aerobic microflora count it seems possible that the antibiotics may have stimulated growth by some other mechanism. Thus, it might be surmised that the antibiotic molecule or a fragment of same might act as a metabolite within the body of the bird.

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Beneficial Effects of Liver on Cortisone Acetate Toxicity in the Rat.* (19236)

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Considerable data are available indicating that, in addition to the known nutrients, substances are present in our diet which may be required in increased amounts during conditions of stress. Such factors are apparently dispensable under normal conditions, or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to such an extent that deficiencies occur, manifested by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient (1,2). Whole liver is a potent source of such unknown nutrients. Thus whole liver or fractions thereof has been shown to counteract the deleterious effects of massive doses of strychnine(3), sulfanilamide(4), promin(5,6), atabrine(7,8) and dinitrophenol(9). Similar results have been observed following the administration of toxic doses of diethyl-stilbestrol(4), alpha-estradiol(10), desiccated thyroid(11-14), and thyroxin, thyroglobulin and iodinated casein(15). In the present communication data are presented on the comparative effects of whole liver and the known B vitamins on cortisone acetate toxicity in the immature rat.

Procedure. Exp. 1. The basal ration employed in the present experiment consisted of sucrose, 61%, casein,† 24%; salt mixture,‡

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5%; and cottonseed oil (Wesson), 10%. To each kg of the above were added the following synthetic vitamins: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; ascorbic acid, 200 mg; 2-methyl-naphthoquinone, 10 mg; and choline chloride, 2 g. To each kg of diet were also added 4000 U.S.P. units of vitamin A§ and 400 U.S.P. units of vitamin D.‖ Each rat also received once weekly a supplement of 4.5 mg of alpha-tocopherol acetate. Thirty male and 30 female rats of the Long-Evans strain were selected at 23 to 25 days of age and an average weight of 46.8 g for the following experiment. Animals were placed in metal cages with raised screen bottoms to prevent access to feces and were fed the following diets *ad lib.* (6 males and 6 females per group): (a) basal ration alone; (b) basal ration plus 200 mg cortisone acetate¶ per kg of diet; (c) basal ration plus 400 mg cortisone acetate per kg of diet; (d) basal ration plus 10% whole liver powder** plus 200 mg cortisone acetate per kg of diet; and (e) basal ration plus 10% whole liver powder plus 400 mg cortisone acetate per kg of diet. The cortisone acetate and whole liver powder were incorporated in the basal ration in place of an equal amount of sucrose. Diets were made up weekly and stored under refrigeration when not in use. Animals were fed on alternate days. All food not consumed 48 hours after feeding was discarded. These measures were employed to minimize oxidative changes in the diet. Feeding was continued for 6 weeks or until death, whichever occurred sooner.

Exp. No. 2. The following 3 diets were employed in this investigation: (a) basal ration; (b) basal ration plus the following additional B vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; biotin, 5 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; and vitamin B_{12}, 150 μg; and (c) basal ration plus 10% whole liver powder. Cortisone acetate was incorporated in the above diets at a level of 100 mg per kg of ration. The whole liver powder and the various supplements were added in place of an equal amount of sucrose. Experiments were conducted with rats fed the above diets and with animals fed the basal ration with cortisone acetate omitted. Twenty-four male and 24 female rats of the Long-Evans strain were selected at 21 to 24 days of age and were fed the above diets *ad lib.* for a period of 6 weeks (6 rats of each sex per group). The experimental procedure was similar to that previously described.

Results. Exp. No. 1. Findings on growth and survival are summarized in Table I. Gain in body weight was significantly reduced in all rats fed cortisone acetate-containing diets. The retardation in growth was particularly marked for female rats and was proportional in general to the level of drug fed. The weight increment of cortisone acetate-fed rats was significantly greater for rats fed the liver-containing diet than for animals fed the basal ration. This occurred at both levels of drug employed and in both male and female rats. The growth-promoting effect of liver was relatively greater in the female than in the male. A beneficial effect of liver was similarly noted in respect to survival. During the 5th and 6th week of feeding a number of deaths occurred in cortisone acetate-fed rats on the basal ration. The mortality incidence was particularly marked at the higher dosage level and was greater in females than in males. No deaths occurred in any of the rats fed cortisone acetate in conjunction with the liver-containing ration.

Alopecia occurred in all cortisone acetate-fed rats on the basal ration, particularly in females. By the end of the first week of feeding, loss of hair was marked between the ears and on the dorsal surface of the neck

† Vitamin Test Casein, General Biochemicals, Chagrin Falls, O.

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ MYVA-DRY Powder, Distillation Products, Rochester, N. Y.

