Arginase Activity in Vitamin E-Deficient Rats.* (20264)

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It is well known that vit. E is necessary for normal gonadal function in certain species, and that in the rat, deprivation of the vitamin produces structural changes in the testes, with characteristic degeneration in the germinal epithelium(1). A similar need for arginine is not as well established, but is indicated by the observation that deficiency of this amino acid results in testicular degeneration resembling that of vit. E-deficiency(2). Moreover, in a patient with myotonia atrophica, associated with testicular atrophy, an abnormally low urinary excretion of 17-ketosteroids was increased following ingestion of arginine(3). Whether the functions of vit. E and arginine are related to each other is not known, but such relationship is at least suggested by the fact that the deleterious effects of vit. E-deficiency on the testes are not seen in the mouse which is able to synthesize arginine more readily than the rat(4). Therefore, it appeared of interest to determine whether the structural changes in the testes of vit. E-deficient rats could be delayed or prevented by the administration of arginine. In addition, since the activity of certain enzymes, for example, transaminase(5) may be altered in vit. E-deprivation, it seemed further of interest to ascertain whether arginase activity also is affected in the tissues of the deficient rat. Definitive data have not heretofore been reported. Williams and Watson(6) studied the arginine content of the tissues and the arginase activity of the liver of partially vit. E-deficient rats, but their results permit no conclusions regarding the possible effects of complete or nearly complete deprivation of the vitamin. More recently, Cruz-Coke and his coworkers(7) found tocopherylphosphate to inhibit the arginase of liver homogenates and of partially purified arginase preparations, but as has been the case with many experiments in which tocopherylphosphate was employed *in vitro* the changes observed probably were due to the phosphate grouping rather than to the tocopherol itself. In another series of investigations(8,9), the same workers observed that tocopherylphosphate given orally to the frog or rat produced no change in the arginase activity of the liver; similarly, administration of arginine was without effect in the rat. The animals employed by these investigators were not maintained on vit. E-deficient diets, and in consequence, the observations are of doubtful pertinence to the problem that served as the basis of the investigations reported on here.

Methods and material. Thirty male rats were segregated into 3 groups at 21 days of age and maintained on diet No. 30 of Mason and Bryan(10). One group was given daily supplements of synthetic α-tocopherol calculated to be sufficient for normal development. The other 2 groups were deprived completely of tocopherol but the diet in one of these groups was supplemented with L (+) arginine monohydrochloride. The dosage of arginine was increased gradually over a period of 6 weeks from an initial level of 100 mg daily to 600 mg per day, the latter supplementation being continued until the animals were sacrificed. The growth of the animals was followed by weekly weighings while the development of testicular changes was ascertained by periodic biopsies(1,10). The histologic examinations were started after the animals had been on the experimental diet for about one month and were continued until the experiments were terminated. At monthly intervals, one animal from each of the 3 groups was selected for biopsy, until all of the animals had been subjected to this study. The composite data gave a representative record of the development of the testicular changes occurring in each group. Liver and kidney arginase was determined by the

* Aided by Grants from the Muscular Dystrophy Associations of America, and the Assn. for the Aid of Crippled Children.

method of Kochakian(11). In this procedure, which employs a veronal-buffered arginine substrate, the urea liberated by the action of the arginase is treated with urease and the resulting ammonia is collected in a boric acid solution. Titration is performed with 0.015 N HCl and estimation of arginase is derived from a calibration curve constructed according to the directions of Edlbacker and Röthler (12). Homogenates of liver and kidney were diluted and aliquots representing 0.38 mg and 15 mg respectively, of wet tissue, were incubated. All incubations were performed in duplicate.

Results and discussion. The growth curves of the animals in all 3 groups were similar. Gross appearance of muscular dystrophy was observed in only a few animals in which weakness of the hind legs developed after deprivation of tocopherol for approximately one year.

Photomicrographs of the testes are not presented here but histologic analysis indicated a normal picture for the control rats, whereas in all of the animals maintained on the vit. E-deficient diet, testicular changes were apparent. In about 70% of these animals, the testicular degeneration was severe and it was evident that the administration of relatively large doses of arginine had afforded no protection against these histologic alterations. As a matter of fact, histologic examination of the seminiferous tubules of the rats that were given arginine suggested a more rapid loss of active sperm and an earlier production of giant cells than in the animals that had been given no supplements of arginine. Sections of skeletal muscle, liver and kidney of the animals in all 3 groups were normal.

Data on the arginase content of liver and kidney tissue for the 3 groups of animals and the wet weight of these organs are given in Table I. It can be seen that liver homogenates from normal, vit. E-deficient and arginine treated E-deficient rats split approximately identical quantities of arginine after one hour of incubation. In conformity with the now commonly used procedure, these results have been expressed in terms of arginase units. Since the total weights of the organ were essentially the same in the animals of all 3 groups, the total arginase activity also was essentially alike.

TABLE I. Average Arginase Units per Gram Tissue.*

Diet	No. animals	Avg total wt, g	Avg arginase per g, units
		Liver	
Normal	7	10.7±1.8†	1264±415.9
Vit. E-deficient + arginine	9	11.1±2.0	1143±189.31
Vit. E-deficient	11	10.9±1.7	1172±290.1
		Kidney	
Normal	7	1.1±0.2	14.6±5.8
Vit. E-deficient + arginine	9	1.4±0.2	15.3±3.0
Vit. E-deficient	11	1.4±0.2	12.5±3.1

* An arginase unit = ml 0.015 N HCl needed to neutralize NH_3 released from urea derived from 1% arginine substrate during one hr incubation at 38°C with 0.38 mg liver or 15 mg kidney (wet wt).
† Stand. dev. from the mean.

Kidney homogenate incubations revealed a similar situation as shown in Table I. Whereas the control animals and those deprived of tocopherol but fed arginine may appear to have decomposed arginine to a slightly greater extent than that exhibited by kidney preparations from E-deficient rats not administered arginine, statistical analysis indicated that such difference was not significant.

Summary. 1. Male rats were raised on a vit. E-deficient diet; some animals were given daily supplements of arginine monohydrochloride. 2. Arginase activity of liver and kidney determined by the method of Kochakian. 3. There was no difference in arginase content of livers and kidneys of animals maintained on a vit. E-supplemented diet, a vit. E-deficient diet and vit. E-deficient diet containing a supplement of arginine. 4. Administration of arginine did not delay or modify the development of testicular degeneration resulting from vit. E-deficiency.

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Received March 16, 1953. P.S.E.B.M., 1953, v83.

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

* This project was supported by a research grant from the U. S. Air Force School of Aviation Medicine, Randolph Field.

† Part of this data presented at April, 1953, meetings of Federation in Chicago.

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