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Effects of Cortexone and Salt on Renin Content, Juxtaglomerular Index and Renal Hypertension in Rabbits. (28818)

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Juxtaglomerular cells have been considered as the source of renin, an enzyme which, acting on plasma angiotensinogen as a substrate, is responsible for the formation of the vasopressor angiotensin. Hypersecretion of renin by ischemic kidneys has been held to be the direct cause of nephrogenic hypertension. More recently, Hartroft and associates have shown that the degree of granulation of the juxtaglomerular apparatus, taken as an indicator of the rate of renin production, decreases after cortexone and salt administration(1) and is directly proportional to the thickness of the zona glomerulosa of the adrenal cortex(2). This finding was corroborated by Gross and associates, who have independently demonstrated that in the rat (3) as well as in the rabbit(4), renal renin content is decreased by cortexone and salt treatment. The significance of the juxtaglomerular apparatus as one part of the homeostatic mechanism governing sodium balance has been confirmed and extended by the demonstration that angiotensin increases the output of aldosterone from the adrenal cortex(5,6).

In view of the dual action attributed to the renin-angiotensin system, it seemed indicated to study the effects of cortexone administration, supplemented by salt on the one hand, and combined with renal ischemia on the other hand. It was reasoned that, were angiotensin the effective mediator of renal hypertension, degranulation of the juxtaglomerular apparatus following cortexone and salt administration, either should be reversed after clamping of the renal artery, or should prevent the rise in blood pressure, which normally follows the latter procedure. The following experiment was performed to test these possibilities. The rabbit was selected as the experimental animal because in this species cortexone is known to have no hypertensive action(7).

Materials and methods. As an initial step, the right kidney was removed in adult male and female rabbits weighing 2-3 kg, who were next randomly divided into 2 groups. The removed kidney was used to determine the normal values for renin content and juxtaglomerular granulation index (JGI). On the day of the nephrectomy the rabbits in one group were given one dose of 0.4 ml per kg body weight of a cortexone trimethylacetate suspension, a commercial preparation supplied by Ciba* under the trade name "Percortène" and containing 25 mg of the active microcrystalline substance per 1 ml of buffered saline. The rabbits of the other group, which served as controls, were injected with 0.4 ml per kg body weight of the same medium but without Percortène, which was also supplied by Ciba. In addition, the test animals were given as a drinking fluid a 0.5% solution of sodium chloride in distilled water. A second similar dose of either cortexone or drug vehicle was given on the 11th day of the experiment. Fourteen days after unilateral nephrectomy, during which interval blood pressure measured at the ear by a sphigmomanometric method(8) was re-

* Ciba, Basle, Switzerland.

TABLE I. Mean Values and Standard Deviation.

Rabbit group	Renal renin content		JGI		Adrenals		Blood pressure rise, mm Hg
	Right, mm Hg	Left, mm Hg	Right	Left	Weight, mg	Width glomer, μ	
I	42 ±17	45 ±18	14.3 ±10.7	25.2 ±30.7	265 ±74	89.7 ± 8.5	+ 1.6 ± 7.2
II	56 ±16	57 ±23	15.9 ±12.9	24.8 ±13.1	257 ±49	87.0 ±15.7	+19.7 ±11.8
III	47 ±17	15 ±12	12.9 ± 9.2	2.3 ± 2.2	208 ±68	75.5 ±12.7	- 2.2 ± 6.0
IV	53 ±19	25 ±10	16.9 ± 7.1	5.5 ± 5.8	268 ±65	75.8 ± 6.3	+22.3 ±12.2

TABLE II. Variance Ratio F.

Source of variation	Left kidney		Blood pressure rise	Adrenals	
	Renin content	JGI		Wt	Width glomer
Renal artery clamping	3.52	<1	38.51†	1.28	<1
Cortexone + salt	28.36†	12.41*	<1	1.00	9.94*
Interaction	<1	<1	<1	2.15	<1

* Probability inferior to .01. † Probability inferior to .001.

corded every other day, each test and control group was further subdivided into 2 subgroups. In one of them, the animals were subjected to simple lombotomy and served as sham operated controls. In the second subgroup, a silver clip, 0.5 mm wide was placed about the left renal artery. Blood pressure was thereafter recorded daily and all animals were sacrificed by nembutal anesthesia on the 8th day after operation. The 32 rabbits which survived the experimental period had been randomly assigned to one of 4 experimental groups of each 8 animals, as follows: Group I, vehicle injected and sham operated animals; Group II, vehicle injected animals with constricted renal artery; Group III, cortexone injected, salt treated and sham operated animals; Group IV, cortexone injected and salt treated animals with constricted renal artery. At autopsy the left kidney, both adrenals, and the heart were weighed; samples for histological study were taken from these organs and fixed for 24 hours in Zenker-formalin. The renin content of kidney extracts was estimated by the Gross and Sulser method (9), using nephrectomized rats as test animals. The results are expressed in mm Hg. The JGI was counted on Bowie stained slides, according to the method advocated by Pitcock and Hartroft(10). Both types of determinations were made by independent investigators, without knowledge of the experimental conditions. Thickness of the zona glomerulosa was measured under a 150 × magnification, in 20 randomly selected sites of each adrenal and the results expressed as the sum of the individual measurements in paired organs. The average of blood pressure readings taken during the 2 preoperative weeks was compared with the similar average of the postoperative week. The statistical significance of the differences between measurements made in the various groups was evaluated by analysis of the variance. The results of our measurements and statistical calculations are summarized in Tables I and II.

