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Received May 5, 1954. P.S.E.B.M., 1954, v86.

Weight, Ascorbic Acid and Cholesterol Changes in Adrenals of Pyridoxine-Deficient Adult Male Rat. (21069)

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Impaired adrenal functions in the pyridoxine-deficient rat have been reported by several workers employing histochemical methods(1), water diuresis tests(2), survival rates during cold exposure(3), and lymphopenic responses (4).

In this study an endeavor was made to measure the functional capacities of the adrenal of the pyridoxine-deficient rat by the responses to two stimuli, a) the administration of adrenocorticotropic hormone (ACTH) in hypophysectomized animals, and b) the exposure of intact animals to hypoxia. Depletion of the ascorbic acid and cholesterol content of the adrenal of the normal rat follows the administration of ACTH(5) or subjection to hypoxia(6,7). This depletion of adrenal ascorbic acid and cholesterol has been widely employed for the assay of ACTH preparations as well as for an index of the functional capacities of the adrenal gland.

Procedure. Long-Evans strain male rats were employed for all experiments. At 103 days of age the animals were placed on a diet of 24% casein, 10% hydrogenated vegetable oil, 4% salt mix(8) and 62% sucrose. A vitamin mixture† was fed 3 times weekly and pyridoxine was omitted from the mixture given the deficient group. Desoxypyridoxine, 30 mg/kg diet, was added directly to the ration of the deficient group. Three groups were studied, deficient, pair weighed and *ad libitum*. During the 8th week on the diet, 60 animals were subjected to 24 hours of fasting. For the last 20 of these hours the rats were placed in individual glass chambers, 30 of which were maintained at a pressure of 349 mm of Hg equivalent to 20,000 ft altitude and the remainder at sea level pressure. All animals received water *ad libitum* through a tube entering each glass jar. *Hypophysectomy* operations were performed on another group of 60 animals at the end of the 7th week on the regimen. For 2 days after the operation they received daily injections of 2 cc of 20% glucose intraperitoneally and their drinking water contained 10 mg % aureomycin and 5% glucose. The glucose intake was then discontinued and all animals were fasted for a total period of 18 hours. Half of these animals were injected during the fasting period with ACTH (Armours, ACTHAR, lot L5072) dissolved in 0.9% NaCl. The remaining animals received equal volumes of 0.9% NaCl.

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† Water soluble vitamins were dissolved in 20% aqueous ethanol and fed 3 times weekly in individual cups. This supplied each rat daily the following amounts in mg; thiamine hydrochloride, riboflavin, pyridoxine and folic acid, .02 each, calcium pantothenate and paraminobenzoic acid, .10 each, niacin amide, .07, inositol, 2.5, biotin, .002, and choline, 5.0. Vit. A and D and mixed tocopherols were dissolved in vegetable oil and were supplied separately in amounts of 1000 I.U., and 100 I.U., and 3 mg/rat/week.

Three doses of ACTH were given, 1 I.U., 7 I.U., and 2 I.U. per 100 g body weight on the zero, 9th and 16th hours respectively. Autopsies were done 2 hours after the last injection using sodium barbital anaesthesia. After exsanguination from the heart, each adrenal was weighed separately. One was homogenized with 5% trichloroacetic acid and the other with a 50:50 mixture of acetone and absolute alcohol. *Chemical methods.* The total adrenal cholesterol was determined by the Schoenheimer and Sperry method(9). The Roe and Keuther method(10) was employed for the determination of adrenal ascorbic acid.

Results. Intact fasted adults at sea level or under hypoxia. The adrenal ascorbic acid contents and adrenal weights at sea level in the deficient, pair weighed and *ad libitum* groups were equal. Hypoxia caused no significant depletion of ascorbic acid or increase in the adrenal weights of any group (Table I). It is surprising that adrenal weight changes were not seen since Langley and Clarke(11) found that maximum adrenal weight increases occurred within the first 2 days during hypoxia exposure. The concentration of adrenal cholesterol in the deficient and *ad libitum* groups at sea level did not differ but both groups had significantly lower values than did the pair weighed group ($p < .05$ and $< .01$, respectively). Significant cholesterol depletion ($p < .01$) followed hypoxia exposure, 23% in the pair weighed group and 43% in the *ad libitum* group. In marked contrast, the cholesterol depletion, by 11%, in the deficient group was not significant. Tepperman and co-workers(7) have reported significant cholesterol depletion without ascorbic acid depletion in normal rats exposed to 25000 ft altitude for 5 hours.

