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Peripheral Arterial and Renal Venous Angiotensin Concentration.* (30715)

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(Introduced by D. H. Blankenhorn)
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Investigations of the renal pressor proteinaceous material called renin have clarified its enzymatic characteristics(1,2). The active product, angiotensin, has been purified, synthesized, and biologically characterized, but its role in health and disease is still controversial(3). Its pressor characteristic has suggested it to be a link in the etiology of hypertension, in particular, renovascular hypertension(4); yet its aldosterone stimulating property has suggested a role in the control of sodium balance(5). If angiotensin is of importance either in hypertension or in salt metabolism, angiotensin blood concentrations would be useful in defining its role. Prior measurements have been made without regard to salt intake largely in patients or animals with various hypertensive disorders(6,7,8,9, 10). Under these conditions, blood concentrations have been reported to be both elevated and normal. The study reported herein was designed to overcome theoretical disadvantages present in other studies by 1) rigorously controlling sodium intake and 2) by sampling renal venous blood, presumably the source of circulating angiotensin. In addition, renal venous concentrations were compared to peripheral arterial concentrations in various patients.

Methods. A. Patient material. Each patient was studied on a metabolic balance ward with the exceptions mentioned. A constant diet containing less than 10 mEq sodium was given throughout the study period. All medications were discontinued and none were administered during study. Patients were either normal (arterial pressures normal; response to sodium restriction normal) or hypertensive. Patients with hypertension had either renovascular lesions demonstrated by aortography or essential hypertension. After each patient came into balance as demonstrated by stable weights and urinary sodium for three days, a percutaneous renal vein catheterization study was performed using Seldinger technique(11) combined with cinefluorography. Catheter location was checked by the rapid injection of 2 ml 50% hypaque which allowed visualization and recording of the renal vein outline (Fig. 1). Fifty to 100 ml of blood were aspirated from each renal vein for angiotensin assay. Afterwards each patient was placed on 12 g of sodium chloride daily (>200 mEq Na) while keeping the diet otherwise constant. On the high sodium intake renal vein catheterization was repeated after body weight and urine sodium were stable. Therefore, in each patient, renal venous samples were analyzed for angiotensin concentration during sodium deprivation and during sodium surfeit.

Peripheral venous blood was obtained twice weekly for blood urea nitrogen, hemoglobin, hematocrit and serum bicarbonate, sodium, potassium, and chloride. An aliquot of 24-hour urine samples was analyzed daily for sodium, potassium and creatinine. Arterial blood pressures were measured every six hours in the recumbent position. In cases identified by Table II a percutaneous renal

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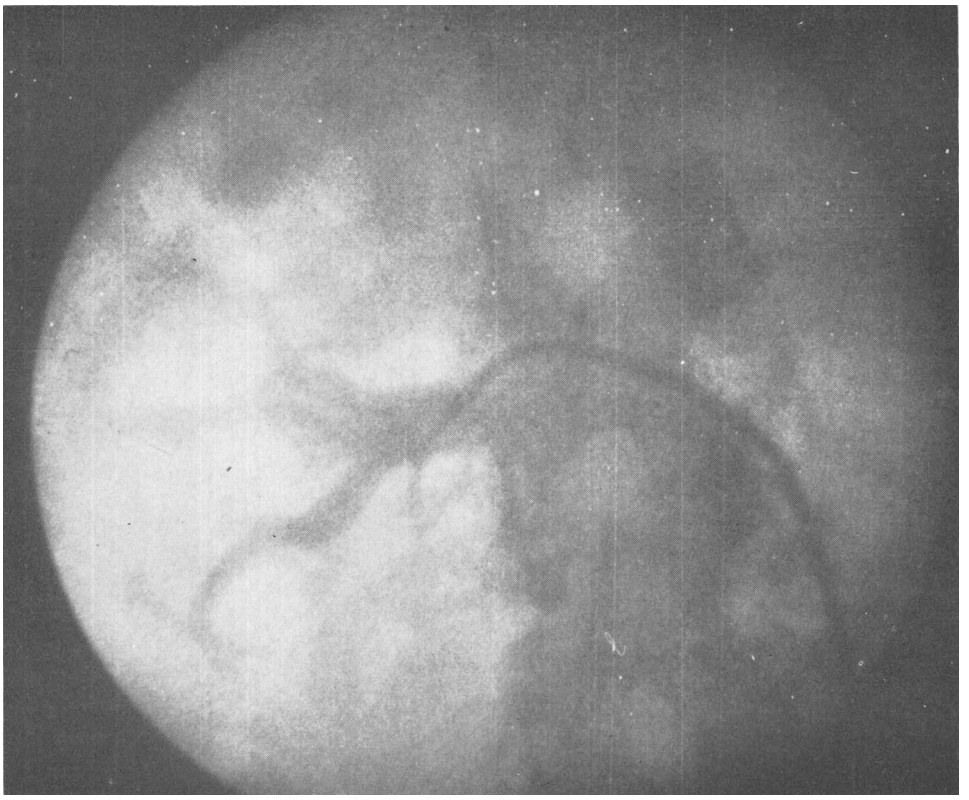

