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jects. The middle bar shows the mean arterial plasma angiotensin concentration from 12 measurements in 11 subjects with hypertension and renal vascular disease; and the last bar represents the mean plasma angiotensin concentration after infusion of relatively small doses of angiotensin II. In this latter group, despite the fact that the mean increase in systolic blood pressure was only 16 mm. Hg and in only one of the subjects did the blood pressure rise to the hypertensive range, the mean plasma angiotensin concentration was significantly higher than the mean plasma angiotensin concentration of the patients with hypertension and renal vascular lesions.

SUMMARY

Data have been presented which indicate that a few patients with hypertension and renal vascular lesions have increased aldosterone production, presumably through the renin-angiotensin system, but that most of these patients have aldosterone excretion and secretion rates and plasma angiotensin II concentrations within the normal range. These data suggest that in the majority of patients with renal vascular lesions and hypertension the renin-angiotensin system is not the mechanism causing the hypertension.

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Vasodepressor Lipid from the Renal Medulla

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THE elucidation of the renal pressor system (renin-angiotensin) in recent years has, perhaps, drawn attention from the possible coexistence of a less well-established renal antipressor function. However, 25 years ago Blalock and Levy,[1] Fasciolo,[2] and Pickering and Prinzmetal[3] were impressed by the marked rise in blood pressure on removing the so-called "protecting" kidney in experimental hypertension due to unilateral renal ischemia. This led to the demonstration by Braun-Menéndez and von Euler,[4] and Grollman, Muirhead and Vanatta[5] of the development of hypertension in the bilaterally nephrectomized dog, termed "renoprival" hypertension. It was later shown by Floyer[6] and Kolff[7] that normal kidneys, grafted into renal hypertensive animals, independent of excretory function, reduced the hypertension. The antihypertensive function of a normal kidney has recently been shown by Tobian, Winn and Janecek[8] to be stimulated by the presence of arterial hypertension.

In 1940, Grollman, Williams and Harrison[9] described an extract of whole kidney which produced a sustained lowering of arterial pressure in animals with experimental renal hypertension. Recently Sokabe and Grollman[10] have presented evidence that the active principle is localized in the renal cortex. However, Muirhead *et al.*[11] have described a lower molecular weight (< 1000) substance, localized in the renal medulla, that produced a sustained lowering of blood pressure in renal hypertensive dogs. Work in our laboratory has demonstrated the presence of a low molecular weight substance in renal medulla that produced an acute and sustained hypotension on injection into normotensive rats and dogs.[12]

Whether these effects are due to the same or different substances cannot be answered until precise chemical identification is accomplished. The present work describes the progress in chemical identification and physiological effects of a potent, vasoactive lipid-like material concentrated in the renal medulla of several species.

A. CHEMICAL ISOLATION AND CHARACTERIZATION

(1) *The bioassay.*—All extracts were tested for vasodepressor activity by injection into 200-250 g., tracheotomized, vagotomized albino rats, prepared with pentobarbital (Nembutal) and pentolinium. The left jugular vein was cannulated with polyethylene tubing for injection of extract from a microsyringe. The right carotid artery was cannulated with polyethylene tubing and the arterial pressure response was recorded on a direct-writing, pen-float mercury manometer.

(2) *Preparation of extract.*—Unless otherwise indicated, extracts were prepared from quick-frozen rabbit kidneys, obtained and kept in the frozen state.* The inner rabbit medulla is readily separated in the frozen state from the outer medulla and cortex by scissors dissection, and yields a wet weight of approximately 1 g. per kidney. Saline homogenates in a concentration of 1 g.

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*Career Development Award Program, U.S. Public Health Service Grant No. HE-K-14, 903-C1.
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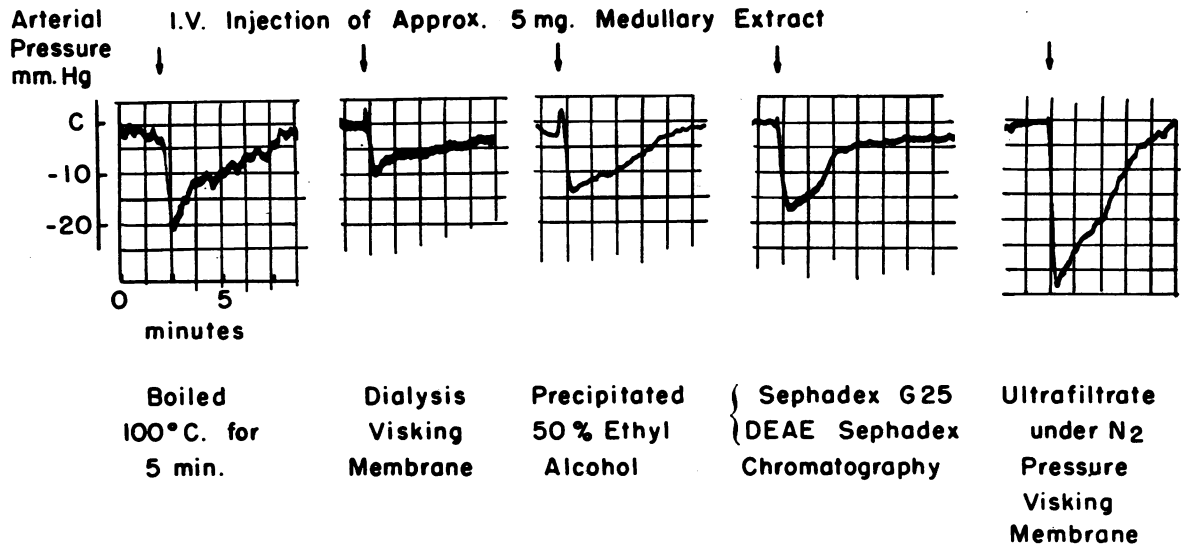

