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Dehydrogenase Enzyme Studies in Experimental Renal Hypertension.* (23298)

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Previous investigations(1,2,3) have demonstrated that the kidney, adrenal, thyroid and pituitary play a role in development and maintenance of experimental renal hypertension. In the present investigation one facet of metabolic activity of these organs was measured in an attempt to determine if an abnormal metabolism within one or more of them could be related to the etiology of experimental renal hypertension, while cardiac muscle was likewise studied to observe any effects of high blood pressure levels on this portion of the cardiovascular system. Since reserpine effectively lowers the hypertensive blood pressure level in experimental renal hypertension(4), it was decided also to study the above mentioned tissues in hypertensive animals under active reserpine therapy. The area of tissue metabolism studied was the endogenous dehydrogenase enzyme activity. This was quantitated employing the tetrazolium technic.

Methods. Both male and female rats of the Denver strain were used. During the years this colony has been employed for cardiovascular problems in our laboratory, the normal, mean arterial blood pressure, measured directly under general anesthesia, has been determined to range from 100-140 mm Hg. With this information available the lower limit for a hypertensive blood pressure level was set at 150 mm Hg. Experimental renal hypertension was produced in 3 different ways. Constriction of the remaining kidney by means of a figure 8 ligature after unilateral nephrectomy(5), feeding a choline deficient diet early in life(6), and encapsulation of one kidney followed by removal of the contralateral kidney(7) were the methods employed. For the latter procedure a butylmethacrylate polymer plastic dissolved in acetone was used to construct the capsule. Blood pressures were measured indirectly using the "Infraton" sphygmograph system(8) and recorded on a direct writing Sanborn E.C.G. Readings were obtained with animals under light ether anesthesia. Measurements using this method were found to agree favorably with simultaneously recorded direct blood pressure measurements. Only animals which were acutely hypertensive were employed for the endogenous dehydrogenase enzyme determinations. Following the first complete week in which an animal presented a consistently elevated mean arterial pressure, as measured every 2 days, the animal was sacrificed or placed upon reserpine therapy for 14 days before being sacrificed. A dose of 100 μg/kg/day of reserpine was necessary to reduce the elevated blood pressure to a stabilized level within 14 days. Pure powdered reserpine was used.‡ It was dissolved in a minimum of glacial acetic acid and brought to the

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TABLE I. Comparative Endogenous Dehydrogenase Enzyme Activity of Tissues from Normal, Hypertensive, and Reserpine Treated Hypertensive Rats.

Exp. procedure	No. of animals	Avg mean arterial pressure of group	Mean values (μg of dye reduced/mg air dried tissue) Kidney	Adrenal	Thyroid	Pituitary	Heart
Normal	17		36.7	22.9	11.0	6.4	17.3
" + reserpine	14		32.6*	23.7	8.2‡	5.6	14.4*
Renal constriction	14	159	32.1*	25.0	10.7	7.6	17.9
Idem	9	193	32.4*	24.6	10.9	7.8	19.7*
Renal encapsulation	11	198	30.6*	21.2	7.9*	4.0	18.1
Idem + reserpine	11	143	30.0	22.4	8.1	6.0	14.2†
Choline deficiency	7	185	34.7	22.6	9.1	7.0	20.8*
Idem + reserpine	6	137	31.5	23.7	9.2	6.6	13.8†

* $P < .01$ when compared with means of normal animals.

† $P < .0001$ when compared with means of animals made hypertensive by the same procedure but receiving no reserpine.