FIG. 1. Renal venous outline with catheter in renal vein.

biopsy was performed, fixed by Zenker's formalin and stained with hematoxylin and eosin.

B. Method of angiotensin assay. Each sample of renal venous blood was collected into 4 volumes of cold 95% alcohol. After precipitation of blood proteins the extract was filtered across Whatman #2 paper on a Buchner funnel and the residue was washed with 80% alcohol. The combined filtrate was acidified by 1 ml glacial acetic acid. The time between collection of the sample and completion of filtration was always less than 30 minutes. The filtrate was evaporated to approximately 100 ml and extracted twice with 1.5 volumes of ether. The aqueous filtrate was further evaporated to approximately 25 ml and its pH was adjusted to 6.0. The solution was passed across a Dowex 50-WX-2 column equilibrated with 0.3 N ammonium acetate (pH 5.95-6.05). The column was eluted with 25 ml 0.1 N ammonium hydroxide. The eluate was lyophilized and the residue was streaked on a line for descending chromatography and run in secondary butanol, isopropyl alcohol, acetic acid and water (7:7:5.2). After running 12-16 hours at 24°C the paper was cut into 2.5 cm segments and eluted with a mixture of ethanol, water and 1 N hydrochloric acid (50:45:5). The eluates were evaporated in a rotary evaporator and the residues were dissolved in 1 ml of normal saline. Each was assayed in a bilaterally vagotomized, pentolinium-treated rat by measuring the response of its carotid arterial blood pressure to an intravenous dose according to a 4-point bioassay design. Synthetic Val-5-angiotensin beta-aspartyl amide (Ciba) was used as a standard. This is essentially the method of Boucher, Biron and Genest(12) with modifications.

The portion of the chromatogram used in the final assay was determined by comparing results obtained from nephrectomized dog blood samples to those from nephrectomized dog blood plus angiotensin (Fig. 2). The en-

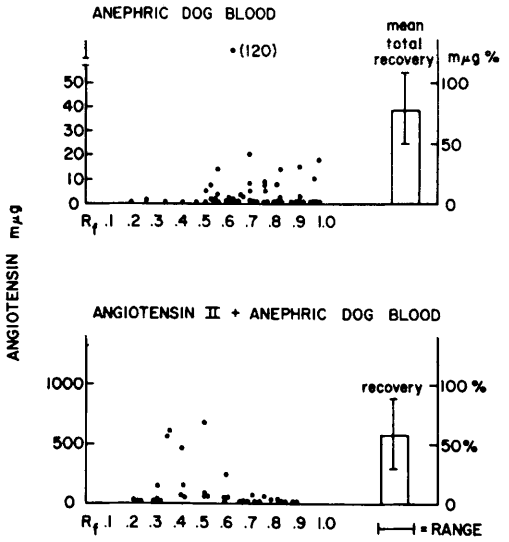

FIG. 2. Chromatogram R_f position of pressor material recovered from nephrectomized dog blood and from nephrectomized dog blood plus added synthetic angiotensin.

