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Influence of the Drug DDD on Adrenal Cortical Function in Adult Rats.*† (20265)

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A recent report by Nichols and Gardner(1) described the effect of the drug DDD (2, 2-bis (parachlorophenyl) 1, 1-dichloroethane) on the adrenal cortex of the dog. These investigators found that the oral administration of the drug for periods up to 8 weeks resulted in a sensitivity to insulin indicating that the adrenal cortex might be involved. This supposition was confirmed by the marked atrophy of the adrenal cortex found on histological examination. In a later report(2) Nichols and Sheehan reported that DDD would alleviate the diabetes produced by alloxan, again implicating the adrenal cortex. However, earlier workers in the field do not agree. Haag *et al.*(3) reported no adrenal damage when DDD was fed at a level of 1200 PPM to rats and Lillie *et al.*(4) found that rabbits did not suffer adrenal atrophy when fed at a level of 0.1% DDD in the diet. In view of the confusion in the literature and the relatively small amount of data available it was decided to test the effect of DDD in rats, determining the effect of the drug on eosinophil count, the level of Na and K in the blood and urine, the insulin sensitivity, and the response to stress.

Materials and methods. Male white rats of the Sprague-Dawley strain averaging about 100 g in weight were used in all experiments. Each animal was tested before use by the blood sugar response to administration of 0.1 unit of insulin per 100 g body weight. The fasting blood sugar was also determined and all animals with abnormally high fasting blood sugar levels or with abnormal insulin sensitivity were eliminated from the experiment. Every animal was also tested for eosinophil response to ACTH before testing for insulin sensitivity. Each animal was given 1 unit of ACTH intraperitoneally and eosinophil counts were made 4 hours following the injection. All rats which did not show a drop in eosinophil count of at least 80% were arbitrarily discarded. The animals were then divided into a normal group and a group receiving 0.1% DDD in the diet. The amount of diet consumed by each animal was determined. Both normal and experimental groups consumed the same amount of diet. At intervals of 1 week, the controls and the group fed DDD were tested for insulin sensitivity by the intraperitoneal administration of 0.1 unit insulin/100 g body weight. The blood sugar was determined on tail vein samples by the Somogyi method at ½, 1, 2, 3 and 4 hours after injection. When the animals developed insulin sensitivity, they were placed in metabolism cages and urine collections were made. After a 3-day collection the animals were starved for 3 days and collections were

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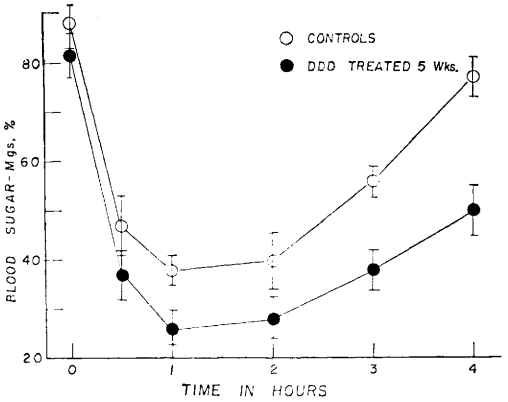

FIG. 1. Influence of DDD on insulin sensitivity curves of adult rats. All animals given 0.1 unit regular insulin/100 g B.W. Bars represent ± 1 S.D. 8 animals used in each group.

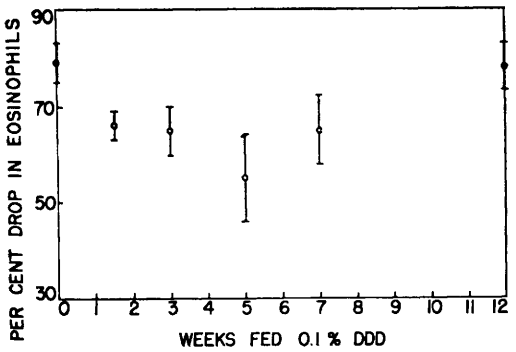

FIG. 2. Relationship between length of time that rats received a DDD diet and decrease in circulating eosinophils when rats were given 1 unit of ACTH intraperitoneally. All counts made 4 hr after inj. Barred lines indicate ± 1 S.D.

again made on the last 2 of the 3 days. The animals were then fed for 3 days and on the fourth day an eosinophil count was made as described above. The phloxine method was used with all due precautions(5). After the count, the animals were etherized, bled by heart puncture and autopsied. Organ weights were determined, histological specimens were taken and the urine and plasma were analyzed for Na and K with the Perkin-Elmer flame photometer. Animals selected for the stress experiments were placed on a diet containing 0.1% DDD for a period of 5 to 6 weeks. At the end of this time the controls and the animals fed DDD were shaved, placed in separate cages in a cold room at 3°C and the survival time of each group was determined. Other groups of controls and DDD treated rats were given 1 unit of ACTH per day for 2 days prior to submission to the cold test.