‡ $P < .02$ but $> .01$ when compared with mean of normal animals.

desired volume with a 10% propylene glycol medium and administered subcutaneously. This same dosage and time schedule in normal rats resulted in some anorexia and weight loss. The endogenous dehydrogenase enzyme activity was quantitated using the method of Kun and Abood(9) as modified for tissue slices(10). Triphenyltetrazolium chloride was used as the indicator in a modified Kreb's solution(11). Incubation was carried out at 37° for 2 hours. The entire pituitary and both lobes of the thyroid were incubated without further sectioning, while the adrenals were carefully divided with a razor blade. Tissue slices approximately 1-2 mm x 1 cm x 1 cm were cut freehand from the kidney and heart. The amount of dye reduced by each piece of tissue was determined colorimetrically using a Bausch and Lomb Spectronic "20", and was then converted to μg of dye reduced/mg of air dried tissue for comparative purposes. Animals made hypertensive by renal constriction with a figure 8 ligature were divided into 2 arbitrary groupings on the basis of level of blood pressure reached. Those animals in the first group had a blood pressure between 150-180 mm Hg, while in the second group the blood pressure was greater than 180 mm Hg for each animal. This was done to observe if any difference in the dehydrogenase enzyme activity of the tissues could be related to the level of blood pressure acutely attained.

Results. The results obtained are tabulated in Table I. In the statistical analysis, the mean values obtained for each tissue in the 2 hypertensive groups treated with reserpine were compared only to the groups in which hypertension was similarly produced but left untreated.

Of the organs studied, the adrenal and pituitary showed no significant change for any of the experimental groups. Although the pituitary appears to have a low value for the group in which hypertension was produced by renal encapsulation, it was not statistically significant.

In the case of the thyroid the results were equivocal. The endogenous dehydrogenase enzyme activity was lower than normal in those groups made hypertensive through feeding of a choline deficient diet early in life and by renal encapsulation. The latter lowering was statistically significant. Since this same condition did not result in the group in which hypertension was produced by renal constriction, it can not be said that any significance can be attached to this lowered activity in relation to the etiology of experimental renal hypertension. Administration of reserpine also lowered the endogenous dehydrogenase enzyme activity of the thyroid. This condition is being further investigated by us to determine the mechanism of action for this reduction.

All of the experimental procedures producing hypertension resulted in a reduction in endogenous dehydrogenase enzyme activity of the kidney. A reduction in succinic dehydrogenase enzyme level of kidney tissue from

animals made hypertensive by partial renal artery occlusion has previously been reported (12). The present investigation further suggests that the kidney is involved in the etiology of experimental renal hypertension at a metabolic level. The exact mechanism whereby the kidney produces its action cannot be determined from this study.

The endogenous dehydrogenase enzyme activity of the heart was raised in the presence of hypertensive blood pressure levels. This appears to have some relationship to the degree of blood pressure attained. Conversely reserpine lowered this activity significantly. Reserpine has been shown to produce a reduction in amplitude and frequency of contractions in the isolated cat and rabbit hearts (13). Thus the dehydrogenase enzyme activity may be a reflection of the work being done by the heart. This would account for the increased enzyme activity of the hypertensive heart. Whether reserpine produces this reduced enzyme activity in the heart directly or indirectly necessitates further investigation. Reduced enzyme activity resulting in reduced cardiac amplitude and frequency of contractions could possibly contribute to the hypotensive action of this substance.

Summary. 1) The endogenous dehydrogenase enzyme activity, as measured by the tetrazolium technic, was determined in kidney, adrenal, thyroid, pituitary and heart tissue from untreated and reserpine treated hypertensive animals. Experimental renal hypertension was produced in rats by 3 different methods. 2) A reduction in endogenous dehydrogenase enzyme activity of kidney tissue was observed in hypertensive animals while the activity of cardiac muscle was increased. Treatment with reserpine resulted in a depression of the endogenous dehydrogenase enzyme activity in cardiac tissue of both hypertensive and normal animals.

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Clotting of Hemophilic Blood with Purified Platelet Cofactor I, Platelet Factor 3 and Threone.* (23299)

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When blood clots a large number of substances are involved in many chemical interactions. These are as numerous and complex as intracellular processes, and one way to satisfy our curiosity about them is to study each chemical reaction separately. Before that can be done the substances involved must first be obtained in purified form. With that accomplished there is an additional opportunity in finding out what happens when such substances are added to blood as found in the

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