tire chromatogram is assayed by 2.5 cm segments. Angiotensin ran with an R_f between 0.3 and 0.6 so that all patient material assayed came from strips of the chromatogram between an R_f 0.3 and 0.6. Rats used in bioassay were discarded if their response was less than a 10-15 mm Hg rise in carotid arterial pressure after 10 mμg angiotensin intravenously. The nature of the rats arterial pressure response is of some importance since several types of arterial pressure curves are observed after an intravenous injection of extract (Fig. 3). In this study we compared the results from angiotensin type pressor response curves to the combined pressor response from all types of curves. In the latter case if there was a measurable pressor response, either typical or atypical, this was taken as evidence of angiotensin activity. Such a comparison revealed no difference between the results from typical angiotensin curves and atypical pressor curves. Furthermore when synthetic angiotensin was mixed with a solution giving an atypical pressor response the character of the pressor response was not altered. Therefore, the results reported herein include all pressor responses taken from the chromatogram between an R_f 0.3 and 0.6.

Sodium and potassium were measured by a Baird-Atomic flame photometer, using lithium as an internal standard. Blood urea nitrogen, chloride and creatinine by autoanalyzer method N-1a (diacetylmonoxime reagent), N-5a and N-11a respectively; hematocrit by Wintrobe tubes.

Results. Recovery. Twelve samples of arterial dog blood (50 ml) were obtained one hour after nephrectomy. Each was collected into alcohol and known amounts of angiotensin were added immediately. The amount of angiotensin recovered was between 30-70% when 100-10,000 mμg% was added. Twelve other samples of nephrectomized dog blood were run as blanks and the results showed a value of 62 ± 28 mμg%. None of the values reported herein are corrected for blank.

Peripheral arterial angiotensin concentration in normals:

Seven patients with normal arterial pressure had peripheral arterial angiotensin concentration of 252 ± 56 mμ% ± SEM. Four of these patients were given an intravenous infusion of angiotensin (0.5-2.25 μg/min) to raise their diastolic arterial pressure 20 mm Hg. An arterial blood sample was obtained before and during the angiotensin infusion. Angiotensin concentration was 198 ± 34 mμg% before the infusion and 1356 ± 785 mμg% during the infusion. Each of these normal patients was on an uncontrolled sodium intake (Table I).

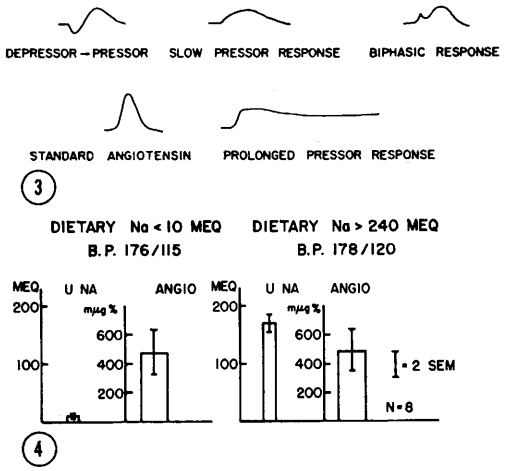

FIG. 3. Characteristics of pressor responses from paper chromatograms of whole blood extracts.

FIG. 4. Urinary sodium and angiotensin during dietary sodium deprivation and excess.

TABLE I. Peripheral Arterial Angiotensin Concentration.

Patient	Angiotensin (mμg %)	Angiotensin during infusion (mμg %)
Normotensives		
T.B.	526	
B.G.	246	
J.C.	130	307
M.E.	267	523
M.F.	170	3447
W.F.	225	1150
C.H.	206	
Mean ± SEM	252 ± 56	1356 ± 785
	(P <.05, paired t)	
Hypertensives		
J.G.*	000	
F.K.	385	
L.H.	353	
R.P.	360	
V.S.*	460	
G.P.*	000	
M.W.*	25	
	226 ± 65	

* Malignant hypertension.