Results and discussions. The data presented in Fig. 1 demonstrate that the animals treated for 5 weeks with 0.1% DDD in the diet develop moderate insulin sensitivity. Animals treated for shorter lengths of time do not develop as marked a sensitivity. The barred lines indicate the range of variation in blood sugar levels and demonstrate significant differences ($P < 0.05$) between the controls and the animals treated with DDD. It is interesting to note that several of the DDD treated group developed convulsions and that one animal died when given a dose of insulin which produced no visible signs in the control group. This insulin sensitivity is characteristic of animals and humans with impaired adrenal cortical function. This indication that the compound might be affecting the "sugar" hormones of the adrenal cortex (*e.g.* cortisone-like substances) was further tested by injecting both the control and DDD treated animals with small doses of ACTH and measuring the per cent fall in eosinophils after a 4-hour interval. The data presented in Fig. 2 reveal that the control animals exhibited the characteristic response to ACTH in that the eosinophils fell an average of about 82% in the 4-hour interval. The eosinophil drop in the animals treated with DDD for 5 weeks was about 54% in the same time interval and with the same dose of ACTH. The difference is significant ($P < 0.05$). This observation indicates that DDD does prevent the elaboration of cortisone-like substances to some degree but that the inhibition is not complete.

Fig. 2 also shows the variation in eosinophil response with time on the diet. The eosinophil response becomes minimum at the same

TABLE I. Effect of Feeding DDD for 5 Weeks on Survival Times of Adult Rats Exposed to 3°C until Death.

Treatment	No. of animals	Survival time in hr
Control	12	10.3 ± .7*
DDD 5 wk	13	6.1 ± .8
" + ACTH	10	5.6 ± 1.2
Normal + ACTH	8	12.3 ± 1.4

* Stand. dev.

TABLE II. Influence of 0.1% DDD on Na and K Excretion, Uric Acid/Creatinine Ratio and Adrenal Weight of the Adult Rat.

Wk on DDD	No. of animals	Uric acid/ creatinine ratio	Urine Vol Ml/day	Urine Na Meq/liter/day	Urine K Meq/liter/day	Plasma Na Meq/liter	Plasma K Meq/liter	Adrenal wt, mg/100 g body wt
Control	18	.78 ± .10*	16	22.4 ± 2.1*	38.5 ± 4.7*	144 ± 2	5.0 ± 1	15.4 ± 1.1*
1½	8	.78 ± .08	17	24 ± 4.6	36.4 ± 5.3	146 ± 1	6.0 ± 1	21.3 ± 1.2
3	8	.48 ± .08	15	28.6 ± 3.7	52.8 ± 8.6	144 ± 3	5.0 ± 1	20.1 ± 1.4
5	12	.45 ± .09	18	19.9 ± 2.6	40.6 ± 7.1	144 ± 2	5.0 ± 1	19.2 ± 1.7
12	8	.56 ± .12	18	17.5 ± 5.2	44.8 ± 7.3	145 ± 2	5.0 ± 1	16.7 ± 1.2

* Stand. dev.

time that the insulin sensitivity is maximum. This would be anticipated on the assumption that DDD is interfering with the production and/or release of certain adrenal steroids. The decrease in eosinophil response declines to a minimum at the fifth week and thereafter the response returns toward normal. The barred lines in the figure indicate the range of the data. It becomes obvious that the difference between the controls and the animals fed DDD for 5 weeks is significant ($P < 0.05$) while the comparison between the controls and the animals fed DDD for longer or shorter periods of time is not so significant.

