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Adrenal Gland Dehydrogenases and Corticosteroid Production in Normal and Arteriosclerotic Female Rats.*† (29844)

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Female rats develop arteriosclerosis when repeatedly bred. The incidence and severity of the arteriosclerosis increases with the number and frequency of the breedings(1). The arterial lesions consist of subintimal accumulations of mucopolysaccharides which become converted into fibrotic plaques. With advancing arteriosclerosis, there is medial pooling of mucopolysaccharides, elastosis, ground substance degeneration and eventual calcification. Droplets of lipid are found at the periphery of intimal plaques, greater quantities of lipid at sites of medial elastic tissue degeneration(1). Cortical hyperplasia and necrosis, lipid depletion, and other indications of adrenal gland exhaustion are also commonly observed in breeder females(2). Also, when maximally stimulated *in vitro* by ACTH, the adrenal glands of the breeder female rats produce significantly less corticosteroid than adrenals from virgin females (3). The evidence that a causal or permissive relationship exists between abnormalities in adrenal function and the pathogenesis of the arteriosclerosis in these animals has been previously discussed(2).

The experiments described here were carried out in an attempt to find the biochemical basis for the reduced cortical steroid production observed in the adrenal glands of breeder female rats. McKerns has published data which indicate that in corticosteroid biosynthesis there is a specific requirement for reduction of TPN by the dehydrogenases of the pentose phosphate cycle. Also, the production of TPNH appears to be a limiting factor in the rate of corticoid synthesis(4). Therefore, it occurred to us that the decreased steroid production by the adrenal glands of breeder rats might be due to a relative deficiency of these dehydrogenases. The data reported here are consistent with this hypothesis.

Materials and methods. The rats used were obtained from the Sprague-Dawley Farms, Madison, Wis. All were females, either 250 ± 10 g virgins or 320 ± 15 g discarded breeders. The animals were killed by decapitation and their adrenal glands were removed and trimmed free of extraneous tissue. The aortas of the breeder animals were examined for evidence of gross arteriosclerosis. The adrenal tissue was then classified according to whether it was derived from virgin animals or from breeder animals with or without gross arteriosclerosis of the aorta. Virgins were the same age as breeder female rats and were uniformly free of arteriosclerosis. Enzyme assays were carried out on supernatants derived from 5% homogenates of the adrenal tissue in distilled water. Particulate matter was removed by centrifugation at 8000 × *g* for 15 minutes. The initial composition of the assay system is indicated in Table I. Measurements were made of the rate of change in optical density of TPNH at 340 μ at one minute intervals using

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TABLE I. Pentose Phosphate Cycle Dehydrogenase Activity in Adrenal Glands of Female Virgin and Breeder Rats.

Source of adrenal glands & No. of determinations		μmoles TPNH produced/min/ mg of tissue protein in supernatant	μmoles TPNH produced/min/ g tissue
Virgin female	(8)	.120 ± .022*	11.99 ± 1.13
Breeder female with no gross arteriosclerosis	(8)	.080 ± .032	8.32 ± 1.77
Breeder female with gross arteriosclerosis	(8)	.072 ± .027	8.46 ± 2.02
P (analysis of variance)†	<	.005	.0005

Assay system: 0.1 M Tris buffer, pH 7.4; 1×10^{-2} M $MgCl_2$, 4×10^{-4} M glucose-6-phosphate; 5×10^{-4} M TPN; either 0.01 or 0.02 ml supernatant. Total volume 1 ml.

* Mean ± standard errors.

† The difference between the 2 classes of breeder females is not significant.

the Beckman DU Spectrophotometer. Enzymatic activities were calculated from kinetic data obtained at 2 enzyme levels and during periods when reaction rates were linear with time(5). Protein in the supernatants was determined by a micro-Kjeldahl method for nitrogen using 6.25 as a conversion factor.

Measurements of rates of *in vitro* steroidogenesis by adrenal tissue were carried out by the methods of Kittinger(2,6). Each incubation flask contained the halved adrenal glands of 2 rats; the pooled tissue weighed approximately 125 mg. The incubations were performed under an atmosphere of 95% O_2 and 5% CO_2 according to the methods of Saffran and Schally(7). During the preincubation and incubation period each flask contained adrenal tissue and 5.0 ml of Krebs-Ringer-bicarbonate-glucose medium, pH 7.4. The preincubation period was one hour. The medium was then discarded and replaced by fresh medium of the same composition. The incubation of the tissue was then continued under the same conditions for 3 hours with porcine ACTH (400 mU) added at the beginning of the first, second and third hour of incubation.