‖ HY-DEE Powder, Standard Brands, New York.

¶ The cortisone acetate was kindly provided by Dr. R. A. Peterman of Merck and Co., Rahway, N. J.

** Desiccated Liver, Armour & Co., Chicago, Ill.

TABLE I. Beneficial Effects of Liver on Growth and Survival of Immature Rats Fed Toxic Doses of Cortisone Acetate (6 Animals per Group).

Dietary group	Initial body wt, g	Gain in body wt after following days of feeding, g 14th	28th	42nd	% surviving
Exp. 1	Males				
BR*	46.3	56.6 (6)	109.7 (6)	160.7 (6)	100
BR + 200 mg CA† per kg of diet	50.3	4 (6)	33 (6)	44.1 (6)	100
BR + 200 mg CA per kg diet + 10% whole liver	47.7	13.7 (6)	48.4 (6)	81.6 (6)	100
BR + 400 mg CA per kg of diet	51.2	—3.1 (6)	3.7 (6)	8 (2)	33.3
BR + 400 mg CA per kg of diet + 10% whole liver	48.3	1.7 (6)	22.8 (6)	43.5 (6)	100
Exp. 2					
BR	41.6	54.8 (6)	115.7 (6)	152.3 (6)	100
BR + 100 mg CA per kg of diet	42.4	30.8 (6)	70.3 (6)	108.3 (6)	100
BR + 100 mg CA per kg of diet + B vit.	42.3	31.8 (6)	72.8 (6)	108.8 (6)	100
BR + 100 mg CA per kg of diet + 10% whole liver	41.8	55.3 (6)	114.8 (6)	161.8 (6)	100
Exp. 1	Females				
BR	43.4	46.5 (6)	83.7 (6)	115.7 (6)	100
BR + 200 mg CA per kg of diet	45.4	—1.8 (6)	7.1 (6)	4 (4)	66.7
BR + 200 mg CA per kg of diet + 10% whole liver	44.5	6.3 (6)	32.4 (6)	55.5 (6)	100
BR + 400 mg CA per kg of diet	46.3	—6.1 (6)	—7 (6)	—14 (1)	16.7
BR + 400 mg CA per kg of diet + 10% whole liver	44.8	.2 (6)	13.2 (6)	29.5 (6)	100
Exp. 2					
BR	40.5	42 (6)	70.5 (6)	96 (6)	100
BR + 100 mg CA per kg of diet	41.7	11 (6)	43.5 (6)	64.5 (6)	100
BR + 100 mg CA per kg of diet + B vit.	41.2	14.5 (6)	47.5 (6)	80 (6)	100
BR + 100 mg CA per kg of diet + 10% whole liver	41	33 (6)	71 (6)	98 (6)	100

The values in parentheses indicate the No. of animals which survived and on which averages are based.

* BR = Basal ration. † CA = Cortisone acetate.

of all the above rats. After 28 days of feeding 4 out of 6 female rats and 1 out of 6 male rats fed cortisone acetate at a level of 400 mg per kg of diet were completely denuded with the exception of several strands of fur along the paws (Fig. 1). Marked alopecia although less extensive than the above also occurred in rats fed this drug at a level of 200 mg per kg of diet and in the remaining animals fed this drug at the higher level. Alopecia was reduced both in incidence and degree in all cortisone acetate-fed rats on the liver-containing ration, particularly in males. Priapism was noted after the 4th week of feeding in a number of cortisone acetate-fed rats both on the basal and liver-containing ration.

During the 6th week of feeding total and differential white cell counts, hemoglobin determinations, total red cell counts and direct eosinophil counts were made on the tail blood of all surviving rats. Differential counts were made on smears stained with Wright's stain, 100 cells on each of 2 slides being employed for each analysis. No significant difference in erythrocyte count or hemoglobin level was observed between the various groups, erythrocytes averaging 7.5 to 8.6 million per cc and hemoglobin 17.1 to 18.4 per 100 cc. A significant reduction in eosinophiles and lymphocytes and an increase in polymorphonuclear leukocytes occurred in all cortisone acetate-fed rats both on the basal and liver-containing ration. Eosinophiles dropped from an average of 350 cells per cc for rats fed the basal ration to less than 50 cells per cc for animals fed cortisone acetate-containing diets; while lymphocytes fell from an average of 7800 per cc to less than 5000 per cc (average range 2100 to 5000 per cc). The reduction in lymphocyte count was greater in females than in males and was proportional in gen-

FIG. 1. Litter mate male rats after 40 days of cortisone acetate administration. The rat in the foreground was fed a purified ration containing 400 mg cortisone acetate per kg of diet. The rat in the background was fed a similar diet supplemented with 10% whole liver powder.

eral to the level of drug fed. Polymorphonuclear cells increased from an average of 2700 per cc for rats on the basal ration to averages exceeding 5200 per cc for animals fed cortisone acetate. Changes in total WBC as a result of cortisone acetate administration were not statistically significant. No significant differences in respect to the above were observed between animals fed the basal ration and those fed a similar diet supplemented with whole liver powder.

