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Research Article

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PLASMA ANGIOTENSINASE ACTIVITY IN PATIENTS WITH HYPERTENSION AND EDEMA *

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An abnormal metabolism of angiotensin II in essential hypertension has been suggested by several studies. Wood (1) reported that the venous blood of hypertensive patients showed a decreased capacity to inactivate an *in vitro* admixture with synthetic angiotensin [1] as judged by the pressor response on reinfusion into the hypertensive donors. Wolf and associates reported a prolonged radiochemical half-life of I^{131}-labeled angiotensin in hypertensive patients after intravenous administration (2), but found a shortened radiochemical half-life in a purely *in vitro* system (3). Klaus (4) described a system in which the measurement of the amount of valine freed from synthetic valyl-5-angiotensin II after an *in vitro* admixture with plasma was equated to angiotensinase activity and reported no significant difference between hypertensive and normotensive plasma.

Thus, in the *in vitro* system employed, there has been suggested an increase, a decrease, and no change in the angiotensinase activity of the hypertensive plasma.[2] Using the pressure response of the subject on reinfusion of an *in vitro* admixture leaves the difficult problem of controlling differences in vascular tone and reactivity between hypertensive and normotensive individuals. The *in vitro* chemical methods leave the difficult problem of equating the chemical end-point to residual biological activity. It seemed that an *in vitro* system employing the loss of pressure response in a standard bioassay rat preparation to the serial injection of an admixture of plasma and angiotensin might obviate some of these difficulties and contribute to an understanding of this intricate problem.

MATERIALS AND METHODS

A. *The assay.* An *in vitro* admixture of synthetic angiotensin II [1] and plasma was studied for the progressive loss of pressor activity by serial injections into a 250-g vagotomized rat prepared with Nembutal and pentolinium. The left jugular vein was cannulated with polyethylene tubing and injected with a microsyringe containing a standard (1 μg per ml) dilution of angiotensin II (preserved as a 20% ethanol solution). The right jugular vein was similarly connected to a microsyringe containing a mixture of 1 ml of the standard angiotensin solution and 1 ml of plasma. A stop watch was started at the time of the admixture, and room temperature was constant at $25 \pm 1°$ C. Alternate equal doses of standard angiotensin solution and standard solution mixed with plasma were administered every 3 to 4 minutes. The right carotid artery was cannulated with polyethylene tubing and the arterial pressure response recorded on a direct-writing pen-float mercury manometer. At the top of Figure 1 is shown the rat's pressure recording in a study on the plasma from a normotensive subject. The hatched spikes represent the serial pressure response of the plasma-angiotensin mixture and show the progressive reduction in the response; the nonhatched spikes represent the serial pressure response of the standard angiotensin solution alone and show full sensitivity of the rat to the serial injection of 10 mμg throughout the study. Variations, particularly a decrease of the standard response by more than 2 mm, are unsatisfactory and indicate that the study should be repeated. A minimal rat sensitivity of 1 mm Hg pressure rise per mμg of injected angiotensin is required. In a given study the serial dose was either 5, 10, or 15 mμg, whichever was necessary to ensure a pressure response in the range of 10 to 20 mm Hg, the same dose being held throughout the study for both the standard and the standard plus plasma

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[1] Ciba Pharmaceutical Co., Summit, N. J.

[2] Plasma angiotensinase activity is used in this paper for convenience to designate the capacity of plasma to inactivate synthetic angiotensin, although a specific plasma enzyme, as such, has not been isolated to date.