Fig. 1.—Evidence of the non-protein nature of rabbit renomedullary vasodepressor factor by the various protein-separation techniques indicated. The sustained hypotension of greater than five minutes following single intravenous injections in the bioassay rat is shown.

medulla in 5 ml. saline, assayed directly, readily show vasodepressor activity in intravenous dosage as small as 0.01 ml. in the bioassay rat.

(3) *Non-protein nature of the factor.*—It was established that the vasoactivity was in the non-protein fraction, since a sustained depressor response was observed in the bioassay rat from extracts which were deproteinized, whether through boiling for five minutes, dialysis through a Visking membrane, ethyl alcohol precipitation, Sephadex-G25, or DEAE Sephadex chromatography, or through ultrafiltration under nitrogen pressure through a Visking membrane. In each instance the arterial pressure in the rat dropped within seconds and gradually recovered over a five- to 10-minute period following injection of deproteinized homogenate (Fig. 1). The Sephadex chromatography indicated a molecular weight of less than 4500 for the vasoactive fraction.

(4) *Non-peptide nature of the factor.*—Extracts were deproteinized by ultrafiltration and incubated two hours at 37° C. at neutral pH with carboxypeptidase and trypsin, retaining full vasodepressor activity. Further, incubation with diazo reagent failed to destroy its activity. Studies on pH stability indicated survival of activity on acid incubation at pH 2 for one hour, but loss of activity on alkaline incubation at pH 11 for one hour. This suggested a lipid.

(5) *Lipid nature of the factor.*—Acid chloroform extraction of the aqueous ultrafiltrate revealed essentially all of the vasoactivity in the chloroform phase (Fig. 2). Evidence of a polar nature of the lipid was its recovery at the front on paper chromatography in a solvent system of isopropanol and butanol with the ninhydrin-positive peptide material behind; peptide-free chloroform extract of the ultrafiltrate gave a positive sudan-black-B stain at the front, where the vasodepressor activity was again recovered on elution.

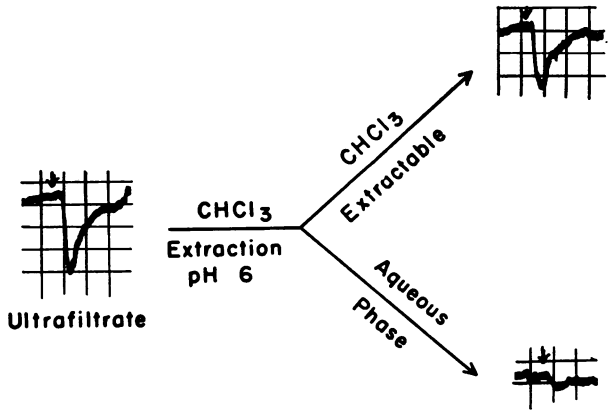

Fig. 2.—Evidence of the lipid nature of rabbit renomedullary vasodepressor factor, showing essentially all of the activity to be extractable from a protein-free solution into acid chloroform.

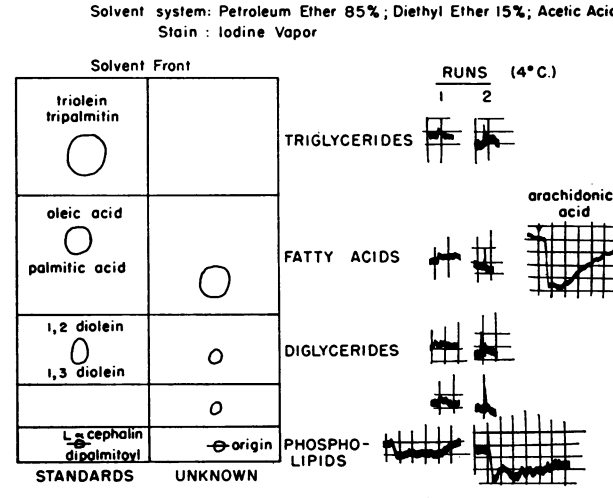

Fig. 3.—Chromatographic characteristics of rabbit renomedullary vasodepressor lipid, showing its highly polar capacity in its failure to move in the solvent system indicated on thin-layer silica gel, unlike the neutral lipid and fatty acid standards.

