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Role of a Pressor Substance in Unilateral Renal Hypertension.* (31295)

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Unilateral renal injury in the rat often leads to the development of hypertension and vascular disease. Cross circulation experiments suggest that the high blood pressure may be humoral in origin(1) although there are findings to the contrary(2,3). Several studies indicate that the juxtaglomerular indices and renin content are increased in the damaged kidney and reduced in the opposite untouched organ(4,5,6,7). The present study was undertaken to determine whether the ischemic kidney of rats with unilateral renal hypertension releases increased amounts of vasopressor material into the circulation and hence whether the high blood pressure could arise on a humoral basis, *i.e.*, the renin-angiotensin system.

Method. Production of hypertension. White male rats weighing 160-200 g were used. Under nembutal anesthesia ischemic atrophy of the left kidney was produced by coarcting the abdominal aorta at a site between the ostia of the two renal arteries(8). The aorta was reduced to 0.25 mm in diameter by ligating the vessel around a stylet of this diameter and then removing the stylet. The animals were then divided into 2 groups. In 90 rats the left kidney remained *in situ* while in 56 rats serving as controls the left kidney was removed immediately after the aorta was constricted. Thirty additional rats were subjected to sham coarctation and formed a second control group.

Determination of blood pressure. This was obtained by inserting a No. 50 polyethylene catheter into the brachial artery and connecting it to a Hg manometer. Control readings were taken prior to operation and at intervals ranging from 4 hours to 5 months after surgery.

Assays for vasopressor material. Rats were assayed for the presence of vasoconstrictor material in their circulation(9). The test samples consisted of 1.0 cc of blood from the brachial artery or 0.1-0.5 cc of blood from the left renal vein (ischemic kidney) or from the right renal vein (untouched kidney). Blood was obtained from each renal vein by veni-

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puncture with a No. 26 needle and syringe. No. 50 polyethylene catheters were inserted into the femoral vein and artery of anesthetized recipient rats which were either normal or given pentolinium tartrate (4 mg/100 g subcutaneously) for ganglionic blockade. An injection of 0.001 μg synthetic angiotensin gave about a 10 mm Hg rise in blood pressure in a normal rat and an 18 mm Hg rise in a pentolinium-treated rat. The test samples were injected into the femoral vein and the response was measured by the rise in blood pressure in mm Hg as recorded by a Statham gauge manometer on a Grass polygraph or read directly from a Hg manometer. Each assay from a hypertensive rat (one with an ischemic left kidney) was controlled by injecting the same recipient with an equal amount of blood from a normotensive animal (one with the left kidney removed or with sham operation) and comparing the response. Each recipient was limited to a maximum of 4 injections. Assays for vasopressor material were made at periods from 4 hours to 5 months after operation. Many animals were tested 2 or 3 times at successive intervals in both the acute and chronic stages of hypertension.

Properties of the vasopressor material. Plasma from the arterial blood or left renal vein blood of acute hypertensive rats was dialyzed against 0.2 M phosphate buffer pH 7.0 and the dialysate was tested for vasopressor activity. Boiling for several minutes failed to destroy the pressor effect of hypertensive plasma. The supernatant obtained after boiling was incubated with chymotrypsin for 1 hour at 37°C and was also mixed with fresh venous plasma in a 3 to 1 proportion and allowed to stand at room temperature for 30 minutes to determine the effect of angiotensinase. In addition the pressor activity obtained with hypertensive plasma in recipient rats which were treated with the adrenergic blocking agent piperoxan hydrochloride (0.75 mg/100 g iv) was compared with that of norepinephrine. As controls each of the above procedures was carried out with normal plasma to which synthetic angiotensin was added.

Results. Blood pressure. Fig. 1 shows the averaged blood pressures of the experimental

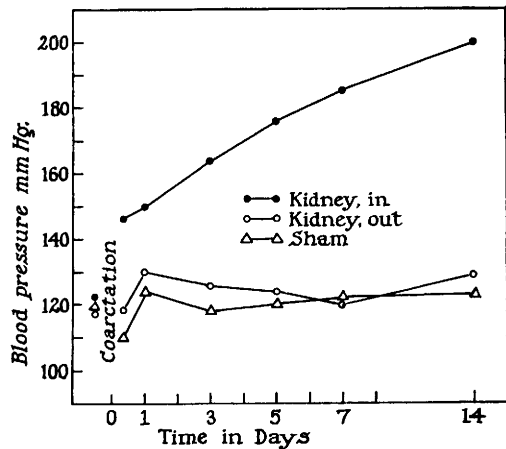

FIG. 1. Blood pressure curves in rats with unilateral renal hypertension.

and control rats during the first 2 weeks after operation. All animals with coarctation and the life kidney *in situ* rapidly became hypertensive. The blood pressure was significantly elevated over the control value at 4 hours postoperative and then rose progressively to reach an average level of 184 mm Hg at one week and 200 mm Hg at 2 weeks. The pressures then remained hypertensive over the next 4 to 5 months, *i.e.*, 160-260 mm Hg. In contrast animals with coarctation and resection of the left kidney or with sham coarctation remained normotensive through the entire period of observation and in the range of 98-140 mm Hg.