Peripheral arterial angiotensin concentrations in patients with essential hypertension:

Seven patients with essential hypertension had peripheral arterial angiotensin concentration of 226 ± 65 mμg% (P>.05 when compared to normotensive patients). Four of these patients had malignant hypertension and in 2 of these angiotensin was not detectable. These patients were on an uncontrolled sodium intake (Table I).

Renal venous angiotensin concentration in essential hypertension:

Eight patients with essential hypertension underwent percutaneous renal vein catheterization while on a low sodium intake and while on a high sodium intake. Each patient had an aortogram which visualized normal renal arteries. Five of these patients had evidence of nephrosclerosis in renal biopsies (Table II). On less than 10 mEq sodium intake renal venous angiotensin was 491 ± 148 mμg% (average of both sides) and on a high sodium intake renal venous angiotensin was 497 ± 177 mμg% (P>.05). Also when the two sides were compared, right and left renal venous angiotensin concentrations were 617 ± 294 mμg% and 333 ± 108 mμg% (low sodium intake, P>.05); 651 ± 288 mμg% and 356 ± 133 mμg% (high sodium intake, P>.05), respectively. The arterial pressure remained the same during each period but urinary sodium reflected intake (Fig. 4).

Renal venous angiotensin concentration in renovascular hypertension:

Six patients with aortographic evidence of main branch renal artery stenosis with poststenotic dilatation and with no vascular abnormality on the opposite side were studied (Table II). The results show that in some cases angiotensin was higher on the side of involvement but in others it was higher on the opposite side. Of interest are the higher angiotensin concentrations on the uninvolved side when the patients were on a high sodium intake. Three of these patients were treated by surgical correction of the renal artery stenosis; only one improved but was not cured (D.C.) after 3 years of follow-up.

Discussion. The great degree of uncertainty about the significance of angiotensin in human physiology is related to the lack of confidence in previously reported measurements. From nephrectomized dog blood synthetic angiotensin II is recoverable to a variable degree (30-70%). Such a variable recovery is

TABLE II. Renal Venous Angiotensin Concentration.

		Low Na		High Na	
Patient		Right	Left	Right	Left
Essential hypertension					
W.G.*		90	80	34	82
O.B.		184	142	52	56
M.G.*		2062	312	398	308
A.B.		762	236	2308	516
V.B.			868	496	246
M.F.*		490	440	914	740
J.S.*		744	588	912	906
V.S.*		000	000	000	000
Mean		617	333	651	356
SEM ±		294	108	288	113
Renovascular hypertension					
Patient	Diet	Involved side		Uninvolved side	
D.C.*	NC	2360		73	
R.L.*	NC	000		000	
A.O.*	Lo Na	506		246	
	Hi "	80		146	
W.M.	Lo "	48		316	
	Hi "	62		316	
E.D.	Lo "	282		632	
	Hi "	162		482	
J.T.*	Lo "	2912		470	
	Hi "	526		1240	
E.M.*	Lo "	000		146	

* Renal biopsy.
NC = Diet not controlled.

TABLE III. Angiotensin Measurements.

Author	Normal dog	Hypertensive man
Gollen, Richardson, Goldblatt 1948, 200 ml blood	.0 mμg %	60-70 mμg %
Scornik, Paladini	1.2-2.6 mμg %	1.6-28.0 mμg % (acute) 1.2- 4.4 " (chronic)
	Normal man	Hypertensive dog
Kahn, Skeggs, Shumway, Wisenbaugh 1952, 250 ml blood	.3-1.6 mμg %	.7- 2.0 mμg % (benign) 2.0-12.0 " (malig)
Genest, Boucher, deChamplain, Veyrat, Chretien, Biron, Roy, Cartier 1964, 50-200 ml blood	7.0 ± 2.0 (SEM) mμg % .0-55.0 (Range)	30.0 ± 8.0 " (benign) *.0-280.0 " 15.0 ± 7.0 " (malig) *.0- 70.0 "
Mulrow 1964, 75-260 ml blood	2.3 ± .3 mμg % (plasma) .0-6.0 "	2.0 ± .3 " (renov) .0- 6.0 "
Genest, *et al*		51 ± 21.0 " (renov) *.0-252.0 "

