may influence the balance between angiotensin production and destruction, and only a portion of arterial blood has come directly from the kidneys. It is not surprising therefore that correlation with clinical hypertensive states is poor, except perhaps when the analysis is performed on renal vein blood. Renin assays on renal vein blood would seem to be less subject to error. More needs to be learned about the factors affecting the action of this enzyme on its substrate, but the conditions of its *in vitro* assay can be controlled. At the present time it appears that aldosterone secretion data may reflect better than anything else the total daily amount of angiotensin generated in patients with renal hypertension.

SESSION 8

Chairman: Professor Alberto Taquini

The Role of the Renin-Angiotensin System in the Hypertension Associated with Renal Vascular Disease

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THE role of the renin-angiotensin system in the etiology of the hypertension associated with renal vascular lesions has been debated for many years. The methods for measuring circulating renin or angiotensin have been fraught with difficulty. Indeed, it is an assumption that the circulating blood level reflects the concentration or quantity of renin and angiotensin at the arteriolar level. The recent demonstration that the renin-angiotensin system can stimulate aldosterone secretion provides another parameter for determining whether excessive amounts of renin or angiotensin are produced. If renin or angiotensin secretion is increased in renal vascular hypertension, the aldosterone production should be increased. Aldosterone excretion appears to be a particularly sensitive index of the renin-angiotensin system, in view of the claims that infusions of small doses of angiotensin II, which produce little or no change in blood pressure, stimulate aldosterone secretion.

The present study, therefore, was designed to determine if angiotensin II is produced in excess in the hypertension associated with renal artery stenosis, by measuring two separate indices of angiotensin production: (1) the measurement of aldosterone excretion or secretion by the isotope dilution derivative method of Kliman and Peterson;[1] (2) the estimation of plasma arterial angiotensin II concentration by the method outlined in Table I.

Arterial blood was rapidly cooled, plasma separated from the cells, the plasma proteins precipitated by trichloroacetic acid (TCA), the TCA and lipids extracted into ether and the aqueous phase containing the angiotensin chromatographed sequentially on IRC 50 and Dowex 50-X2 resin columns. The angiotensin-like material in this purified extract from the last column was estimated by assay at two or more dose levels in a rat bioassay that can be sensitive to 0.2 mμg. of synthetic aspartyl angiotensin II.

TABLE I.—METHOD FOR ESTIMATION OF PLASMA ARTERIAL ANGIOTENSIN II CONCENTRATION

Blood cooled rapidly
Plasma separated at —2° C.
TCA precipitation
TCA extracted with ether
Ion exchange chromatography
(a) IRC—50
(b) Dowex 50-X2
Rat bioassay

We measured angiotensin II rather than renin because angiotensin II is the active principle in this system. An elevated plasma renin value would not necessarily mean that angiotensin II was also elevated. A record of a typical dose response to angiotensin is illustrated in Fig. 1. The doses of angiotensin II employed varied from 0.4 to 1.2 mμg. The blood pressure was recorded from the carotid artery by means of a Statham strain gauge and Offner recorder. The numbers on top of the tracing indicate the mm. rise on paper following the injection of the standard. An 8-mm. rise is equivalent to about 5 mm. Hg. At times we can double the sensitivity by increasing the gain of the recorder.

Fig. 2 shows the results from an assay of angiotensin II-like material extracted from the plasma of one subject. Doses of standard angiotensin are indicated in mμg.; doses of unknown in microlitres. The figures in parentheses are the quantities of angiotensin calculated to be present in the dose of the unknown. Each dose of unknown is bracketed by doses of standard angiotensin that give slightly less and slightly greater pressor responses. The pressure recording was actually continuous from the upper left to the lower right. The shape of the pressor response to the plasma extract is similar