Analysis of results. As shown in Table I, clamping of the renal artery in unilaterally nephrectomized rabbits of Group II was followed by a sustained rise in blood pressure. The increment reached a mean value of 19.7 mm Hg, which represented a 28% elevation above the normal preoperative level of 71 mm. Renin content of the ischemic kidney did not vary. A rise in JGI was observed in this group but did not exceed a similar change, which occurred in control Group I. In Group III, administration of cortexone supplemented by salt resulted in a highly significant decrease of both renin content and JGI. Blood pressure on the other hand was not influenced. In Group IV, where cortexone and salt administration was combined with renal ischemia, blood pressure rose in the

same measure as in Group II where the animals were subjected to isolated renal artery constriction. In addition, renin content and JGI were decreased to the same extent as in sham operated cortexone and salt treated rabbits of Group III. A close and direct correlation was found between renin content and JGI ($r = 0.561$, $p<.001$).

The width of the zona glomerulosa was significantly reduced under the influence of cortexone and salt administration and remained unaffected by renal artery constriction. In comparison with vehicle injected controls of Groups I and II, the cortexone and salt treated rabbits of Groups III and IV showed a 15% reduction in the mean thickness of their zonae glomerulosae, a highly significant decrease.

Discussion. The results obtained in Groups II and III, where unilaterally nephrectomized rabbits were subjected respectively to clamping of the renal artery and to cortexone treatment supplemented by salt, were those to be expected. In particular, the absence of any change in renin content following renal artery constriction is in agreement with similar observations recorded in unilaterally nephrectomized rats by Gross and associates (11). They found that an elevation of renin content in the ischemic kidney occurs solely when a contralateral intact kidney is present. In that case, it is accompanied by a proportional decrease on the intact side, bringing total renin content close to normal. In contrast with the lack of variation in renin content, elevation of the JGI was seen in rabbits subjected to isolated renal artery clamping. This change, however, was not statistically significant. Furthermore, a comparable rise was found in the sham operated and vehicle injected controls.

The most interesting observations were made in Group IV, where the possibly conflicting effects of cortexone supplemented by salt and of renal artery constriction were combined. Our results show that each experimental procedure maintained its own characteristic and full effect without interference from the other. Clamping of the renal artery did not alter the depressed level of renin content and JGI and the reduced renin formation did not prevent induction of hypertension. From the lack of correlation between the parameters indicative of renin formation and hypertension, as demonstrated in the present investigation, it may be concluded that, within the limits of our particular experimental conditions, renin does not represent a necessary factor in the mechanism responsible for induction of renal hypertension. Exogenously administered cortexone supplemented by salt resulted in a combined reduction of renin content, JGI and thickness of the zona glomerulosa without preventing the development of hypertension after renal artery constriction. Our observations confirm the fact that in the rabbit, in contradistinction to the rat, mineralocorticoids and salt have no hypertensive activity. In all other respects, however, renal reactions to cortexone and to arterial clipping are comparable in the 2 species. The absence of any correlation between mineralocorticoids and hypertension, which we found in the rabbit may therefore logically be assumed to have a general significance in the pathogenesis of renal hypertension. Our view is strengthened by two recent studies published after the completion of the present work. Both studies concern rats subjected to renal artery constriction. In the experiments of Bing(12), the animals were treated by cortexone and salt and it was concluded that "renal hypertension could be produced in renin depleted as well as in normal rats." This conclusion, like the evidence on which it rests, is essentially similar to ours. Bing's results should, however, be interpreted with some caution, as clamping of the renal artery in the rat might simply have enhanced the well known pressor activity of cortexone and salt, so that the rise in blood pressure which was obtained could well have an endocrine, rather than a renal origin. Fisher and Klein (13) have made the complementary observation that sodium depletion does not influence the rise in blood pressure which results from renal ischemia.

Summary. In unilaterally nephrectomized rabbits, cortexone and salt administration reduces renin content and the juxtaglomerular granulation index of the kidney and does not

provoke hypertension. Renal artery clamping induces a rise in blood pressure without causing changes in renin content nor JGI, which remain normal. Combination of these 2 experimental procedures is followed by hypertension and a decrease in renin content and JGI. The width of the adrenal zona glomerulosa is reduced under the influence of cortexone and salt, and remains unaffected by renal artery constriction. In the rabbit, therefore, mineralocorticoids seem to play no major role in induction of renal hypertension. Neither does renin appear as a necessary agent in the mechanism responsible for the development of renal hypertension.

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Some Physical-Chemical Variables Affecting Insulin Migration *in vitro* I. Electrophoresis.* (28819)

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Berson and Yalow(1), using filter paper electrophoresis, have reported that tracer amounts of insulin-I^{131}, alone or added to serum from control subjects (patients never having received insulin), were quantitatively bound to Whatman 3 mm paper at the site of application. However, if the paper was soaked in an insulin-containing solution prior to electrophoresis to saturate the insulin-binding sites on the paper, the radioactivity migrated in the inter alpha-1-albumin area whether or not plasma was present. By contrast, radioactivity in insulin-treated subjects migrated in a zone between the beta and gamma globulins.

It was later reported by Randle and Taylor(2) and Antoniades *et al*(3) that "free" insulin in serum or plasma migrated in this inter alpha-1-albumin zone.

Using similar *in vitro* electrophoretic techniques, we have previously shown that tris (hydroxymethyl) aminomethane (TRIS) buffer was able to release insulin bound to beta-gamma globulins in the sera of treated and insulin-resistant diabetics(4).

To the best knowledge of the authors insulin electrophoresis has not been performed on cellulose acetate, a supporting media introduced by Kohn(5). This material is homogeneous, microporous, and contains extremely low concentrations of heavy metals. In addition the carboxyl groups are acetylated.

Because of these characteristics, it was felt that the use of this paper would minimize insulin-paper interaction. We, therefore, elected to extend our studies and compare

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