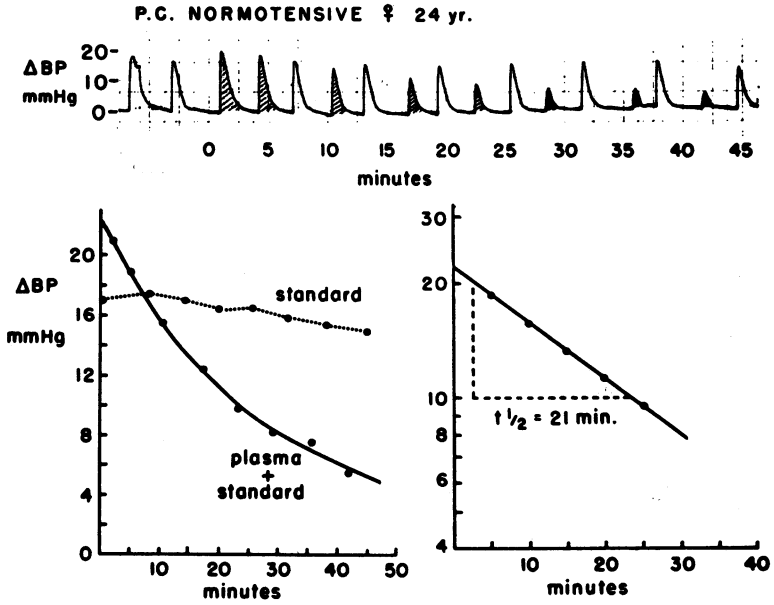

FIG. 1. ARTERIAL PRESSURE TRACING IN THE BIOASSAY RAT PREPARATION, SHOWING PROGRESSIVE LOSS OF RESPONSE (HATCHED AREAS) TO THE PLASMA-ANGIOTENSIN MIXTURE COMPARED WITH THE FULL RESPONSE (NONHATCHED AREAS) TO THE ANGIOTENSIN STANDARD. Shown below is the plot of the data and the determination of the biological half-life of angiotensin in human plasma.

mixture. At this low dose range, the steep or linear part of the logarithmic relationship between dose and response pertains. Figure 2 demonstrates this linearity in 16 different test rats, representing the rise in pressure to

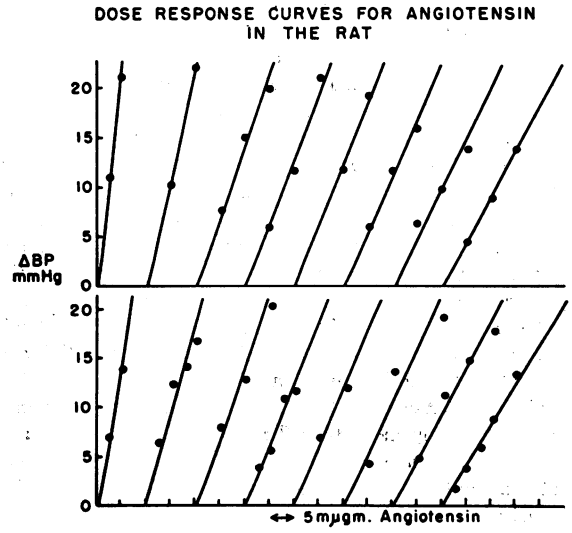

FIG. 2. LINEAR RESPONSE OF ARTERIAL PRESSURE TO SMALL INCREMENTS IN THE ANGIOTENSIN DOSE ADMINISTERED (0 TO 15 MμG) IN 16 DIFFERENT BIOASSAY RAT PREPARATIONS.

increments in the angiotensin dose from 0 to 15 mμg. Thus, a given reduction in pressure response during the assay for angiotensinase activity may be equated to a proportionately equal reduction in the amount of angiotensin remaining in the plasma-angiotensin mixture. The regression curve for this progressively smaller pressure response is shown on the lower left of Figure 1, indicating first-order kinetics, which has been the characteristic response in all plasma samples tested. This was corrected to the starting point of the standard response before plotting to compensate for any slight variation in the standard curve, and plots as a straight line against a logarithmic ordinate, shown on the lower right of Figure 1. From this, the *in vitro* biological half-life of synthetic angiotensin II in plasma may be read as the minutes required for a 50% reduction in pressure response; the shorter the biological half-life, the greater the plasma angiotensinase activity presumed to be present.