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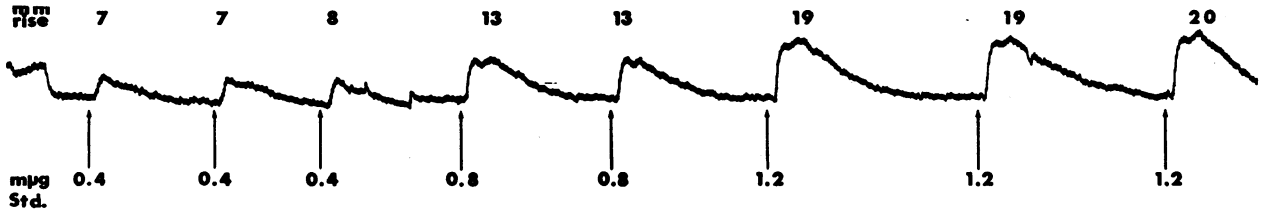

Fig. 1.—Typical dose response to angiotensin II.

to that following standard angiotensin, and the dose response to the different doses of plasma extract is fairly linear.

Table II shows the results of recovery experiments. From 10 to 150 mμg. of stable aspartyl angiotensin II and/or 2 to 4 mμg. of tritium-labelled aspartyl angiotensin II were added to plasma. The recovery of the stable angiotensin ranged from 36 to 59% and the recovery of the radioactive angiotensin from 51 to 73%. The mean recoveries and their standard errors are shown. We have no ready explanation for the discrepancy between the stable and radioactive angiotensin II. Part of this may well be due to the fact that the radioactive angiotensin was extensively purified before use while the stable angiotensin was not and may have contained some impurities. In any event, these experiments indicate that the method is reasonably consistent for the measurement of small amounts of angiotensin II.

The next step was to define the plasma concentration of angiotensin II in normal subjects. These results are shown in Fig. 3. The bar represents the mean angiotensin concentration, 2.3 mμg./100 ml. of plasma, in 22 control subjects. The S.E. is ± 0.3. The dotted line represents the range of angiotensin concentration from undetectable quantities to 6 mμg./100 ml. of plasma. The volume of shed blood that was analyzed varied from 75 to 260 ml.

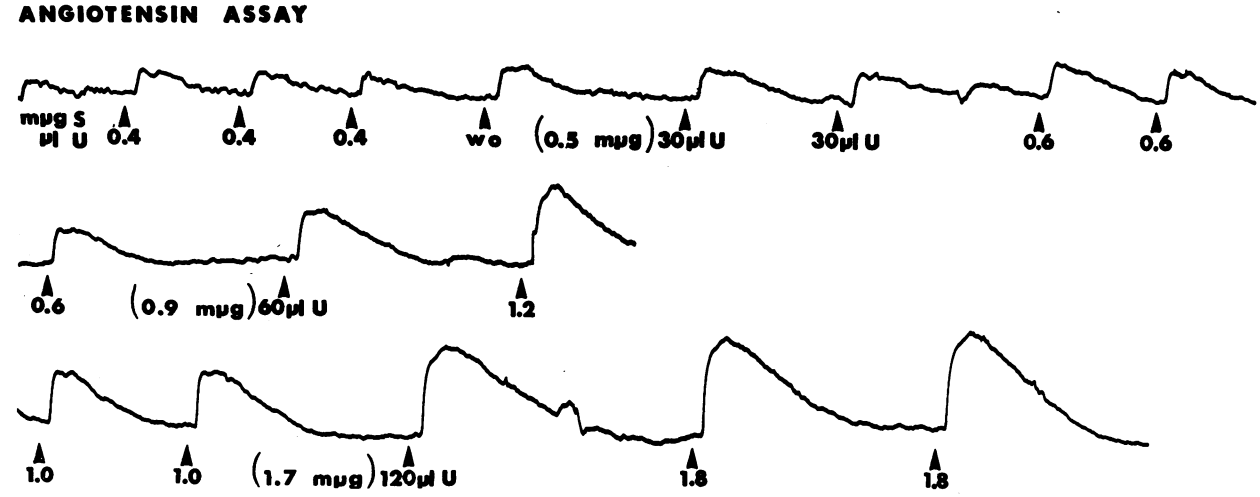

Fig. 2.—Dose response to standard angiotensin II in anesthetized, nephrectomized, pentolinium tartrate (Ansolysen)-treated male rat.