* Range = SEM × n.

in contrast to both the results reported by Boucher(12) where blood was obtained from humans for recovery studies and the results reported by Paladini(13) who subtracted blank values to ascertain recovery from non-nephrectomized dog blood. Both suggested that from 90-100% of added angiotensin was recoverable, although Paladini used "prepared angiotensin" as a standard instead of synthetic angiotensin II. Our studies show that a blank value exists in nephrectomized dog blood. Interpretation of patient data should consider this background value. Most of our patient data exceeds this value, but in some instances the patient value was even less than mean blank. Of course with recoveries as low as 30% a single measurement could be far less than that actually present so that changes in concentration might be obscured by the poor recovery.

Our values are higher than most of the values reported in the literature. This may be partly due to our use of total pressor response as a measure of angiotensin in activity. Table III shows data(6,7,8,9,10) converted from Goldblatt units or rat units by a conversion factor of 3.2-3.5 Goldblatt units/μg(14) or 200-250 rat units/μg of angiotensin(13). In fact nephrectomized dog blood samples contain enough pressor material to account for most of these reported values. An appreciation of this fact might be enhanced by consideration of an example. If 5 chromatogram strips are assayed it is not unusual to find that 2 or 3 of the strips will cause an increase in arterial blood pressure by 5 mm Hg. If the rat responds to 10 mμg of angiotensin by an increase in blood pressure by 10 mm Hg, then a close estimate of the angiotensin equivalent will be 5 mμg/strip. When this is multipled by 5 then a total of 25 mμg is found in a 50 ml sample of blood (starting sample) or 50 mμg%. This is the order of magnitude found by most investigators, yet our values from patients were one order of magnitude higher. This by no means supports the specificity of the method in our hands but highlights the difficulties in interpretation of prior data.

Data from Kahn *et al*(6) suggest that peripheral arterial angiotensin is elevated in malignant hypertension, but not in benign hypertension. Conversely, Genest *et al*(8) report peripheral arterial angiotensin concentration to be elevated in benign hypertension, but not in malignant hypertension. We were unable to demonstrate a difference in peripheral arterial angiotensin concentration between normotensive and hypertensive patients. Furthermore the concentrations were considerably less than the amount found during an infusion sufficient to elevate arterial blood pressure ($P<.05$). This was so even in renal venous blood obtained from patients

with essential hypertension. In fact, renal venous and peripheral arterial samples showed no significant difference in angiotensin concentrations.

In the dog arterial blood concentrations of angiotensin have been reported to be increased in the salt-deprived state(15,16). However, we could not find a similar change in angiotensin during the two states in hypertensive humans. The renal venous levels were the same on a high sodium intake as they were on a low sodium intake. Renin activity is reported to be increased in peripheral venous blood of salt-deprived man(17). How to reconcile the elevated renin activity without an increase in angiotensin activity is not clear but it could be that the rate of synthesis and destruction of angiotensin in the circulation is such that a change in circulating angiotensin is not detectable while the amount utilized during salt deprivation is increased. In the salt-deprived state, for example, angiotensin could be present in the adrenal gland in greater concentration but still not be elevated in the peripheral circulation. Bumpus *et al*(18) do not control salt intake in their tritium angiotensin localization studies, but it is known that the aldosterone stimulating properties of renin administered intravenously last for hours(19) and that renin might be stored(20).

