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Changes in Renin Content in Kidneys of Renal Hypertensive Rats. (27129)

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In earlier studies on rats with renal hypertension due to unilateral clamping of the renal artery, the renin content of the clamped kidney was found to be increased, while renin had nearly disappeared from the untouched kidney(1). This unequal distribution of renin was also observed in rats, in which the unilateral clamping had not led to hypertension(1), and also in other species like the rabbit, the cat or the dog, in which it is generally necessary to place clips on both renal arteries in order to obtain hypertension. If both renal arteries are clamped in the rat, the renin content is always lower in the heavier kidney than in the lighter one, even if the difference in weight between the 2 kidneys is small(2). In the above studies the renin content of the kidney was estimated semiquantitatively by injecting a freshly prepared kidney extract into a nephrectomized assay rat. A more precise estimate of renin concentration in kidney tissue, as used here, may be obtained by the indirect assay method, *i.e.*, by measuring the amount of angiotensin liberated from a given excess of angiotensinogen (rat plasma) by the renin contained in a fixed volume of kidney extract. By this procedure it is possible to follow quantitatively the changes in renin concentration in the clamped and unclamped kidney when either the one or the other kidney is removed. The present report concerns a) the influence of unilateral clamping of a renal artery on concentration of renin in the clamped and unclamped kidneys, b) the influence of one kidney on concentration of renin in the other, c) time necessary for the increased or decreased renin content of a kidney to return to normal after removal of its partner.

Methods. Male white rats averaging 160-180 g in weight were made hypertensive by placing a silver clip (0.2 mm I.D.) on either one or both renal arteries. The following groups were investigated 3 to 4 weeks after clamping or after unilateral nephrectomy: a) Unilateral (left) clamping of renal artery, the other kidney being untouched (50 animals); b) clamping both renal arteries (8 animals); c) clamping the left renal artery immediately after removal of the right kidney (16 animals); d) unilateral nephrectomy (25 animals); e) control animals (80 animals).

In a second series of experiments, the time necessary for the renin content to return to normal was investigated. Renin concentration in the kidney was determined before unilateral nephrectomy and at intervals of 2, 8, and 24 days after operation. The following groups were studied: f) Unilaterally nephrectomized normotensive animals; g) renal hypertensive animals after removal of the clamped kidney; h) renal hypertensive animals after removal of the unclamped kidney.

Each group consisted of 15 animals. Subgroups of 5 animals each were sacrificed at the given time intervals.

Renin was determined indirectly accord-

ing to a modification of Schaffenburg's method(3) in homogenates of whole rat kidneys.

Results. a. *Renin content of kidneys in renal hypertension.* In a normal kidney the content of renin determined by the indirect method corresponds to 40 ± 6 μg (mean ± S.E.) angiotensin per gram fresh tissue (80 kidneys from 40 animals). In intact animals both kidneys contain the same concentration. After clamping one renal artery the renin concentration in the "clamped" kidney increases to twice that in the intact kidney, while renin disappears nearly completely from the untouched kidney (Group a). Total renin content of both kidneys of these animals, therefore, is the same as in intact rats. Clamping both renal arteries (Group b) also induces a marked difference in renin content between the kidneys. Under these conditions, blood flow to both kidneys is never impaired to the same degree. Because of the unequal blood flow one kidney is generally lighter than the other. The concentration of renin in the lighter kidney is about 5 times that in the heavier kidney. In the otherwise intact animal after unilateral nephrectomy the renin content in the remaining kidney does not change. Clamping of the artery of the remaining kidney, however, is not followed by an increase in renin content, though it induces hypertension. Thus, the presence of an unclamped kidney is a prerequisite for an increase in renin content of the clamped kidney (Fig. 1).

b. *Influence of one kidney on renin content of the other.* In an intact kidney renin concentration does not change during 24 days after unilateral nephrectomy. If, however, in a renal hypertensive animal with an uneven distribution of renin the clamped

ANGIOTENSIN γ/g kidney	40 ±6	40 ±6	1 ±0.1	80 ±7	20 ±10	100 ±20	40 ±5	40 ±4
BP mm Hg	105 ±2		180 ±2		185 ±4		105 ±4	195 ±4

FIG. 1. Renin concentration expressed as μg angiotensin/g whole fresh kidney tissue under various experimental conditions. From left to right: Intact animals; experimental renal hypertension due to unilateral clamping of renal arteries; the same due to bilateral clamping of renal arteries; unilateral nephrectomy; clamping the remaining renal artery after unilateral nephrectomy.