Assays for vasopressor material. Positive assays were obtained only with hypertensive rat blood from the left renal vein or from the brachial artery (Fig. 2). When injected into recipient rats such blood caused a distinct rise in blood pressure which exceeded that given by the same amount of blood from normotensive rats by 8-42 mm Hg. The type of response was like that observed with synthetic angiotensin. The blood pressure of the recipient began to rise promptly, *i.e.*, within several seconds, reached a peak within 1 minute and then returned to the initial baseline in about 3-5 minutes. Renal vein blood from the right kidney of hypertensive rats or arterial blood from normotensive rats (coarctation of aorta and left kidney removed or sham operation) elicited either a minimal response

TABLE I. Increments in Pressor Response of Hypertensive Over Normotensive Rats.

		Increments (mm Hg)						
	No. assays	−4 to −1	0	1-4	5-9	10-19	20-29	30-45
Renal vein blood								
Acute hypertension	44	2	1	5	10	16	8	2
Chronic "	43	10	19	8	2	3	1	0
Arterial blood								
Acute hypertension	30	0	0	2	8	16	3	1
Chronic "	29	9	14	6	0	0	0	0

(2-8 mm Hg) or none at all (Fig. 2).

Table I shows the results of 87 assays of renal vein blood obtained from the injured left kidney of rats with unilateral renal hypertension. All bloods withdrawn up to 2 weeks after renal injury were placed in an acute hypertensive group while those withdrawn after this time, *i.e.*, 2 weeks to 5 months, were placed in a chronic hypertensive group. In each recipient the increment in pressor response in mm Hg of the hypertensive over the normotensive control blood was obtained by subtraction and the values were then assembled numerically as shown in the Table. Thus of 44 renal vein bloods from rats with acute hypertension 10 elicited a response 5-9 mm greater than the corresponding controls, 16 gave a response 10-19 mm greater, 8 a response 20-29 mm greater, etc.

In contrast renal vein blood from the ischemic kidney of chronic hypertensive rats generally failed to yield a significant pressor response. Of 43 such samples, 19 appear in the zero column of the Table indicating there was no difference in the reactions elicited by the hypertensive and normotensive control blood. In 18 additional assays there is about the same number of small positive increments (to the right of the zero column) as negative increments (to the left of the zero column). Six of the 43 assays were positive and of these 5 represented blood samples removed between 2 and 5 weeks after renal injury while one positive result was obtained at 7 weeks after renal injury. Thereafter samples of renal vein blood removed from each of 20 hypertensive rats between 2 and 5 months following renal injury gave negative assays.

The results of 59 assays of blood from the brachial artery of rats with acute or chronic renal hypertension are also shown in Table I. Positive assays for vasopressor material were limited to the acute hypertensive stage, *i.e.*, up to 2 weeks after renal injury. After this time the assays were negative.

In several animals with acute hypertension test samples obtained simultaneously from the left renal vein and brachial artery showed greater pressor activity in the former (Fig. 3). In 10 additional acute hypertensive rats assays of blood from the left renal vein (ischemic kidney) were uniformly positive, while those removed almost simultaneously from the right renal vein (untouched kidney) were negative or yielded only a minimal rise in blood pressure. Assays of blood from the untouched right kidney of 16 chronic hypertensive animals were uniformly negative for vasopressor material.

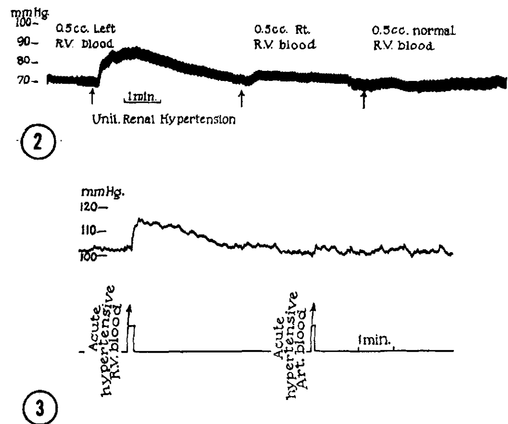

FIG. 2. Pressor response of 0.5 cc left renal vein blood (ischemic kidney) and 0.5 cc right renal vein blood (untouched kidney) withdrawn almost simultaneously from a hypertensive rat (BP 190) 3 days after constriction of aorta. Negative response to 0.5 cc renal vein blood from a normal rat is also shown.

FIG. 3. Pressor response of 0.1 cc left renal vein blood and of 0.1 cc arterial blood withdrawn almost simultaneously from a hypertensive rat (BP 170) 2 days after constriction of aorta.

Properties of the vasopressor material. The properties of the pressor agent in hypertensive plasma were similar to those of angiotensin and were characteristic of a polypeptide. The agent was heat stable and also dialyzable against a semi-permeable membrane. After boiling the plasma the pressor substance contained in the supernatant was inactivated by chymotrypsin and also by normal rat plasma. The pressor response of recipient rats to an injection of active plasma was not altered by treatment of the animals with piperoxan hydrochloride whereas the response to norepinephrine was largely eliminated.