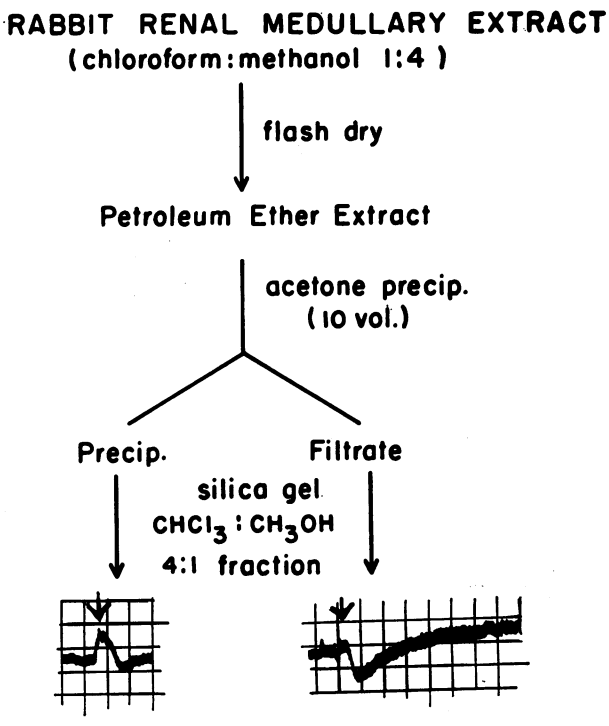

Fig. 4.—Evidence of the non-phospholipid nature of rabbit renomedullary vasodepressor lipid. On acetone precipitation of phospholipids from petroleum ether extracts, vasopressor activity is shown in the phospholipid fraction; all of the vasodepressor activity is in the non-phospholipid fraction.

(6) *Thin-layer silica gel chromatography of lipid extract.*—Lipid chromatography on thin-layer silica gel in a solvent system of petroleum ether 85%—diethyl ether 15%, stained with iodine vapour, left all of the vasoactivity recoverable at the origin, where phospholipid standard remained, whereas diglyceride, fatty acid and triglyceride standards moved up from the origin (Fig. 3). Arachidonic acid, which has been shown to be vasodepressor in the rabbit,[13] was also run. All of the vasodepressor activity was recovered toward the front, with none at the origin (Fig. 3).

(7) *Non-phospholipid nature of the factor.*—While the behaviour of the extract on thin-layer chromatography suggested a phospholipid, concentrated extract (37° C. *in vacuo*) was extracted into petroleum ether, and phospholipids were removed as acetone-insoluble material, as described by Hanahan, Dittmer and Warashina.[14] As shown in Fig. 4, all of the vasodepressor activity was recovered in the non-phospholipid fraction. An interfering vasopressor factor was recovered in the phospholipid fraction, as shown.

(8) *Pevikon electrophoresis and gas chromatography.*—Further purification was carried out by block electrophoresis of lipid extracts of rabbit renal medulla on Pevikon, a copolymer of polyvinyl chloride and polyvinyl acetate. Runs were made at pH 8.6 with triethylamine buffer. The vasoactive fraction moved toward the anode in such a way that all of the vasodepressor activity was isolated in a single 2-cm. cut, 25 cm. from the origin (Fig. 5). Thin-layer silica gel chromatography on the eluate of the active Pevikon cut in a solvent system of chloroform 65 ml.—methanol 25 ml.—water 4 ml. revealed a solitary spot under iodine vapour which had run at the front, where vasopressor activity was recovered. Subsequent gas-liquid chromatographic analysis of this fraction failed to reveal any significant fatty acid content

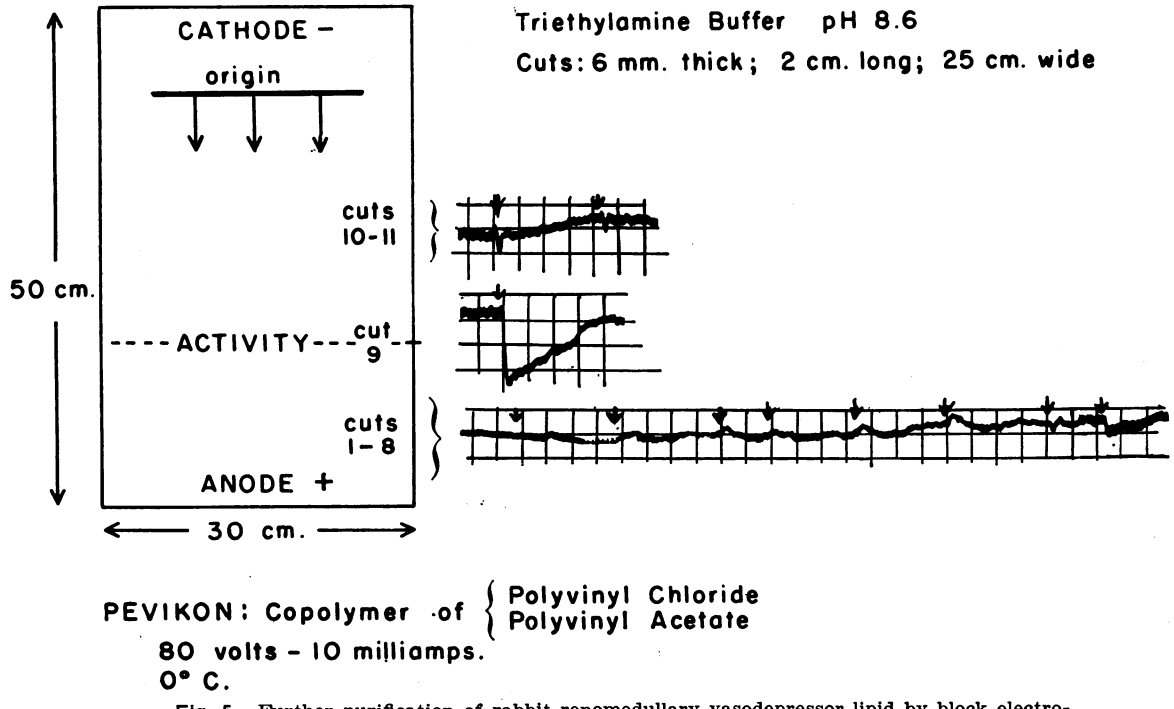

Fig. 5.—Further purification of rabbit renomedullary vasodepressor lipid by block electrophoresis. Its sharp movement toward the anode in an alkaline pH is evidence of its homogeneity and of its polar and acidic capacity.