TABLE II.—PLASMA ANGIOTENSIN II RECOVERY EXPERIMENTS

	Addition (mμg.)	*No.*	*% Recovery (mean ± S.E.)*
Stable	10 to 150	8	46 ± 2
Radioactive	2 to 4	10	63 ± 3

Recovery of angiotensin II added to plasma. Stable indicates valine-5, aspartyl angiotensin II; radioactive indicates tritium-labelled valine-5, aspartyl angiotensin II. Under the first column is shown the quantity of angiotensin II that was added in mμg. No. means the number of experiments. In six experiments, stable and radioactive recoveries were estimated simultaneously.

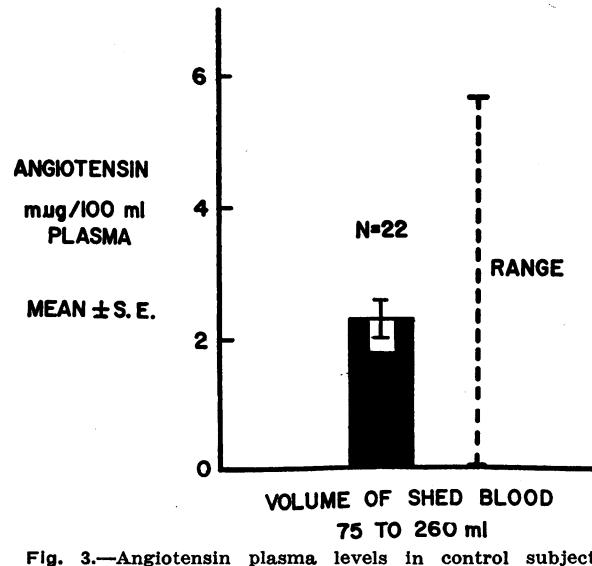

Fig. 3.—Angiotensin plasma levels in control subjects. Assay of human plasma extract for angiotensin II.

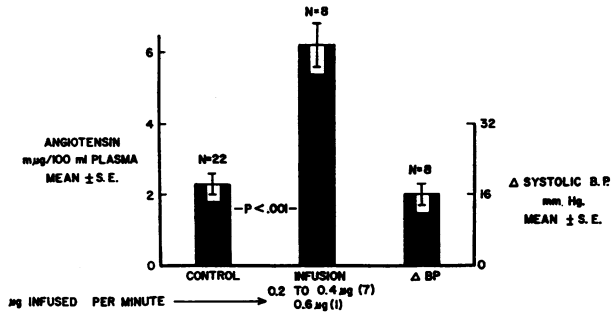

Fig. 4.—Plasma angiotensin during angiotensin infusion. Volume of shed blood indicates the volume of blood collected for analysis.

The next step was a really important one. Could this method detect an increase in plasma angiotensin concentrations during the infusion of small doses of angiotensin? In order to study this, angiotensin was infused at the rate of 0.2 to 0.4 μg. per minute in seven subjects, and 0.6 μg. per minute in one subject. The infusions were carried out for from 50 to 100 minutes. During the latter part of the infusion, after the blood pressure had stabilized, a sample of arterial blood was collected for the measurement of angiotensin II.

Fig. 4 illustrates the results of these experiments. On the left is the scale for angiotensin in mμg./100 ml. of plasma. On the right is the scale for the change in systolic blood pressure in mm. Hg. The mean plasma angiotensin concentration in the subjects receiving infusions of small doses of angiotensin is significantly different from the mean angiotensin concentration in the control subjects.

We then studied a group of 16 patients with mild to moderate hypertension and associated renal vascular disease. The types of renal vascular lesions ranged from bilateral and unilateral renal artery stenosis, aneurysms, branch renal artery lesions to intrarenal vascular anomaly. All the patients were on *ad lib.* sodium diets and were not receiving antihypertensive or diuretic therapy, and had normal serum potassium concentrations at the time of study.

