

tein depletion diet increases very markedly the susceptibility of the kidney to the nephrotoxic action of ethionine in male and female rats. Methionine inhibits the renal toxicity of ethionine while glycine and alanine are without effect.

The authors are indebted to Dr. Jean Oliver, professor of pathology, State University of New York, College of Medicine at New York City, for his cooperation and invaluable help.

Received June 29, 1951. P.S.E.B.M., 1951, v77.

Differences of Cortical/Medullary Ratio in the Adrenals of Normal Rats, Normal Rats treated with ACTH, and Renal Hypertensive Rats.* (18880)

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The existence of a direct relationship between heart weight and adrenal weight in male rats made hypertensive by the removal of one kidney and constriction of the other has been reported(1,2). It was shown that the relative degree of enlargement of the medulla exceeded that of the cortex, although by far the greater part of the weight increase of the whole gland was due to increase in size of the cortex(2).

The assumption that enlargement of the adrenals under conditions of stress is due to the effect of hypophyseal corticotrophin has been generally accepted(3). If increase in the size of the adrenals in severe or prolonged stress is due solely to the effect of hypophyseal corticotrophin, and if enlargement of the adrenals in renal hypertensive rats is an instance of the reaction to stress, then it would be expected that the administration of adequate doses of ACTH would bring about changes in the adrenals of normal rats similar to those found in renal hypertensive rats. The aim of the present report, in part, is to show that such is not the case. ACTH was given to normal rats and found to induce cortical but not medullary growth, within the limits of accuracy of the method. If any change in medullary size occurred it was very small. In contrast, the adrenal medulla of the renal

hypertensive rat has been shown to increase to as much as 4 times the normal size. This is due to both cell hypertrophy and cell hyperplasia(2).

Experimental procedure. Four male rats of the Stanford colony were given 0.5 mg of ACTH (Armour) intramuscularly 4 times daily to a total dose of 25 mg each. They were then killed by exsanguination from the aorta under ether anesthesia and their adrenals weighed to the nearest milligram on a torsion balance. Seven normal control rats were killed similarly, as were 6 renal hypertensive males. Cortical/medullary volume ratios were determined by dividing each adrenal into 3 discs, embedding these in paraffin after fixation in Bouin's fluid and dehydration in dioxane, and filling several slides with sections. The slides were projected onto paper at 37 diameters magnification, drawn in outline, and the areas of cortex and medulla measured with a planimeter. A correction was made for vascular space in the medulla only. The individual cortical/medullary ratios given are based on a minimum of 100 sections through the adrenals and their statistical accuracy has been tested and verified by plotting the course of cumulative ratios(2).

Results. The data are given in Table I. The 32% increase of the ACTH treated rats over the normal rats in adrenal weight expressed as percent of a predicted value based on body weight, is statistically significant ($P < 0.02 > 0.01$). No significant change in medullary weight seems to have been produced

* This investigation was supported by USPHS Grant No. H-811.

1. Rather, L. J., *Stanford Med. Bull.*, 1950, v8, 119.
2. Rather, L. J., *J. Exp. Med.*, 1951, v93, 573.
3. Sayers, G., *Physiol. Rev.*, 1950, v30, 303.

TABLE I. Cortical/Medullary Ratios and Calculated Weights of Cortex and Medulla in Normal Male Rats, Renal Hypertensive Male Rats and Normal ACTH Treated Male Rats.

Body wt, g	Adrenals wt, mg	% normal*	Cortical/medullary ratio	Cortex, mg	Medulla, mg
Normal male rats					
320	43	119	14.1/1	40.2	2.8
312	26	74	10.2/1	23.7	2.3
312	40	114	13.8/1	37.3	2.7
308	34	97	9.7/1	30.8	3.2
193	31	112	14.7/1	29.1	1.9
200	24	86	12.2/1	22.2	1.8
200	28	99	13.7/1	26.1	1.9
Mean		100	12.6/1		
ACTH treated rats					
303	48	139	15.4/1	45.1	2.9
275	43	130	13.5/1	40	3
170	37	140	13.1/1	34.4	2.6
175	32	121	16 /1	30.1	1.9
Mean		132	14.5/1		
Renal hypertensive male rats					
162	59	232	10.7/1	53.9	5.1
255	96	302	8.4/1	85.8	10.2
207	77	268	6.7/1	67	10
145†	55	229	10.1/1	50.1	4.9
246†	96	302	8.8/1	86.2	9.8
162†	62	248	8.1/1	55.2	6.8
Mean		264	8.8		

* Predicted from body wt according to equations of Addis and Gray(5) based on data from this colony.