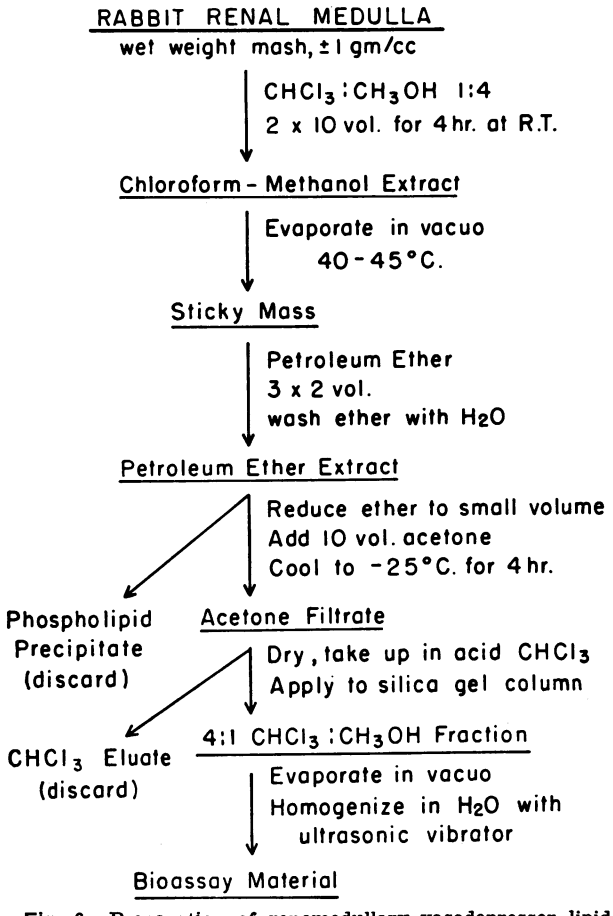

Fig. 6.—Preparation of renomedullary vasodepressor lipid in batch, the method showing the recovery of activity with techniques that tend to eliminate the ordinary classes of lipids (phospholipids, triglycerides, simple fatty acids).

in the C_{12}-C_{20} series. Similarly, microphosphate analysis on the Pevikon cuts failed to reveal any significant content in the vasoactive fraction.

(9) *Silica gel column graded solvent elution.*—At this point the evidence from this work seemed to be against the active fraction's being an ordinary fatty acid, neutral triglyceride or phospholipid. Thus, our current procedure for preparing extract in batch (Fig. 6) involves, first, the removal of phospholipids, as described above, followed by extracting the dried non-phospholipid fraction into acid chloroform, which is then added to a silica gel column for graded elution as described by Hanahan, Dittmer and Warashina.[14] The first eluent, chloroform, removes much of the fatty acid and neutral lipid components; no significant vasoactivity is found in this fraction, which is discarded. The next eluent, 4:1 chloroform-methanol, removes nearly all of the vasoactive fraction, consistent with its polar nature, and is the fraction preserved. Only a trace more of vasodepressor activity is recovered in the subsequent elutions with increasing content of methanol in the chloroform-methanol elution mixtures. The 4:1 eluate is flash-dried and homogenized into a 20% ethanol solution by an ultrasonic vibrator for subsequent assay and physiological studies. It may be stored in the frozen state without loss of vasoactivity.

(10) *Infra-red absorption studies.*—Infra-red absorption studies were performed on medullary extract as purified by the method outlined above (Fig. 6), and revealed a long carbon-chain compound (in the range of C_{26}), with a high absorption in the range for keto and carboxylic acid radicals, and particularly in the range for free hydroxyl groups. This is evidence that the lipid could be in the special group of long-chain hydroxy fatty acids.

B. Apparent Localization of the Lipid from Various Species in the Renal Medulla

Methanol-chloroform extracts from the renal cortex and medulla of several species were compared for vasoactivity in the bioassay rat. In the rabbit, rat, dog and human, vasodepressor activity lasting more than five minutes was found in the medullary extracts. No such activity was found in the corresponding cortical extracts, except in the human autopsy material.

Other tissues of the rabbit (liver, spleen, small intestine, heart, lung, muscle, adipose tissue and plasma) were similarly extracted and assayed; no sustained vasodepressor activity was found comparable to that of the renal medulla.

It would appear, then, that the lipid-like material may be localized in, or present in significant quantity only in, the renal medulla, although the findings in the human kidney suggest either less sharp localization of the medulla or autopsy artefact.

C. Physiological Studies in the Rat and Dog

Precise dosage in terms of weight of vasoactive material administered remains speculative until absolute purification is certain; but it is fair to say that distinct physiological activity appears to be present in microgram quantities of our purest preparations to date.

In a previous report,[15] before the lipid nature of the substance was established, the following two pharmacological properties were noted: (1) absence of the development of tachyphylaxis or untoward reaction on repeated dose administration in the bioassay rat; (2) progressively large fall in arterial pressure in the bioassay rat with progressively large dose administration until a maximum was reached, beyond which larger doses failed to produce a further depression of pressure, indicating a logarithmic relationship between dose and response, characteristic of a direct-acting, vasoactive substance.