Fig. 5 shows the results of these studies. The dots represent individual measurements. Twenty-two aldosterone excretion rates were measured in 16 patients. In six patients, aldosterone secretion rates were measured. Plasma arterial concentration of angiotensin was measured 12 times in 11 patients. One patient had two separate measurements, three months apart.

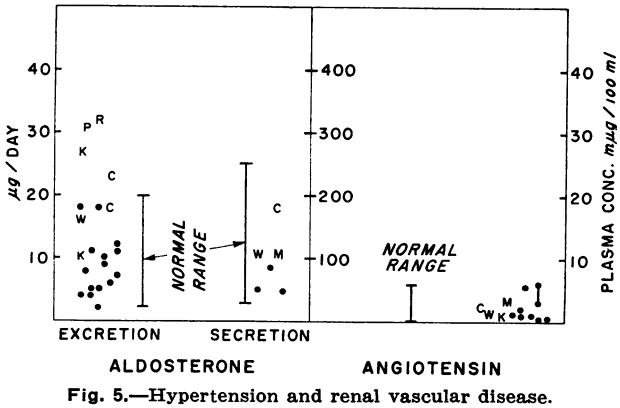

Fig. 5.—Hypertension and renal vascular disease.

The letters indicate the patients cured by surgery. Fourteen of the 16 patients underwent surgery. Six were cured or improved. The follow-up in some is too short to be certain of the total picture. Most of the aldosterone excretion rates and all of the secretion rates were within the normal range. The arterial plasma angiotensin concentrations were within the normal range. Two were at the upper limits of normal. In the "cured" patients, the evidence is strong that the hypertension was related to the renal vascular lesion. Subjects P and R were studied before we were in a position to estimate plasma angiotensin and did not have angiotensin measurements. R was a 5-year-old boy with moderately severe hypertension.

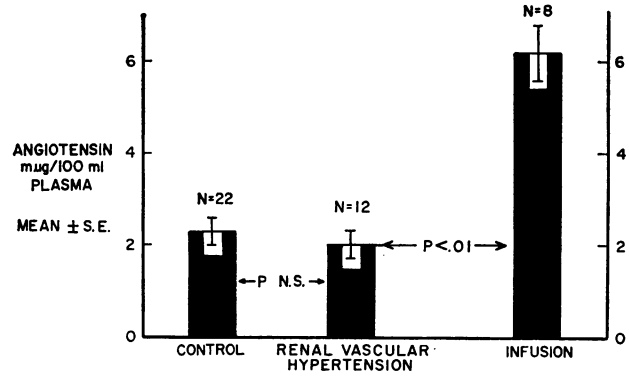

Fig. 6.—Comparison of the plasma angiotensin concentrations in control subjects, patients with hypertension and renal vascular lesions and in normal subjects during infusions of small doses of angiotensin II.

The aldosterone excretion is very high for his age group. After operation it fell to 0.8 μg./24 hours. After surgery subject P had excretion rates of 6 and 8 μg./24 hours. Subject K had two separate measurements of aldosterone excretion. One was moderately elevated, at 27 μg./24 hours; the other was definitely normal. Her blood pressure did fluctuate considerably and on one occasion, following mild sedation when her blood pressure was normal, the plasma angiotensin concentration was normal. Subject C had one normal excretion rate, one normal aldosterone secretion rate and a normal arterial plasma angiotensin concentration. Subjects W and M had normal aldosterone excretion and secretion rates and plasma angiotensin concentration. Although the cured patients tended to have higher aldosterone excretion rates, this was by no means consistent or universal. Except for the excretion rate of the 5-year-old boy, the highest aldosterone excretion rates were not very high and much less than the excretion rates found after six days of salt depletion.

Fig. 6 presents a summary of the angiotensin results. The first bar shows the mean ± S.E. of the plasma angiotensin concentration of 22 normal sub-

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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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This work was supported by the John A. Hartford Foundation and U.S. Public Health Service Grant No. HE-07521-01.
*Career Development Award Program, U.S. Public Health Service Grant No. HE-K-14, 903-C1.
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