Effect of Cortisone Acetate on Aspartic-Glutamic Transaminase Activity of Mouse Tissues.* (20874)

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The aspartic-glutamic transaminase activity of the liver, kidney and muscle of the rat is reported(1) to be unchanged after adrenalectomy and replacement doses of cortisone. On the other hand, arginase activity of the liver and kidney is reduced after adrenalectomy(2-4) and restored to and above normal by cortisone(2,3). The stimulatory effect of cortisone on the arginase activity of the liver and kidney is also demonstrable in normal mice but only after about 2 days of treatment (5). Since arginase and transaminase are closely related in their role in the intermediary metabolism of protein, it seemed important to reinvestigate the transaminase activity of the liver and kidney under conditions in which cortisone produced a definite increase in arginase activity(5). Furthermore, since cortisone seems to influence the metabolism of the heart and this tissue contains the highest activity of transaminase(6) it also was studied.

Procedure. Mice of the dba-1, 20C57B1 line 6, and BBF strain (Jackson Laboratory)† were maintained at not more than 5 in a glass jar containing wood shavings. They were fed *ad libitum* Purina laboratory chow checkers or a prepared diet composed of casein 16.7, sucrose 61.2, hydrogenated vegetable oil (Primex) 7.4, dry Brewers' Yeast (Fleischmann's No. 2019) 9.2, Celluflour 1.8, Wesson's salt mixture 3.7 and, 3 times/week a supplement of 2 drops of cod liver oil and 1 drop of 40% tocopherol concentrate of vegetable oils diluted 10 fold with Wesson oil. No difference in results between strains of mice or due to the use of the two diets was detected. *Hormone.* The cortisone acetate‡ was prepared in cylindrical pellets weighing approximately 15 mg each and implanted subcutaneously. The average amount absorbed decreased as the duration of the experiments was increased, (Table I)(5). *Autopsy. Preparation of tissue.* Food, but not water, was removed from the mice 19 to 24 hours before autopsy. The mice were killed by spreading the vertebrae at the neck to sever the spinal cord and were immediately bled to death by cutting the blood vessels of the neck. The entire liver, kidneys, and heart were removed for the enzyme determination, except in cases in which a small section of the heart was used for histological preparations. The organs were weighed on Roller-Smith torsion balances, and immediately placed in ice-cold water, homogenized in an all-glass apparatus and diluted to volume. *Enzyme determination.* The activity of the aspartic-glutamic transaminase was determined by the procedure of Tonhazy *et al.*(7) but the values are expressed on the basis of fresh tissue weight.

Results. Body and organ weights. The body, spleen, thymus and liver weights decreased and the kidney weight increased (Table I) as previously reported(5). The heart also increased in weight. The younger mice, however, did not show these changes in heart and kidney weight (Table I).

Transaminase activity. The activity of the transaminase decreased in the liver both on a total and a u/g basis. (Table II). The decrease was evident only after 14 days when it was maximal. On the other hand, the activity of this enzyme in the heart and kidney increased. The above changes did not appear until between 2 and 7 days of treatment. Similar results were obtained in another series of experiments of 4 and 10 days duration§

* Investigation supported by a grant-in-aid from the American Heart Assn.

†Mice made available from funds provided by the Cooper Foundation to the Roscoe B. Jackson Memorial Laboratory.

‡ Cortisone acetate was provided by Merck and Co. through the courtesy of Dr. Elmer Alpert.

§ Adenosinetriphosphatase activity was also determined. Total units in liver decreased 25 and 41%, in kidney 14 and 13% and in heart no change for 4 and 10 day experiments respectively.

TABLE I. Effect of Cortisone Acetate on Body and Organ Weights of Mice.

Cortisone Treatment, days	Cortisone Absorbed, mg/day	No. mice	Body wt, g Initial	Body wt, g Change	Spleen, mg	Thymus, mg	Liver, mg	Kidney, mg	Heart, mg
Control		24*	25.1	+2.5*	133 ± 9.3	40 ± .1	1230 ± 48	249 ± 6.6	99 ± 3.4
2	1.0	6	24.8		44 ± 2.5	9 ± .1	1230 ± 79	231 ± 17.6	93 ± 6.7
7	.5	6	23.8	—1.4	61 ± 12.6	5 ± 1.4	1110 ± 78	284 ± 12.0	116 ± 4.3
14	.4	6	23.8	—2.3	62 ± 5.7	0	1090 ± 47	295 ± 14.3	112 ± 3.5
21	.35	6	24.3	— .2	50 ± 3.8	0	1090 ± 43	338 ± 18.7	118 ± 10.0
Control		10†	15.3	+1.5‡			810 ± 25	208 ± 8.	81 ± 2.9
4	.5	10†	15.2	+ .1			720 ± 16	199 ± 9.	83 ± 1.9
10	.3	5†	14.5	—1.8			520 ± 40	217 ± 15.	81 ± 4.3

* Values of the 6 controls of each period averaged and presented with the stand. error of the mean, $\frac{\sqrt{\frac{\overline{X^2}}{N} - \overline{X}^2}}{\sqrt{N-1}}$. Body wt change is for 21 day group.

† 5, 8, and 2 females in respective groups.

‡ Represents change in 10 days.

(Table II). The higher control values obtained in this series are very likely due to the younger age of these animals. Both male and female mice of the dba-1 strain were used in the latter study but no difference due to sex was detected.