(1) *Isolated rat hind-limb and kidney perfusion studies.*—When the isolated rat hind-limbs were perfused with Ringer's solution under constant pressure and the flow was recorded with a drop counter, histamine was vasodilator, as indicated by an increase in flow (decrease in excursion of the drop-counter recording), whereas rabbit renomedullary extract was vasoconstrictor, as were

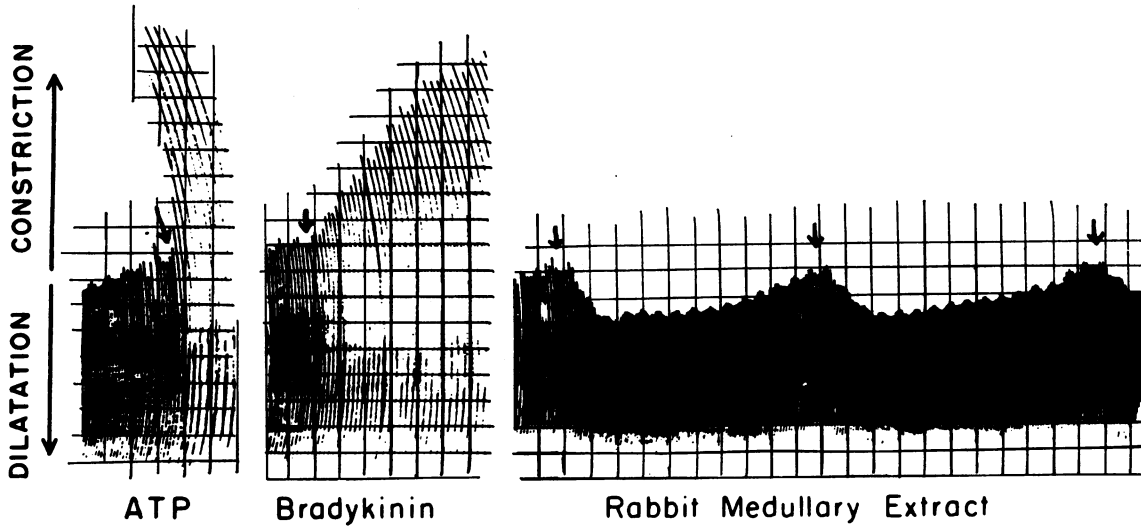

Fig. 7.—Vasomotor effect of rabbit renomedullary extract on the isolated rat kidneys perfused with Ringer's solution at constant pressure. The fall in amplitude of excursion of the drop-counter recording shown after each of three injections of medullary extract (to the right) indicates a direct renal vasodilatation, to be contrasted with the vasoconstrictor effects of ATP and bradykinin, indicated by an increase in the amplitude of excursion of the drop-counter recording.

angiotensin and bradykinin, as indicated by an increase in the drop-counter excursion. However, when the isolated rat kidneys were similarly perfused (Fig. 7), the medullary extract was clearly vasodilator in contrast to ATP and bradykinin, which were vasoconstrictor. In these two systems, the extract manifested direct myotropic activity.

(2) *Cardiovascular effects in the anesthetized dog.*—Ten mongrel dogs have been studied to date for the cardiovascular effects of intravenously administered rabbit renomedullary extract. As shown in Fig. 8 (top), the material produced a prompt and sustained fall in systolic and diastolic aortic pressure, with narrowing of the pulse pressure, the effect wearing off gradually after a period of over 30 minutes. Creatinine and PAH clearance studies and electrolyte excretion studies performed during the period of hypotension in one dog failed to reveal any significant change over the control period. As in the rat, progressively large doses failed to lower arterial pressure below a maximum, and no harmful effects were observed. At the bottom of Fig. 8 is shown the pressure response in the renal artery of the same dog on intravenous injection of extract. The renal blood flow was kept constant with a steady-flow pump at 156 ml./minute. The fall in renal artery pressure shown, with widening of the pulse pressure, indicates an active renal vasodilatation, consistent with the findings in the isolated perfused rat kidneys. In another dog in which continuous cardiac output and aortic pressure were simultaneously monitored electronically, the extract produced a moderate rise in cardiac output (30%), coincidental with a fall in mean aortic pressure, giving a large fall in calculated peripheral resistance (Fig. 9). The three