† On high protein (60% casein) diet. All other rats on stock diet containing 18% protein and .3% NaCl.

by the injections of ACTH. When the mean cortical/medullary ratio of the ACTH treated rats (14.7/1) is compared with that of the renal hypertensive rats (8.8/1) a highly significant difference ($P < 0.01$) is evident. Calculation of the weight of the medulla on the assumption that cortex and medulla are of equal density shows a great increase in medullary size in the renal hypertensive rats, which is due to cell hypertrophy and hyperplasia (2). Intracytoplasmic bodies were found in the cortical fasciculata in Bouin-fixed paraffin-embedded sections of the adrenals of all rats with chronic severe renal hypertension. These spherical droplets varied from 2 to 8 μ in diameter and often several were present in

a single cortical cell. The nuclei of such cells usually showed nucleolar enlargement. The intracytoplasmic bodies stained brilliantly with leukofuchsin-sulfite after periodic acid oxidation. This positive PAS reaction could be blocked with the use of the technic described by McManus(4) consisting of treating the sections with acetic anhydride in pyridine, and restored by hydrolysis in dilute NaOH. The bodies were not digested by diastase. The solubility characteristics and the above histochemical reactions suggest that the material is a glycoprotein. At least the presence of a 1, 2 hydroxy grouping is to be presumed. Such bodies were very rarely present in the ACTH treated rats and not found at all in the controls.

Comment. This difference in the behavior of the adrenal in response to injections of ACTH and to the physiological situation of experimental renal hypertension can only mean that factors in addition to ACTH are concerned in the growth of the adrenal in the latter condition. The nature of these factors and the significance of the medullary enlargement is unknown. The hyperplastic, hypertrophic medulla is most probably producing an increased amount of hormones. It is possible, of course, that a dissociation of growth and hormone output has occurred, analogous to that seen in the thyroid after the administration of thiouracil. An explanation of this, however, would seem to be even more remote than one assigning to an increased production of medullary hormones some significance in the pathogenesis or maintenance of the elevated blood pressure in the renal hypertensive rat.

Summary. In the enlarged adrenals of renal hypertensive rats there is a relative increase in medullary size, whereas in the enlarged adrenals of rats treated with ACTH there is a relative decrease in medullary size. Large numbers of PAS positive intracytoplasmic bodies are found in the cortical fascicular zone in the adrenals of rats with severe chronic renal hypertension, but were not produced in comparable numbers with ACTH. It is con-

5. Addis, T., and Gray, H., *Growth*, 1950, v14, 81.

4. McManus, J. F. A., and Cason, J. E., *J. Exp. Med.*, 1950, v91, 651.

cluded that factors in addition to ACTH are concerned in the growth of the adrenal in renal hypertension.

The author wishes to acknowledge the technical assistance of Mrs. Elizabeth Jensen.

Received June 29, 1951. P.S.E.B.M., 1951, v77.

Hepatic Artery Ligation in Rabbits Followed by Thoracic Caval Constriction.* (18881)

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Acute ligation of the hepatic artery, as shown by Markowitz(1), is compatible with life in dogs if an antibiotic such as penicillin is given in the postoperative period. This discovery has led to new approaches to the problem of ascites in animals and in man.

It has been indicated by Reinhoff(2), in cases of portal hypertension, that hepatic artery ligation might prevent the formation of ascites. Perfusion experiments have shown that blood flow through the hepatic artery in cirrhosis encounters less resistance than in the normal liver. The higher pressure of blood flow through the hepatic artery, with less resistance, tends to increase the resistance to the flow through the liver from the portal vein. The rationale of ligation of the hepatic artery in these cases was based upon the fact that lowering the intra-arterial pressure also lowers that in the portal vein. In a previously reported experiment(3), it was found that the ascites formed in rabbits by thoracic caval constriction was not affected by previous 2-stage portal ligation. The present experiment, as a continued study, was carried out to determine what effect hepatic artery ligation

would have on the type of ascites produced by experimental constriction of the thoracic inferior vena cava. The fluid formed by such a procedure is believed to be the result of venous congestion in the liver. McKee(4,5) produced ascites in dogs within a few days by means of an adjustable aluminum band placed on the thoracic inferior vena cava midway between the heart and diaphragm. Volwiller(6) and Volwiller and co-workers(7), also working with dogs, used a cellophane band placed around the thoracic segment of the inferior vena cava. The fibrous reaction of the cellophane would cause a progressive constriction of the vessel lumen to approximately one-quarter of its original diameter within three to four weeks. This resulted in marked ascites.

Rabbits weighing 3 to 4 kg were used in this experiment. The diet consisted of stock rabbit pellets containing 17% protein, oats and water. All surgical procedures were executed under aseptic operating conditions. In the cases in which thoracotomy was performed, local anesthesia and positive endotracheal oxygen pressure were utilized. Postoperative intake and output were not measured.

Method and results. Ten control rabbits were subjected to hepatic artery division and ligation just distal to the gastroduodenal

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1. Markowitz, J., Rappaport, A., and Scott, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v70, 305.

2. Reinhoff, W. F., *Bull. Johns Hopkins Hosp.*, 1951, v88, 368.

3. Milnes, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1951, v76, 147.

4. McKee, F. W., *Surg., Gyn. and Obst.*, 1950, v89, 529.

5. McKee, F. W., *J. Exp. Med.*, 1948, v87, 457.

6. Volwiller, W., *Proc. Staff Meetings, Mayo Clinic*, 1950, v25, 31.

7. Volwiller, W., Grindley, Bollman, *Gastroenterology*, 1950, v14, 40.