Finally several studies suggest that the renin-angiotensin system is the cause of renovascular hypertension(4,9). In fact it is suggested that when there is unilateral renal venous angiotensin elevation, surgical removal of that kidney will effect a cure of the hypertension(9). We were unable to correlate the presence of a clear-cut lesion with renal venous angiotensin concentration and, in fact, the highest concentration might occur opposite the side of involvement (patient J.T.). Since we have not studied any patients whose hypertension was cured by surgery, it is unfair to exclude the possibility that angiotensin measurements will not be of help in predicting surgical results. Likewise our studies do not exclude the possibility that renin measurements might be abnormal in essential hypertension or in renovascular hypertension. Yet Scornik and Paladini(16) in dogs, and Blaquier, Bohr and Hoobler(21) in rats have accumulated evidence against a humoral pressor component in chronic renovascular hypertension.

The results indicate a ceiling for angiotensin-like activity: in arterial blood 252 ± 56 mμg%, in renal venous blood 356 ± 108 mμg%. If physiologic variations occur they must clearly exist below these concentrations. The present method fails to show significant changes in angiotensin concentration in response to changes in dietary sodium intake or renal artery constriction.

Summary. Nephrectomized dog blood contains pressor material in very low concentrations (62 ± 28 mμg%). Recovery of synthetic angiotensin II was variable (30-70%). Peripheral arterial angiotensin concentration was elevated by an infusion of angiotensin, but patients with essential hypertension had angiotensin concentrations lower than during the infusion and their angiotensin concentration was the same as in normotensives. Sodium intake did not influence angiotensin concentration in renal venous blood. The presence of renal artery stenosis did not elevate renal venous angiotensin concentration on the affected side.

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Hemagglutinins of Human Parotid and Submandibular Secretions.* (30716)

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Various investigations have indicated that antibodies may be present in human and animal saliva. Salivary proteins, serologically related to plasma albumin and α, β, and γ globulins, have been identified(1-5). It has been claimed that diphtheria antitoxin(6) and agglutinins against *Brucella melitensis*(7) are present in human mixed saliva. Kraus and Konno(8), in a study of the capability of indigenous human oral bacteria to stimulate the production of antibodies, have stated that antibodies appeared in whole saliva only when they were present in serum. Utilizing parenterally administered I^{131} labelled proteins, some of which subsequently appeared in saliva, Tung and Schein(9) have concluded that proteins are transferred from blood to saliva.

These findings imply that the salivary glands may permit passage of certain serum proteins. The present effort was directed toward obtaining further knowledge as to the origin of salivary proteins.

Materials and methods. Human parotid fluid, submandibular fluid, and serum were assayed for tetanus and diphtheria antibodies using the hemagglutination procedure described by Levine *et al*(10). The cells were prepared by suspending defibrinated horse blood in 10 volumes of sterile saline, mixing and centrifuging (5 minutes at 2,000 rpm) 3 times. One milliliter of the washed, packed cells was diluted with 40 ml buffered saline (pH 7.2) to obtain a 2.5% suspension. The cells were tanned by adding one volume of 1:25,000 reagent grade tannic acid in saline to an equal volume of 2.5% red cell suspension in pH 7.2 buffer and the mixture incubated at room temperature for 10 minutes. This was followed by a 10-minute centrifugation at 1500 rpm and 2 washings with pH 7.2 buffered saline. The cells were then resuspended to 2.5% and separate portions sensitized to diphtheria and tetanus toxoids as follows: 4 volumes of pH 6.4 buffered saline containing sufficient toxoid such that the final mixture was 30 Lf per milliliter was added to one volume of 2.5% tanned cells. The incubation was at room temperature, followed by centrifugation at 1,500 rpm for 5 minutes and one washing with 1:250 normal rabbit serum in saline which had been inactivated at 56°C for 30 minutes and filtered.

The samples to be tested were also inactivated at 56°C for 30 minutes and dilutions made in saline containing 1% normal rabbit serum so that 2-fold serial dilutions would bracket the end point. The 2-fold serial dilutions in 1% NRS were performed to leave

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