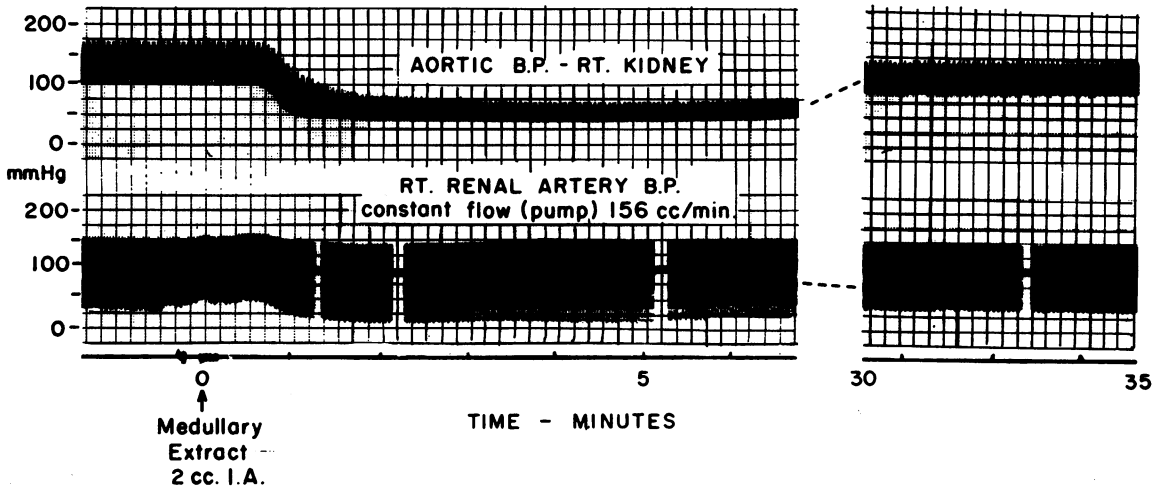

Fig. 8.—Hemodynamic response to rabbit renomedullary extract in a 50-kg. dog anesthetized with pentobarbital (Nembutal). At the top is shown a sustained (30 min.) depression of aortic pressure after a single intravenous injection. At the bottom is shown a fall in right renal arterial mean pressure with widening of its pulse pressure with the same injection. Since the right kidney blood flow was kept constant with an infusion pump, the pressure changes are indicative of an active renal vasodilatation.

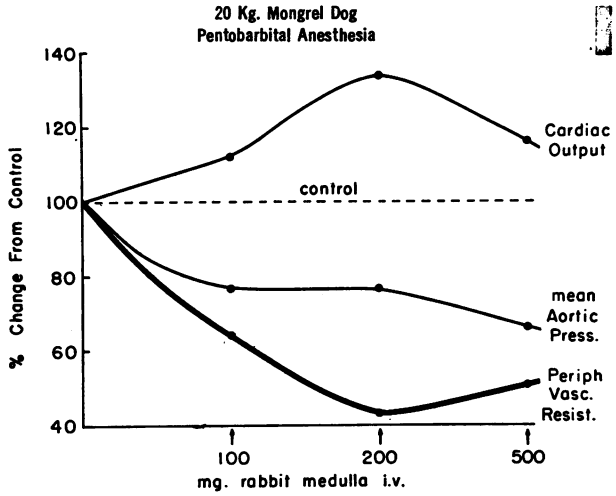

Fig. 9.—Cardiovascular effects of rabbit renomedullary extract in a 20-kg. dog anesthetized with pentobarbital, with constant monitoring of cardiac output and mean aortic pressure by electronic instrumentation. The simultaneous rise in cardiac output and fall in mean aortic pressure indicate a large fall in calculated peripheral resistance. Increasingly large doses of extract, expressed as milligrams of rabbit medulla from which each of the three injections was made, failed to increase these changes beyond a maximum, as shown, suggesting a physiological mechanism of action.

progressively large doses of extract, given as shown in Fig. 9, are expressed as milligrams of rabbit medulla from which each of the three injections was made.

(3) *Further observations on possible mechanism of action.*—Preliminary studies (direct myocardial strain gauge recordings in the dog) suggested a positive inotropic effect. The apparent simultaneous inotropic and vasodilator effects suggested beta-adrenergic receptor stimulation, akin to the effects of isoproterenol. However, the intravenous injection in the bioassay rat of 1 mg. of a beta-adrenergic receptor blocking agent* completely abolished the vasodepressor effects of a subsequent injection of isoproterenol, but only a slight reduction in the vasodepressor response to medullary extract was observed.

*Pronethalol (Nethalide), Ayerst Laboratories, Inc.

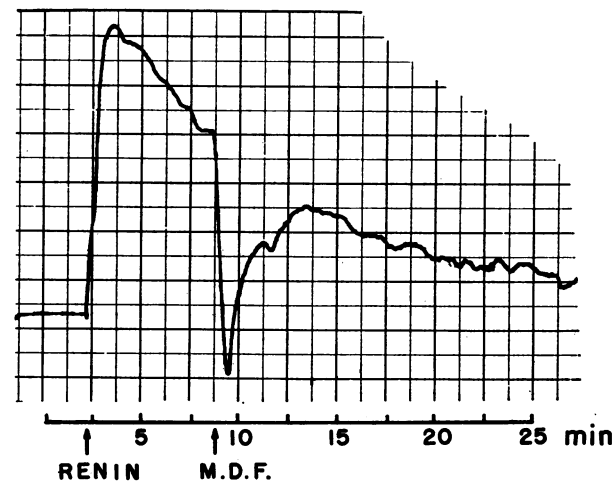

Fig. 10.—Capacity of rabbit renomedullary extract to neutralize transiently the vasopressor response to intravenously administered renin in the bioassay rat.

Medullary extract will completely neutralize the pressor response in the bioassay rat to intravenously administered renin, as shown in Fig. 10. However, after the acute vasodepressor response to the intravenous injection of extract had worn off in the bioassay rat, only a moderate reduction (approximately 25%) in the pressor response was observed on the subsequent injection of angiotensin, noradrenaline and renin (Fig. 11). Thus, blocking of a highly significant degree and duration after recovery from the vasodepressor phase of medullary extract was not observed against angiotensin or noradrenaline.

D. Discussion

The presence of vasoactive and myotropic lipids in animal tissue has been recognized for many years. In a recent review, Vogt[16] detailed at least two main groups of substances with such properties.