Hypophysectomized adults, saline or ACTH treated. In the 3 saline treated groups the adrenal weights and ascorbic acid concentrations did not differ (Table I). The cholesterol concentrations in the saline treated deficient and pair weighed as well as the deficient and *ad libitum* groups were equal, but the pair weighed group had a higher cholesterol value ($p < .01$) than the latter group. All groups treated with ACTH as compared with their respective saline injected groups showed a distinct decrease ($p < .01$) in adrenal ascorbic acid, 31% to 45%, a marked increase, 20 to 25% in the adrenal weights ($p < .01$) but no decrease in adrenal cholesterol. The adrenal cholesterol in the ACTH treated *ad libitum* group was even increased by 18% ($p < .01$) above its saline value. The reason for the latter finding is not apparent.

TABLE I. Weights, Ascorbic Acid and Cholesterol Contents of Adrenals of Intact and Hypophysectomized Pyridoxine-Deficient Rats. (8 to 12 rats in each group.)

Conditions	Adrenal wt, mg	Adrenal ascorbic acid, mg %	Adrenal cholesterol, %
I. Intact animals			
At sea level			
Deficient	46.7±2.4*	410±19	2.19±.14
Pair weighed	42.8±1.4	415±21	2.65±.20
Ad libitum	45.4±1.9	419±12	1.95±.13
At 20000 ft			
Deficient	50.6±2.9	465±26	1.96±.12
Pair weighed	40.4±2.7	464±24	2.03±.11
Ad libitum	49.6±1.9	360±41	1.01±.11
II. Hypophysectomized animals			
Saline inj.			
Deficient	34.5±1.8	336±19	2.56±.14
Pair weighed	32.7±1.6	307±11	2.64±.12
Ad libitum	32.2±1.3	336±18	2.24±.07
ACTH inj.			
Deficient	43.4±2.5	183±15	2.59±.19
Pair weighed	39.3±1.8	215± 8	2.63±.25
Ad libitum	38.7±1.3	184± 7	2.65±.12

* In all cases mean and stand. error.

Discussion. The failure of adrenal cholesterol depletion during hypoxia is clearly due to the lack of pyridoxine apart from inanition. Several factors may have caused the observed impairment. Failure of release of sufficiently increased quantities of ACTH or adrenal cortical steroid hormones or both may have occurred. Butler and Morgan(12) concluded that the pyridoxine-deficient intact rat did liberate augmented amounts of ACTH and adrenal 11-oxysteroid hormones under hypoxia since maximum eosinopenic responses occurred. But in the absence of pyridoxine the increased release of these hormones may have been inadequate for chemical evidence of adrenal cholesterol depletion.

The adrenal of the pyridoxine-deficient, hypophysectomized animal displayed no impairment in ascorbic acid depletion and

adrenal weight increase following ACTH administration. A number of workers(13-15) have reported a lack of correlation in the biological activities of various ACTH preparations. These findings have led to the recent viewpoint(15) that there are several adrenocorticotropic hormones having different biological activities. Until this question is resolved, conclusions are not justified concerning the pituitary or adrenal origin of the impairment in adrenal cholesterol depletion observed in the pyridoxine-deficient rat. One may not at present regard the depletion of ascorbic acid and cholesterol of the adrenal and its weight increase as comparable biological activities measuring the same functional activity of the gland. Better understanding is needed of the physiological processes involved in the production of adrenal cholesterol and its relationship to hormone production.

Summary. Two days after hypophysectomy, the pyridoxine-deficient rat treated with ACTH had the same amount of adrenal ascorbic acid depletion and adrenal weight increase as did the pair weighed and *ad libitum* controls but like these controls no decrease in adrenal cholesterol. When intact animals were subjected to hypoxia, however, the pyridoxine-deficient group, although able to maintain adrenal weights and adrenal ascorbic acid content as did the pair weighed and *ad libitum* groups, clearly failed to show the adrenal cholesterol depletion characteristic of the latter groups. The depletion of adrenal cholesterol is apparently not controlled by the same mechanism involved in adrenal hypertrophy and adrenal ascorbic acid depletion.

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Received May 7, 1954. P.S.E.B.M., 1954, v86.

Carcinogenic Activity of Radioactive Phosphorus.* (21070)

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The development of malignant tumors has been described in rats which survived a dose of radioactive phosphorus in the LD_{50} range, *i.e.,* 4.5 μc per gram body weight(1). The present study indicates that even when substantially smaller doses are used, P^{32} is still a potent carcinogenic agent.

Method. A total of 123 adult white male rats of the Wistar strain, weighing about 150 g each, obtained from Carworth Farms, were used. These were divided into 6 groups in which each animal received a single dose of 0.25, 0.5, 1.0, 2.0, 3.0 or 3.5 μc of P^{32} per

* Work performed under Contract between the U. S. Atomic Energy Commission and Western Reserve University.