Discussion. It is of interest that the decrease in transaminase activity of the liver occurred under circumstances which had produced an increase in arginase activity(5). In both instances, the change in enzyme activities did not appear until after the initial protein catabolic phase had disappeared.

The change in the activity of the transaminase of the liver apparently is related to the excess cortisone administration. Neither adrenalectomy nor replacement therapy with cortisone affect the activity of this enzyme in the rat(1). If protein depletion is related with the changes in the activities of these two enzymes, it is peculiar to the action of cortisone. Loss of protein by dietary means does not result in changes in the activities of these enzymes(8).

In contrast to the liver, the kidney responds to an overdosage of cortisone by an increase in both the transaminase and arginase activities with a concomitant small increase in weight. Furthermore, the heart responds in a manner similar to the kidney.

Summary. Cortisone acetate produces in the mouse after 7 days a decrease in the aspartic-glutamic transaminase activity of the liver but an increase in that of the kidney and heart.

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TABLE II.*
Effect of Cortisone Acetate on Aspartic-Glutamic Transaminase Activity of Mouse Tissues.

Treatment, days	Liver Total u	Liver u/g	Kidney Total u	Kidney u/g	Heart Total u	Heart u/g
Control	118 ± 3.2	97 ± 3.0	15.0 ± 2.7	58 ± 1.9	13 ± 1.5	132 ± 4.7
2	116 ± 4.7	95 ± 4.6	13.0 ± 1.2	54 ± 1.5	11 ± 1.0	116 ± 3.9
7	115 ± 4.6	104 ± 12.8	23.3 ± 1.8	81 ± 3.3	18 ± .5	153 ± 5.6
14	79 ± 1.4	74 ± 3.3	18.0 ± .1	61 ± 2.4	17 ± 1.5	147 ± 9.9
21	84 ± 5.6	80 ± 5.2	23.0 ± 2.7	73 ± 4.1	22 ± 1.3	193 ± 10.4
Control	163 ± 5.4	202 ± 8.3	27 ± .8	128 ± 2.5	20 ± 1.0	246 ± 5.9
4	138 ± 5.4	192 ± 5.8	32 ± .9	159 ± 3.6	24 ± .7	288 ± 5.9
10	81 ± 6.8	157 ± 8.3	30 ± 4.0	144 ± 4.5	25 ± 1.7	308 ± 6.0

* See Table I and text for details.

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Received January 4, 1954. P.S.E.B.M., 1954, v85.

Prevention by Vitamin B_{12} of Protein Catabolic Action of Cortisone.* (20875)

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Large doses of vit. B_{12} partially counteract certain catabolic effects of cortisone in young rats fed diets deficient in this vitamin(1-3). Reductions in body, hair and thymus growth caused by cortisone were partially overcome by feeding 10 times the normal requirement for this vitamin. The beneficial effects were invariably accompanied by increases in food intake and by greater efficiency in converting food into body weight gains. It was suggested that (a) large doses of cortisone may increase requirements for vit. B_{12} and (b) administration of the vit. may enhance the availability of protein. The first was partially corroborated by Wahlstrom and Johnson(4), who reported that large doses of cortisone injected into baby pigs increased urinary excretion of vit. B_{12}. The results of the present experiments demonstrate that when young rats are fed *ad libitum*, vit. B_{12} can prevent an increase in urinary nitrogen losses produced by injecting large doses of cortisone.

Methods. Two similar experiments were performed in 90 rats, but since the results were essentially the same in both, only one will be reported. Fifty young male Carworth rats were divided into 5 uniform groups of 10 each, and fed the vit. B_{12}-deficient, corn-soybean diet previously described(1) for 20 days. After this depletion period, each group was treated as follows for 30 days: 1) no vit. B_{12}; 2) 200 μg vit. B_{12}‡/kilo of diet; 3) cortisone‡; 4) cortisone and 200 μg vit. B_{12}/kilo of diet; 5) same as 4 but pair-fed to group 3. Groups 3, 4 and 5 received one mg of cortisone acetate daily by subcutaneous injection for the first 10 days, 2 mg daily for the second 10 days, and 4 mg daily for the third 10 days. During the initial depletion period, three 24-hour urine specimens were collected at approximately weekly intervals and analyzed for total nitrogen by the micro-Kjeldahl procedure(5). After this, urinary nitrogen was determined on all rats of each group on the 5th and 10th days of each 10-day treatment with a particular dose of cortisone. Measurements of body weight and food consumption were made every 2 days. The rats were housed in metal cages with raised screen bottoms in an air-conditioned room at a temperature of 78 $\pm$ 1°F.

Results. The 50 rats averaged 58.3 g each in body weight at the beginning and 94.9 g each at the end of the 20-day depletion period. Body growth trends after this period are shown in Fig. 1. Rats which were continued on the vit. B_{12}-deficient diet (group 1) reached a final average body weight of 140.0 g as compared to 192.4 g each for the rats fed vit. B_{12} (group II). Growth of rats given cortisone but no vit. B_{12} (group III) was completely suppressed by one and 2 mg of hormone daily, and loss of body weight re-

* Published with approval of Director of Michigan Agric. Exp. Station as Journal Article No. 1588.

† Part of these data were presented by the senior author before the Conference on Food and Nutrition of the Gordon Research Conferences, Aug. 10-14, 1953.

‡ Crystalline vit. B_{12} and cortisone acetate (Cortone) were furnished through the kindness of Dr. L. Michaud of Merck and Co., Rahway, N. J.