(1) *Phospholipids.*—In general, Vogt noted that acidic phospholipids contract smooth muscle and that the neutral phospholipids do not. Further,

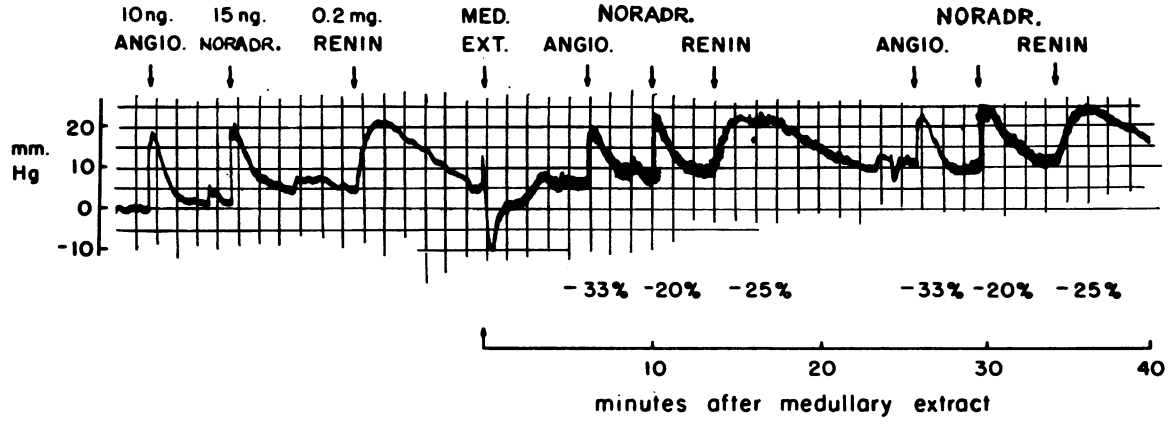

Fig. 11.—Effect of rabbit renomedullary extract on pressor response to angiotensin, noradrenaline and renin in the bioassay rat. After recovery from the vasodepressor response to injected extract, the vasopressor response to the subsequent injection of angiotensin, noradrenaline and renin was only moderately reduced, suggesting that the extract does not act, at least to a very significant degree or for a very significant duration, by blocking these normally circulating vasopressor factors.

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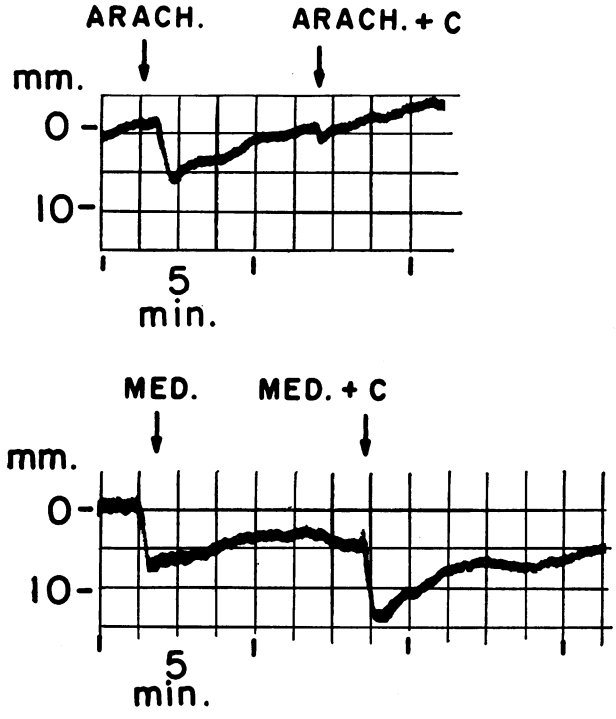

Fig. 12.—A comparison of the effect of ascorbic acid added *in vitro* (50 mg./ml. of solution) to arachidonic acid and rabbit renomedullary extract on vasoactivity on subsequent injection into the bioassay rat. As shown, arachidonic acid lost nearly all of its vasodepressor activity, whereas medullary extract did not show any loss, suggesting that the development of peroxides in the arachidonic acid may account for its activity but not for the activity of medullary extract.

increasing acidity and water solubility increase the myotropic effects of phospholipids, lysophosphatidic acid being the outstanding example of this group, having some 10 times the smooth-muscle-stimulating activity per unit weight as the next most potent myotropic phospholipid, phosphatidic acid (from lecithin). Further, the recent identification of the vasopressor factor liberated in incubated plasma as most likely being a lysophosphatidic acid[17] is consistent with this formulation. In our work, when the phospholipid fraction was removed from the vasodepressor-containing fraction of renomedullary lipid extract, the finding of an interfering vasopressor substance was shown (Fig. 4). Further studies have shown it to be extractable into water from petroleum ether, similar to the plasma factor reported by Khairallah and Page.[17] The vasodepressor effect invariably predominates over the vasopressor one in the chloroform-methanol extracts of rabbit renal medulla, but in some of the other species tested, notably dog and man, a slight pressor phase often preceded the predominant vasodepressor effect in the bioassay rat. *This serves to emphasize that any attempt at precise quantitation of the vasodepressor factor in renal medulla should include the removal of the phospholipid fraction prior to bioassay to eliminate interference from the pressor activity of acidic phosphatides.*

(2) *Simple unsaturated fatty acids.*—It has been thought that certain simple unsaturated fatty acids are also myotropic, notably arachidonic acid, which has been shown to induce a sustained vasodepressor response on administration to rabbits and dogs.[13] However, it has recently been demonstrated by Dakhil and Vogt[18] that such fatty acids derive their myotropic activity from oxidative contamination with hydroperoxides. This is quite consistent with our finding (Fig. 12) that the sustained vasodepressor response to arachidonic acid administration in the bioassay rat (approximately 1 mM I.V.) was almost completely abolished by reduction of peroxides through the *in vitro* admixture of the arachidonic acid standard with ascorbic acid (50 mg./ml. of solution). By contrast, the failure of ascorbic acid to modify the vasodepressor response of medullary extract (Fig. 12) would indicate that the activity was independent of any artefactual lipid peroxidation product.