The medium from each incubation was decanted into a ground glass stoppered centrifuge tube. The tissues were washed with a small volume of saline and the washings combined with the medium. Adrenal tissue was discarded. The combined fluids were extracted 3 times with 2 ml of dichloromethane using centrifugation to separate the solvent layers. Quantitative determinations of the individual steroids in the extracts were carried out by methods previously described(6).

Results and discussion. The activity units in Table I are a measure of the maximum capacity of the 2 linked enzyme systems, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase to produce TPNH from enzyme saturating concentrations of glucose-6-phosphate and TPN. Activity units in the first column are based upon actual enzymatic activity in the supernatant protein. Data in the second column are calculated with the assumption that no activity was lost during centrifugation(4,5). An analysis of variance shows that significantly less dehydrogenase activity was found in adrenal extracts obtained from female breeder rats when compared with extracts obtained from virgin rats. There were no significant differences observed between the enzymatic activity of adrenal glands removed from the breeder rats with and without gross arteriosclerosis of the aorta. However, microscopic arteriosclerosis of the aorta can be demonstrated in virtually 100% of the breeder females without gross arteriosclerosis(1). It may be of interest that the aortas of arteriosclerotic breeder rats also contain decreased concentrations of pentose phosphate cycle dehydrogenases(5). These observations suggest the possibility that the arteriosclerosis and the abnormal adrenal function are both consequences of a defect in protein synthesis of a more general nature.

The data in Table II show that *in vitro* steroid production is significantly decreased in both classes of breeder females when compared to virgin females. The rates of production of "total steroids," "blue tetrazolium positive steroids" and corticosterone production are all reduced.‡ The reduction in blue tetrazolium positive steroids is almost entirely due to a decreased production of corticosterone, the most abundant corticoid se-

‡ These terms are defined in reference(6).

TABLE II. Corticosteroid Production by Adrenal Glands of Female Virgin and Breeder Rats.

Source of adrenal glands & No. of determinations	μg/hr/100 mg adrenal		
	Corticosterone	Blue tetrazolium positive steroids	Total steroids
Virgin female (12)	9.39 ± 1.99	12.41 ± 2.21	19.40 ± 4.08
Breeder female with no gross arteriosclerosis (10)	7.41 ± 1.18	10.31 ± 1.55	15.25 ± 1.99
Breeder female with gross arteriosclerosis (10)	7.14 ± 1.07	10.37 ± 1.60	15.89 ± 2.62
P (analysis of variance)*	< .05	.05	.05

* The difference between the 2 classes of breeder females is not significant.

creted by the rat adrenal. Production of this steroid is strongly influenced by ACTH stimulation(7).

These results support the hypothesis that, under the conditions of the experiment, production of cortical steroids is rate limited by TPNH production. It seems unlikely that corticosteroidogenesis is rate limited by the concentration of endogenous steroid substrate in the adrenal glands. For example, concentrations of free and esterified cholesterol, which serve as precursor material for corticosteroids, are higher in the adrenal glands of breeder females than in the glands of virgin females(3).

In ACTH stimulated glands the rates of steroid production may well be controlled by the concentration of a critical enzyme or enzymes. Ferguson has shown that puromycin, an inhibitor of protein synthesis, strongly inhibits the adrenal gland response to ACTH (8). Kittinger has shown that puromycin will inhibit the production of certain of the cortical steroids even in the absence of ACTH stimulation(9). If *de novo* protein synthesis is involved in regulation of corticosteroid production, it is reasonable to suspect that in such cases the kinetics are controlled by the effective concentrations of certain enzymes. The pentose phosphate cycle dehydrogenases would seem to be likely sites for such control because of their key importance in production of the TPNH necessary for the several hydroxylations involved in the conversion of cholesterol to cortical steroids(10). Therefore, the observation of decreased cortical steroid production and decreased dehydrogenase concentrations in the adrenals of breeder rats prone to arteriosclerosis is of considerable interest. The question arises as to whether the lack of response of the glands to ACTH is actually due to a defect in *de novo* protein synthesis or, more specifically, to a defect in the synthesis of pentose phosphate cycle dehydrogenases. These possibilities will be investigated.

Summary. The adrenal glands of female breeder rats contain significantly less pentose phosphate cycle dehydrogenase activity than do the glands of virgin females. Consequently, less enzymatic potential for TPNH production is present in the adrenal glands of breeder rats. Since TPNH production may be a rate-limiting factor in cortical steroid synthesis, comparisons were also made of the *in vitro* steroidogenic capacity of virgin and breeder adrenal glands. When the glands are maximally stimulated by ACTH, *in vitro* cortical steroid production is again less in breeder females compared to virgin animals. It is suggested that these two observations may be causally related and due to a decreased capacity for TPNH production by these dehydrogenases.

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