Since there was no observed pressor response to administration of plasma alone in the volumes involved in the assay (.003 to .075 ml), it is assumed that there was no error introduced by the possible presence of endogenous renin or angiotensin in the plasma being assayed.

Evidence suggesting that the reaction may be enzymatic in nature is cited as follows. Chilling the angiotensin-plasma mixture in ice water almost completely inhibited the degradation of the angiotensin, and preheating the plasma at 57° for 30 minutes before adding

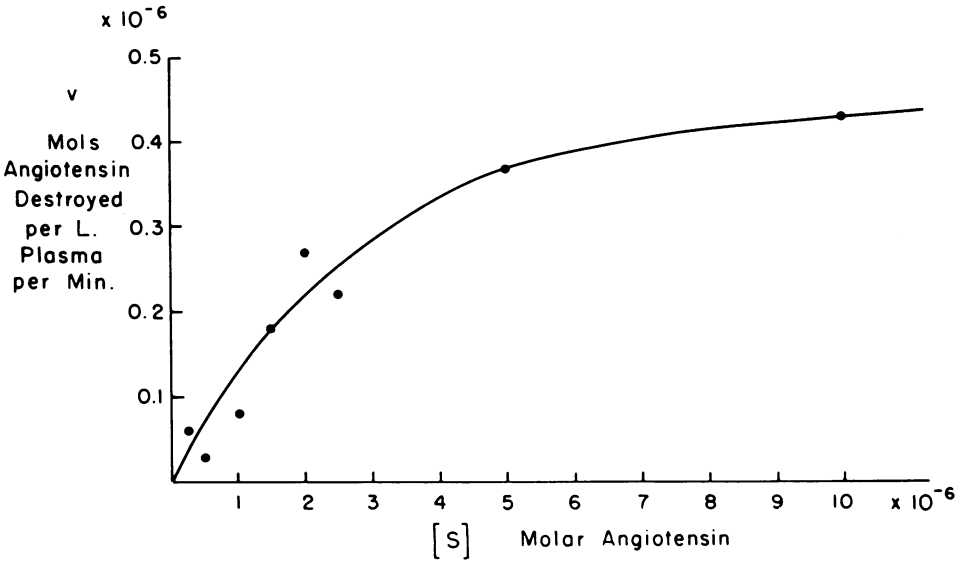

FIG. 3. DEMONSTRATION OF THE PHENOMENON OF SUBSTRATE SATURATION, EVIDENCED BY THE PLATEAU IN THE VELOCITY OF THE ENZYMATIC REACTION (MOLES ANGIOTENSIN DESTROYED PER LITER PLASMA PER MINUTE) WHEN THE SUBSTRATE CONCENTRATION REACHES THE RANGE OF 5×10^{-6} M ANGIOTENSIN.

angiotensin markedly decreased its capacity to inactivate the angiotensin. The velocity of the reaction was studied with increasing concentrations of added angiotensin solutions (1 ml of solution added to 1 ml of plasma) in one normal subject. An increasing rate of angiotensin inactivation was found up to a concentration of 10 mμg per ml added to the plasma, above which concentration the rate leveled off sharply in a manner characteristic of the phenomenon of enzyme saturation. This relationship is plotted in the conventional fashion in Figure 3, showing substrate concentration as moles per L angiotensin in the final solution and enzyme velocity as moles of angiotensin destroyed per L plasma per minute. Finally, the effect of varying enzyme concentration on the reaction velocity was studied by varying the plasma concentration in the final mixture with angiotensin standard solution between 25 and 60%. In the two plasmas so studied, a linear relationship was found between the plasma concentration and the percentage of angiotensin destroyed (Figure 4). This is characteristic of an enzyme reaction, and the linearity of the response is evidence against the presence of an enzyme inhibitor or activator.