(3) *Unsaturated hydroxy fatty acids.*—The final group of myotropic lipids to be considered are the unsaturated hydroxy fatty acids, which recently have been reviewed in detail.[19] This group includes the prostaglandins, which have been structurally elucidated, and which have been characterized as "a group of hormonal compounds of widespread occurrence".[20] A review of their biological effects in man and experimental animal preparations includes a prolonged lowering of arterial blood pressure, stimulation of most smooth muscles such as those of the intestine and uterus *in vitro* and *in vivo,* and inhibition of the motility of the nonpregnant human myometrium.[21] Eliasson[21] states that a number of experiments "support the hypothesis that prostaglandin is present in the tissue in a bound, inactive state and liberated by an enzymatic process".

Our chemical work to date raises the consideration that the active principle of the renomedullary extract may be in the group of myotropic, unsaturated, hydroxy fatty acids, although this must remain inferential until final identification is accomplished.

It is quite possible that the presence in the renal medulla in high concentration of such a vasoactive lipid, perhaps stored in a bound, inactive state, and which may be released under an appropriate physiological stimulus, could be indicative of a local renovascular (? medullary vasodilatation) and systemic cardiovascular homeostatic mechanism. Such a hypothesis would support the concept of a possible dual role of the kidney in blood pressure homeostasis. This will have to await extensive study for proper evaluation.

SUMMARY

A lipid, present in high concentration in the renal medulla of several species, including man, is described, with direct vascular smooth muscle effects, that produces a sustained fall in arterial pressure on intravenous administration in the bioassay rat and labora-

tory dog. In the rat and dog evidence of active renal vasodilatation by this substance is cited.

A similar factor could not be shown to be present, at least in such significant concentration, in a variety of other tissues, including the renal cortex, similarly extracted in the rabbit.

Chemical characterization to date would indicate that this is probably not an ordinary lipid (neutral fat, diglyceride, simple fatty acid, or phospholipid). Evidence for the possibility of its being in the class of myotropic, unsaturated, hydroxy fatty acids (e.g. the prostaglandins) is discussed.

A possible relationship between the presence of this factor in the renal medulla and the evidence suggesting an antipressor function of the normal kidney is considered.

The authors wish to express their appreciation to Dr. David Turner, Sinai Hospital, Baltimore, Maryland, for performing gas chromatographic analysis, and to Dr. Robert Griesemer and Dr. Robert Sheuplein of the Dermatology Research Laboratory, Massachusetts General Hospital, Boston, for performing infra-red absorption studies on several of our rabbit renomedullary extracts.

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Acute Effects of Angiotensin on Calcium, Phosphorus, Magnesium and Potassium Excretion

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WHEN angiotensin became available, we initiated studies on the effect of acute intravenous infusions of the hormone on urinary sodium and potassium excretion. Because of the reported relationship of urinary calcium to urinary sodium excretion,[1] and in order to obtain a spectrum of the effects of angiotensin on electrolyte excretion, measurements were also made of urinary and serum calcium, phosphorus, and magnesium.

METHODS

All studies were carried out on persons on an unrestricted sodium intake. After an overnight fast the subjects drank 1000 to 1200 ml. of water at 7:00 a.m. and an additional 200 ml. per hour until the completion of the study. Some of the subjects were studied in both erect and supine positions. Those studied in the erect position lay down one-half hour before the termination of the angiotensin infusion, to prevent postural hypotension. All the studies reported were carried out while the subject was supine unless otherwise indicated. Urines were collected every 30 minutes by voiding before, during, and over a one- to three-hour period following the infusion. The infusion, prepared by dissolving 0.5 mg. of valine-5 angiotension II in 1000 ml. of 5% dextrose in water, was begun by gravity drip into a forearm vein between 8:30 and 9:30 a.m. The rate was adjusted to deliver approximately 0.5 μg. per minute for the first hour, 0.8 μg. per minute for the second hour and 1.2 μg. per minute for the third hour. Blood pressure was determined frequently by a sphygmomanometer. Blood samples were obtained at the beginning and at the end of the infusion.

Eleven studies were performed using nine normal subjects. Two patients with primary hyperparathyroidism were studied and one of these was restudied after removal of a parathyroid adenoma. One patient with a pheochromocytoma was studied both preoperatively and postoperatively. One patient with primary aldosteronism was studied while untreated, during spironolactone therapy, and after removal of an adrenal adenoma. Two patients with essential hypertension were studied while supine and erect. One normal subject was studied while erect. Sodium nitroprusside (60 mg. in one litre of 5% glucose in water) was given intravenously at the same time as the angiotensin infusion in one normal subject to prevent a rise in blood

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