The data in Table I indicate that those animals which have received DDD for 5 weeks have a greatly decreased response to stress as measured by the cold test. Normal control animals can survive extreme cold for a period of about 10 hours. The animals treated with DDD on the other hand can survive the same degree of cold for only 6.5 hours. This difference is statistically significant ($P < 0.05$). Any single test would not be considered of great importance, but when animals fed DDD for 5 weeks have a decreased response to cold, a decreased eosinophil response and an increased insulin sensitivity and when all of these responses are assumed to be under the control of the "gluco-steroids" the evidence becomes more conclusive. The adrenal gland is no longer able to produce the material which enables the organism to respond normally to the above tests. The site of destruction or lack of production must be at the adrenal level because the administration of ACTH does not restore the animal to normal in the stress test (Table I).

Further evidence of the impairment of adrenal cortical function was found in the uric acid/creatinine ratio (Table II). Selye (6) has stated that an increase in the production of steroids (*e.g.* under stress) will produce a rise in uric acid excretion and an increased ratio. Conversely, a decrease in the normal level of steroid production should produce a decrease in this ratio. Such a decrease is found in animals treated for 5 weeks with DDD.

In order to test the ability of the animals treated with DDD to conserve Na and excrete K, the rats fed DDD for 5 weeks were starved during urine collection periods. The data in Table II demonstrate that both groups were essentially equal in their ability to excrete K and conserve Na. The plasma Na and K were within normal limits in both the control and DDD treated groups.

Nichols and Gardner observed that the adrenals of dogs fed DDD atrophy in the inner zones of the cortex with little or no change in the zona glomerulosa. The data obtained in the rat in the present experiments do not confirm this observation. The data in Table II on the weight of the adrenal glands in rats fed DDD show clearly that the adrenal increases in size from the normal to a maximum size after 1½ weeks on the DDD diet, after which time the adrenals decrease in size. This is in direct contradiction to the data obtained in the dog but confirms the report of Haag *et al.*(3) that DDD fed to rats produced no adrenal change. Haag did not investigate the physiological changes in his animals and determined the adrenal weight only after the animals had been on the diet for several weeks. Histological sections of the adrenals in our experiments show no significant change in cell structure, despite the alteration in size and function and they indicate that there is no

change in the size of the glomerulosa.

Summary. Adult rats fed the drug DDD rapidly develop signs of some adrenal dysfunction; decreased eosinophil response, decreased uric acid/creatinine ratio, increased insulin sensitivity and decreased response to stress of cold. The urinary and plasma Na and K are not affected.

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Localization of Glycolytic and Respiratory Enzyme Systems on Isolated Mouse Brain Mitochondria.* (20266)

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The Krebs cycle has been demonstrated using cyclophorase preparations, *i.e.*, suspensions of washed homogenate pellets(1). On the other hand, the investigators who have worked with isolated mitochondria prior to this time have demonstrated many of the Krebs cycle reactions, but never the malonate-sensitive oxidation of pyruvate denoting the complete cycle(2-9). These findings are in accordance with the fact that isolated mitochondria have not previously been reported to show the reversible staining with Janus green B which is characteristic of mitochondria *in vivo*(10). The absence of this special staining reaction indicates that the functional intactness of these mitochondria was apparently of too low a degree for them to carry the complete Krebs cycle. Glucolysis has been studied in extracts of various tissues(11-15). This process has, however, never before been demonstrated for isolated cell constituents used singly or in combination. Glycolysis, with hexose-diphosphate in the presence or absence of glucose as substrate, has been reported to occur in the supernatant(16-18); supernatant plus submicroscopic particles(6); or in the supernatant, in the submicroscopic particles, and in the mitochondria(19). No report on the Janus green B staining reactions of these preparations was given. The present paper briefly presents data which demonstrate that both these enzymic sequences occur in balanced quantities on mouse brain mitochondria isolated and washed in 0.25 M sucrose.

Materials and methods. Webster strain mice were killed by cervical dislocation and the entire brain immediately removed. The tissue was collected in ice-cooled 0.25 M sucrose solution at pH 7.4-7.7. Razor-cut slices were made from brains in a small amount of fresh sucrose medium in a petri dish set in ice. Slices were drained of solution, weighed and placed directly in manometric vessels. Brain tissue used to prepare mitochondria and supernatant was homogenized in a pyrex glass test tube fitted with a plastic plunger, and suspended in 9 volumes of fresh sucrose medium. It was then subjected to one 45-60 second clearance run at up to 7000 g, in a refrigerated centrifuge, to remove nuclei, whole cells and cell fragments.

* These data are from a thesis to be presented to the Faculty of the Graduate School of Georgetown University by M. L. Hesselbach in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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