A simplified calculation of rate of angiotensin destruction as a measure of angiotensinase activity has been adopted, which obviates the necessity of correcting the regression curve back to the starting point to compensate for slight variations in the standard response and then plotting on semilog paper. At 20 minutes of incubation at room temperature the slope of the regression curve is still relatively steep (Figure 1), and a measure of the angiotensinase activity is given by determining the percentage of difference between this curve and the standard curve at precisely 20 minutes. Thus, for the clinical material to follow, the percentage of destruction of angiotensin in 20 minutes is calculated and used as the index of plasma angiotensinase activity. By comparing the mean value for a group of normal subjects with that of the groups of patients studied, an index is then obtained of the percentage of increase (or decrease) in plasma angiotensinase activity above (or below) the normal mean. The reaction may be stopped at the 20-minute point by placing the plasma-angiotensin mixture in boiling water prior to assaying.

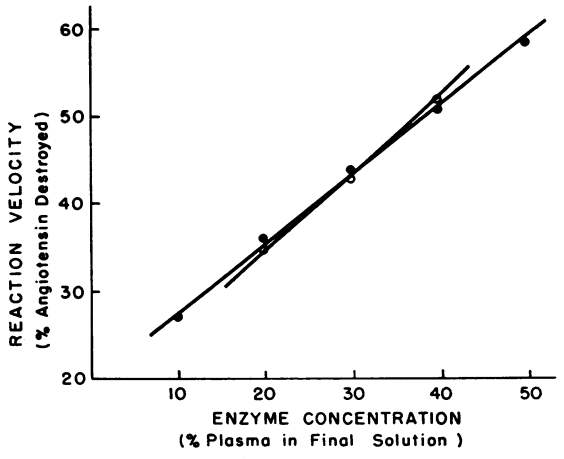

FIG. 4. DEMONSTRATION OF THE LINEAR RELATIONSHIP BETWEEN PROGRESSIVE ENZYME (PLASMA) DILUTION AND LOSS OF ENZYME ACTIVITY (IN TERMS OF PERCENTAGE OF ANGIOTENSIN DESTROYED) IN THE PLASMA FROM TWO SUBJECTS.

TABLE I

*Clinical and angiotensinase data in essential hypertension**

Patient	Age	Sex	Race	Admission BP	EKG	Chest film	Albuminuria	BUN	Optic fundi	Destruction of anigotensin	Change in anigotensinase activity from normal mean	Severity of hypertension
								mg/100ml	*grade*	*% in 20 min*	*%*	*class*
H.V.	25	F	W	180/115	N	N	Trace	15	1	8	−70	A
L.C.	46	F	W	220/110	N	N	0	16	1	13	−52	A
N.K.	28	M	W	160/100	N	N	0	15	1	18	−33	A
V.L.	37	F	W	140/100	N	N	0	11	2	21	−22	A
C.Mc.	28	F	W	220/110	RBBB	N	0	23	2	24	−11	A
P.S.	37	M	W	160/120	N	N	0	21	1	32	+19	A
H.C.	55	M	W	175/95	N	N	0	18	1	32	+19	A
A.D.	47	F	W	180/130	N	N	1+	20	2	32	+19	A
G.S.	28	F	W	150/90	N	N	0	18	1	35	+30	A
A.R.	38	M	W	200/120	N	N	0	15	2	40	+48	A
R.C.	38	M	W	165/115	N	N	0	16	2	44	+63	A
V.B.	36	M	W	170/120	N	N	0	17	2	49	+82	A
M.G.	61	M	W	200/105	N	LVE	0	25	2	12	−56	B
S.G.	37	F	N	220/120	LVH	N	0	10	2	23	−15	B
S.J.	60	F	N	200/120	Old MI	LVE	0	14	2	25	− 7	B
E.P.	31	M	N	225/135	LVH	N	1+	17	2	26	− 4	B
C.C.	54	F	W	215/125	RBBB	LVE	0	15	2	30	+11	B
D.S.	28	F	W	200/145	LVH	N	Trace	14	2	35	+30	B
T.S.	44	F	W	200/120	Myocard. ischemia	LVE	0	18	2	37	+37	B
J.M.	56	M	W	240/130	RBBB Old MI	LVE	0	19	2	42	+56	B
A.G.	49	M	W	240/150	LVH	N	2+	16	2	52	+93	B
J.H.	60	M	W	195/115	LBBB	LVE	0	15	2	52	+93	B
G.S.	63	F	W	200/120	LVH	LVE	0	18	2	57	+111	B
M.S.	30	F	N	180/120	LVH	LVE	Trace	17	2	59	+119	B
P.L.	57	F	W	160/90	LVH Old MI	N	0	13	2	75	+175	B
J.R.	47	M	W	260/130	LVH	LVE	2+	80	4	31	+15	C
B.T.	49	M	W	200/110	LVH	LVE	1+	35	3	46	+70	C
F.H.	43	M	W	175/110	LVH	LVE	0	46	4	47	+74	C
G.S.	33	M	W	200/140	Old MI	LVE	0	28	3	52	+93	C
C.K.	53	M	W	170/100	LVH	LVE	Trace	11	4	52	+93	C
J.C.	44	M	W	220/110	Sinus bradycardia	LVE	2+	28	4	57	+111	C
T.M.	52	M	W	220/130	LVH	LVE	Trace	28	4	71	+163	C
C.D.	30	F	W	200/140	LVH	LVE	Trace	17	3	77	+185	C