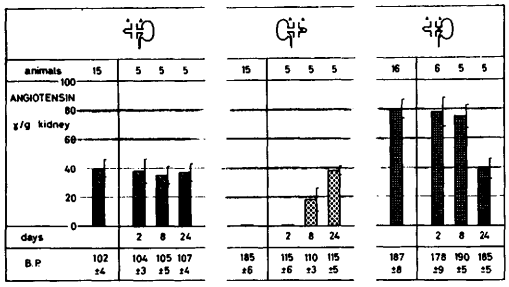

FIG. 2. Renin concentration expressed as μg angiotensin/g whole fresh kidney tissue in intact kidney (left), unclamped kidney (middle), and clamped kidney (right) after removal of the contralateral kidney. Determinations were made before (controls) and 2, 8, and 24 days after nephrectomy.

kidney is removed, renin concentration in the unclamped (renin depleted) organ increases to about half the normal value within one week and was found normal at the end of 24 days. A normal renin content is reached, therefore, some time between the 8th and the 24th day after operation (Fig. 2). When the unclamped kidney is removed, the elevated renin concentration in the clamped kidney comes down slowly. While one week after operation it is still nearly twice as high as in the intact kidney, it decreases to normal within 24 days, possibly earlier. Blood pressure remains high after removal of the unclamped kidney. A high blood pressure may, therefore, be maintained independently of an increase in renin concentration of the clamped kidney (Fig. 2).

Discussion. In intact as well as in unilaterally nephrectomized animals with experimental renal hypertension there is no direct correlation between the height of blood pressure and renin concentration found in the kidneys. Although in the presence of both kidneys there is an about 2-fold increase in renin concentration in the clamped kidney, total renin content does not exceed normal values, as the reduction in the unclamped kidney approximately compensates for the higher amount in the clamped kidney. In the unilaterally nephrectomized animal renin content in the remaining kidney does not differ from normal and is not influenced by clamping the renal artery.

It may be argued that the tissue concentration of a humoral factor does not give in-

formation about its rate of secretion, but recently Omae, Masson, and Page(4) showed that the secretion of renin as observed in a grafted kidney corresponds well with the concentration found in renal tissue. If such results hold for kidneys *in situ,* changes in renin content may reflect changes in secretory rate, albeit with a certain delay. From earlier experiments on renin depletion elicited in rats by overdosage of cortexone or aldosterone and salt, as well as from the present observations, it is evident that the period necessary for adjustment of the renin content in the kidney is about 2-3 weeks. It is, however, probable that changes in the secretory rate of renin occur more rapidly than alterations in the renin concentration.

It is not surprising that upon removal of the clamped kidney not only does blood pressure decrease rapidly but the renin content in the unclamped kidney is also restored. This repletion occurs much more slowly than the fall in blood pressure. It was, however, an unexpected finding that excision of the unclamped kidney also influences renin content in the clamped kidney. As the presence of the unclamped kidney is not necessary for maintenance of hypertension, the effect on renin content must be independent of blood pressure. The present data demonstrate, however, that renin concentration in the kidneys does not change independently, but that some factor must regulate the renin concentration so that the amount present in one kidney is influenced by that present in the other. How this information is transmitted is not clearly understood. The following assumption would, however, account for all the changes observed so far. A reduction in blood perfusion pressure as produced by an arterial clip may enhance renin secretion by the kidney. Increased secretion of renin by one kidney induces 2 different trains of events: a) The other kidney ceases to secrete renin, with the consequence that its renin content drops to values near zero within 3 weeks. The depression of renin secretion in the unclamped kidney is probably not a direct consequence of an increase in amount of circulating renin or angiotensin. It may be assumed to be caused by sodium retention. This interpretation is in agreement with the reduction of renin content of both kidneys in animals treated with large doses of aldosterone or cortexone(5) or overloaded with salt, or, conversely, the increase in renin content in kidneys of salt-depleted rats(6). In the animal with renal hypertension sodium retention may, in turn, be caused by oversecretion of aldosterone, since angiotensin is known to stimulate aldosterone secretion(7,8). b) The normal untouched kidney exposed to renin and/or angiotensin reacts by either inactivating these substances or by secreting another humoral factor which antagonizes either renin or angiotensin in the same or in the clamped kidney. Such a mechanism stimulates increased secretion of renin/angiotensin in the clamped kidney either directly or indirectly. Only in the presence of this additional stimulation does renin oversecretion by the clamped kidney persist long enough to cause a detectable increase in renin concentration. Clamping the one renal artery in a unilaterally nephrectomized rat, therefore, does not cause a detectable increase in renin concentration of the kidney. This mechanism, however, is not as such responsible for maintenance of hypertension.