Exp. No. 2. Findings are summarized in Table I. Cortisone acetate at a level of 100 mg per kg of diet significantly reduced the gain in body weight of immature male and female rats fed a purified basal ration. Supplements of the known B vitamins were without significant effect. Desiccated whole liver at a level of 10% of the diet completely counteracted the above retardation in growth. Neither alopecia nor priapism occurred in any of the rats fed this level of cortisone acetate.

The mean arterial pressure was determined by the direct method in 3 male and 3 female rats in each group on the 43rd day of feeding.†† The rats were anesthetized with ether and the abdominal aorta was exposed and punctured with a 24-gauge needle connected to a mercury manometer of 1 mm bore tubing. The puncture was made during light anesthesia and with the mercury column in the manometer raised to a level of 90-100 mm Hg. No significant difference in mean arterial pressure was observed between the various groups (values averaged between 72 and 76 mm Hg for the different groups with an individual range between 68 and 81 mm Hg).

Discussion. The toxic effects of large doses of cortisone acetate were largely counteracted in the rat by the administration of desiccated whole liver. Liver, however, did not counteract the effect of the drug on the leukocyte count. The beneficial effect of liver was apparently not due to its content of known B vitamins. This is indicated by the fact that supplements of all the known B vitamins were without significant effect on the gain in body weight of immature rats fed 100 mg per kg of diet of cortisone acetate. Available data do not indicate, however, whether the protective effect of liver was due to an unidentified factor(s) or to its content of other known nutrients. Since liver also contains a factor (distinct from any of the known B vitamins) which counteracted the toxic effects of thyroid(11-14), promin(5,6) and atabrine(7) administration in the rat, further work is indicated to determine what relationship if any exists between the "antitoxic factor of liver"(2) and the factor or factors indicated above.

Summary. The administration of toxic

†† We are indebted to Dr. Sheldon Rosenfeld, Dept. of Physiology, University of Southern California, for the blood pressure determinations.

doses of cortisone acetate to immature rats maintained on a synthetic diet resulted in retarded growth, alopecia and death. These effects were largely counteracted by the administration of desiccated whole liver when fed at a level of 10% of the diet. The protective factor(s) in liver was distinct from any of the known B vitamins.

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Further Studies on Anti-Thyroxine Effects of Various Compounds.* (19237)

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The extension by Woolley(1) of the specific metabolic inhibitor principle into thyroxine analogues initiated an interest which has been taken up by other laboratories, reports of whose work have appeared during the past few years. Frieden and Winzler(2) expanded the variety of substituted ethers of 3,5-diiodo-4-hydroxybenzoic acid, demonstrating special activity in 4-benzyloxy-3,5-diiodobenzoic acid and 4-benzyloxy-3,5-diiodophenylalanine in preventing the thyroxine-induced acceleration of tadpole metamorphosis. Maclagan, Sheahan, and Wilkinson(3) noted interference with increased metabolic rates caused by thyroxine in mice when 4-benzyloxy-3,5-diiodobenzoic acid was injected, as well as the dimethyl acetal of 3,5-diiodoanisaldehyde. As was also pointed out by Barker *et al.*(4), the amounts of inhibitors required for mammalian experiments were greater than for amphibia. Cortell(5) was able to cause a considerable reversal of the thyroxine depression of thiouracil-induced hypertrophy of rat thyroid glands by the use of 2',6'-diiodothyronine. Barker and coworkers(4) presented the results of 3 different approaches to quantitative evaluation of thyroxine inhibition, two involving measurement of changes in metabolism and the third employing the depression of response to standard estrogen dosages produced by thyroxine. It was concluded that the thyroidectomized rat maintained at an approximately normal metabolic level by daily injections of 12 μg of DL-thyroxine† offered a highly sensitive preparation for evaluation of antithyroxine effects. This technic was used by Klitgaard and coworkers(6) in their study of iodinated phenoxyacetic acids, several of which were found to antagonize the energy metabolism control of the thyroid hormone, with greatest activity when iodine was in the 2, 4, and 6 positions in the phenyl ring.

The present investigation was concerned with a varied group of substances which either have been reported to show thyroxine inhibition or might be expected to do so.

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† DL-Thyroxine was made available by Dr. K. W. Thompson, Organon, Inc., Orange, N. J.