* BP, blood pressure; EKG, electrocardiogram; RBBB, right bundle branch block; LVH, left ventricular hypertrophy; MI, myocardial infarction; LBBB, left bundle branch block; LVE, left ventricular enlargement; BUN, blood urea nitrogen; optic fundi, grades 1–4, Keith-Wagner classification; class of severity: A, labile; B, hypertensive cardiovascular disease of moderate severity; C, severe hypertensive cardiovascular disease.

TABLE II

*Clinical and angiotensinase data in renal hypertension**

Patient	Age	Sex	Race	Diagnosis	Admission BP	EKG	Chest film	Albuminuria	BUN	Optic fundi	Destruction of angiotensin	Change in angiotensinase activity from normal mean	Severity of hypertension
									mg/100ml	*grade*	*% in 20 min*	*%*	*class*
J.W.	39	M	W	Chronic pyelonephritis	170/90	N	N	1+	150	1	33	+ 22	A
J.D.	63	M	W		210/110	LVH	N	3+	33	2	23	− 15	B
R.K.	49	M	W		205/100	N	LVE	2+	19	1	28	+ 4	B
D.D.	43	M	W		230/140	LVH	LVE	1+	80	2	28	+ 4	B
G.L.	60	M	W		220/120	LVH	LVE	2+	40	2	37	+ 37	B
J.L.	36	M	W		190/120	LVH	LVE	Trace	10	2	39	+ 44	B
E.F.	44	M	W		250/130	LVH	LVE	2+	92	3	11	− 59	C
R.M.	38	F	W		270/130	LVH	LVE	4+	120	4	31	+ 15	C
M.K.	71	F	W		240/120	LVH Old MI	LVE	2+	59	3	33	+ 22	C
I.H.	25	F	W	Unilateral pyelonephritis	160/110	N	N	Trace	15	2	35	+ 30	A
J.W.	28	M	W		160/110	N	N	0	12	1	46	+ 70	A
M.R.	47	F	N		240/130	LVH	LVE	1+	19	2	15	− 56	B
J.M.	40	F	N		210/120	N	LVE	0	16	2	45	+ 67	B
S.B.	58	M	W	Chronic glomerulonephritis	200/100	Old MI	N	1+	46	2	44	+ 63	A
A.B.	50	F	W		250/130	LVH	LVE	2+	125	2	33	+ 22	B
J.G.	61	F	W		210/110	LVH	LVE	3+	150	2	33	+ 22	B
E.K.	20	M	W		165/100	K-intox.	LVE	2+	235	3	48	+ 78	C
W.R.	43	M	W		220/140	LVH	LVE	Trace	25	3	49	+ 82	C
J.M.	28	M	W		210/120	LVH	LVE	2+	250	4	50	+ 85	C
M.D.	24	M	W		200/100	LVH	LVE	2+	150	4	54	+100	C
J.S.	10	F	W	Renal artery stenosis	180/120	N	N	0	15	1	60	+122	A
E.Mc.	44	F	W		220/110	LVH	N	1+	13	2	47	+ 74	B
C.M.	65	M	W		230/130	LVH	LVE	2+	31	2	49	+ 82	B
F.M.	57	M	W		200/100	LVH	LVE	1+	13	4	52	+ 93	C
S.S.	30	F	W		230/170	LVH	LVE	1+	20	4	54	+100	C