Discussion. Our results show that hypertension will develop in rats subjected to coarctation of the abdominal aorta only if there is an ischemic kidney distal to the coarctation. This agrees with the work of previous investigators(10,11,12,13). Mechanical obstruction of the aorta *per se* did not lead to hypertension.

The high blood pressure was probably initiated on a renal humoral basis. A potent vasopressor substance was present in renal vein blood from the ischemic kidney and in arterial blood in reduced concentration, while it was absent in renal vein blood from the opposite untouched kidney. This correlates with studies of unilateral renal hypertension showing normal or increased juxtaglomerular indices and renin content in the damaged kidneys and reduced values in the opposite untouched organs. The physical and chemical properties of the vasopressor substance were similar to those of synthetic angiotensin.

In our study a vasopressor agent was found in the circulation of hypertensive rats for a period of 2 or 3 weeks after unilateral renal injury. Thereafter, for reasons not entirely clear, there appeared to be a progressive decline in output of this agent by the damaged kidney. Only a very occasional positive pressor response was elicited with renal vein blood obtained between the second and fifth weeks of the hypertensive state and thereafter the assays were uniformly negative even though the blood pressures of the animals remained at very high levels. Thus there was an 'acute' stage of hypertension characterized by a readily demonstrable vasopressor substance in the circulation and a 'chronic' stage in which no circulating pressor substance was detectable.

The amounts of vasopressor material detectable in the circulation in chronic renal hypertension by means of bioassay are at best so small that the high blood pressure cannot be attributed to a direct constrictive effect on the blood vessels. It is still possible that the damaged kidney continues to release minute amounts of angiotensin which exceed the usual normal production and yet are not detectable by present methods of assay. Such amounts might sustain peripheral arteriolar contraction indirectly by enhancing the activity of the sympathetic nervous system(14) or by inducing cerebral vasoconstriction which in turn renders the medullary vasomotor center more active(15). Dickinson and Lawrence (15) doubt that resetting of peripheral arterial baroreceptors is an important item. Ledingham and Cohen(16) proposed that in chronic renal hypertension vascular constriction is maintained through myogenic autoregulation of blood flow brought about by the elevated blood pressure. This is similar to Folkow's postulate(17) that increased resistance to flow depends on secondary hypertrophy of the vessel walls as a result of adaptation to sustained hypertension. However, Nolla-Panades(13) reported an increase in the hindlimb perfusion pressure of rats with coarctation of abdominal aorta above the renal arteries. This was not secondary to hypertension *per se* since the vascular bed in the hindlimbs was not exposed to a high blood pressure. The increased perfusion pressures were associated with enhanced reactivity of the hindlimb vessels to norepinephrine. However, from available evidence it appears doubtful that the vascular bed in chronic renal hypertension is more sensitive than usual usual to exogenous pressor agents.

Summary. Unilateral renal hypertension was produced by constricting the aorta of the rat between the ostia of the main renal arteries. The acute stage of hypertension probably depended on a renal humoral mechanism since during the first 2 or 3 weeks of the hypertensive state, blood from the renal vein of the ischemic left kidney con-

tained a potent vasoconstrictor substance, probably angiotensin, which was also present in arterial blood but not in renal vein blood from the untouched right kidney. After 3 weeks the left kidney ceased to release a pressor agent and in addition no such agent was demonstrable in the general circulation by bioassay. Hence the chronic stage of unilateral renal hypertension could not be attributed to the direct vasoconstructive effect of a humoral pressor agent.

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Mast Cell Population Density in Rat Skin. (31296)

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The relationship of the mast cell, its presence and distribution within the skin, to the general vitality of skin has yet to be defined. Part of this difficulty is due to insufficient information about the distribution of mast cells within skin. Some investigators have determined the distribution as a function of surface area of tissue examined, and others have used subjective and nonparametric systems(2,5,8,9,12). It is obvious that in order properly to appreciate the role of the mast cell in skin, distribution within the tissue should be realistically determined. Using a statistical cell counting method of Floderus (7), we previously(3) were able to study mast cell distribution in the liver. Applying this method to the problem of determining mast cell distribution in the skin of rats, a series of interesting observations has been collected and statistically analyzed for this report.

Materials and methods. Eight Sprague-Dawley rats, males and females, 3 to 4 months of age and *ca.* 350 g were used. The animals were noninfected, untreated, and were considered normal. Tissues were fixed in 10% neutral formalin and prepared by the paraffin technique. Five micron sections were stained in toluidine blue (0.05% in 70% ethanol, pH 4.4) for one minute followed by extraction in 4 changes of 95% ethanol for 15 seconds each. Microscopic examination was by bright field at 640×. In each rat 10 different skin regions of dermis were studied; *viz.*, lip, cheek, distal ear (*i.e.*, free end of pinna), dorsum, ventrum, footpad, and tail. In 3 of these regions, lip, dorsum, and footpad, the counts were extended to include superficial and deep portions of the connective tissue. A total of 20 fields were counted for each specimen, *i.e.*, 1600 fields in all.

The method developed by Floderus(7) for cell counting was used to make the mast cell population counts. The formula is: