats to partially overcome inhibition of growth and appetite induced by feeding large doses of diethylstilbestrol or thyroprotein(11). In general, these results are believed to indicate that marked changes in estrogen, thyroid or cortisone levels within the body may alter the nutritional requirements of the organism.

The mechanisms by which vit. B_{12} and aureomycin protected the rats against cortisone in these experiments remain to be elucidated. It is pertinent, however, that vit. B_{12} has been shown to favorably influence protein synthesis(17,18) and to increase nitrogen retention(19), while aureomycin may increase the availability of vit. B_{12}(20,21) and niacin(22) to the organism.

Summary. (1) Cortisone was injected in daily doses of .5 mg for 30 days into 130 immature male albino rats on a vitamin B_{12}-deficient diet. The depressions of body, hair, and thymus growth which resulted were completely or partially prevented by incorporating 200 μg of vit. B_{12} pr kilo of ration or .005% aureomycin. (2) Vit. B_{12} was more effective in these respects than aureomycin, and the combination of the two substances was more effective than either alone. (3) The favorable actions of the vitamin and antibiotic were accompanied by an increase in food consumption and greater efficiency in converting food into body weight gains. (4) It is concluded that when large doses of cortisone are injected into rats on a vit. B_{12}-deficient diet, the deficiency becomes aggravated and thus accounts at least in part for the observed inhibitions of body and hair growth. The mechanisms by which vit. B_{12} and aureomycin protect the thymus against cortisone action are as yet unknown.

Addendum. While this paper was in press, Wahlstrom and Johnson (PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 112) independently reported that injections of relatively large doses of cortisone into baby pigs fed a vit. B_{12}-deficient diet, aggravated the deficiency. Body growth and survival rate were reduced and excretion of vit. B_{12} into the urine was increased. This is believed to confirm the opinion that large doses of cortisone increase requirements for vit. B_{12} and possibly other factors.

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