Summary. The renin content of fresh kidney tissue was assayed indirectly by a modification of Schaffenburg's method(3) in rats with experimental renal hypertension, under a variety of conditions. Clamping one renal artery caused an increased concentration of renin in the clamped kidney, and a fall in the contralateral kidney. Clamping both renal arteries also caused an increase in renin content of the more efficiently clamped kidney and a decrease in the contralateral kidney. Renin content in the one remaining kidney was not influenced by unilateral nephrectomy in normal rats. Clamping the one remaining renal artery in unilaterally nephrectomized rats induced hypertension, but did not result in an increase in renin content of the kidney. Similarly, removal of the unclamped kidney in rats with hypertension due to a unilateral renal artery clip resulted in a slow decrease of renin content in the clamped kidney to normal values. Removal of the clamped kidney in such animals caused a rapid fall of

blood pressure and a subsequent slow increase of renin content in the remaining unclamped kidney. A unifying hypothesis to explain these changes is presented, involving a concept of renin inhibition or inactivation.

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Effect of Water-Miscible Preparation of Vitamin D_2 on Metamorphosis of Thiourea and Citrate Treated *Rana pipiens* Larvae. (27130)

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Vitamin D_2 (calciferol) in moderate doses has been shown to enhance metamorphosis in tadpoles(1,2). Since calciferol has been demonstrated to have a thyroid-enhancing effect(3,4,5) it was thought interesting to study its effect when combined with other substances which may affect thyroid function.

Immersion in thiourea inhibits tadpole metamorphosis(6) presumably by interfering with the oxidation of iodide in synthesis of thyroxine(7). Therefore, it was decided to determine the effect of calciferol on the metamorphosis of thiourea-treated tadpoles.

Furthermore, an increase in serum and tissue citrate levels with Vit. D_2 has been demonstrated in mammals(8). Although the thyroid has an unusually high citrate concentration(9,10), it does not synthesize citrate at all, but does degrade it to a moderate degree(11). Since the decrease in thyroidal citrate can be correlated with an increase in the gland's oxidative metabolism(9,12) the possibility exists that citrate is captured by the thyroid, perhaps adventitiously with calcium, and is either utilized as fuel or released in the course of metabolic activity. Therefore, it was decided to determine the effect of immersion in citrate plus Vit. D_2 on tadpole metamorphosis.

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Methods. *R. pipiens* tadpoles obtained by induced ovulation were immersed at the hind-limb bud stage in thiourea solution (.033%), sodium citrate (.05%), thiourea (.033%) and calciferol (1000 I.U./l), sodium citrate (.05%) and calciferol (1000 I.U./l). Control tadpoles were also reared. Hind-limb lengths were measured prior to immersion in experimental media and after 63 days. The larvae were placed in fresh solutions on alternate days. On the 35th day of experiment the level of calciferol was increased to 2500 I.U./l, when preliminary observations indicated an incipient difference between control and experimental groups.

Results. The results summarized in Table I, subjected to a "t" analysis showed a significant difference between Groups I and II (95-98% level) and II and V (90-95% level).

One may conclude that Vit. D_2 has a metamorphosis-enhancing effect; it does not counteract the action of thiourea; the effect of Vit. D_2 is greater than that of Vit. D_2 plus citrate.

Discussion. Although these results cannot be fully explained there are several speculative possibilities. The enhancing effect of Vit. D_2 on thyroid function may be explained: by its citrate effect (reference to