* LVH, left ventricular hypertrophy; MI, myocardial infarction; LVE, left ventricular enlargement; BUN, blood urea nitrogen; optic fundi, grades, 1–4, Keith-Wagner classification; class of severity: A, labile; B, hypertensive cardiovascular disease of moderate severity; C, severe hypertensive cardiovascular disease.

Duplicate determinations have been performed on 20 patients. The same plasma sample was used for the repeat determination in 11 instances, and a new plasma sample drawn for the repeat determination in 9 instances (frequently on a later day). The same range of variation between the 2 duplicate determinations was found in both groups (0 to 11%, average 3%) in terms of the percentage of angiotensin destroyed in 20 minutes. Replicate determinations were performed 15 times on the same plasma sample, giving a mean of 38.4% with SD of ± 4.0%. This gives a relative SD (coefficient of variation) of 10.4%. However, angiotensinase activity of red blood cells is higher than that of plasma (5), and immediate and complete separation of red cells from the heparinized venous blood sample without hemolysis is essential; a centrifuged but unseparated blood sample standing at room temperature for 4 hours showed a 20-minute angiotensin destruction of 44% on the initial assay and 60% after standing. Plasma samples may be stored at 4° C without affecting the angiotensinase activity, but storing plasma at − 18° C has consistently resulted in a depression of activity and should be avoided. Heparin per se has no effect on the pressor activity of standard angiotensin.

B. *Clinical material.* Several groups of subjects have been studied for plasma angiotensinase activity: 18 normal control subjects, 33 patients with essential hypertension, 26 patients with renal hypertension, and 4 patients with adrenal hypertension (2 with Cushing's syndrome and 2 with pheochromocytoma). Two groups of patients with conditions commonly associated with secondary hyperaldosteronism have been studied as well: 5 with refractory edema (6–8) (nephrosis, 3; cirrhosis with ascites, 1; congestive heart failure with anasarca, 1), and 5 with pregnancy (9) (normal third trimester, 2; toxemia, 2; hypertension and pregnancy, 1). One Addisonian patient who was deficient in steroid replacement therapy at the time of study is included.

The normal control subjects were all healthy individuals (6 females, 12 males) ranging in age from 24 to 38 years. The clinical data for the patients with essential hypertension are shown in Table I. Each patient was subjected to an extensive hospital study to rule out an identifiable cause before being classified as essential. The group is divided into labile hypertension (Class A), hypertensive cardiovascular disease of moderate severity (Class B), and severe hypertensive cardiovascular disease (Class C). The labile group had blood pressure readings fluctuating between normotensive and hypertensive levels and was completely lacking in evidence of cardiac, cerebral, or renal complications. Twelve of the patients with essential hypertension fulfilled these criteria. The group with hypertensive cardiovascular disease of moderate severity all had evidence of cardiac hypertrophy by electrocardiogram or cardiac enlargement on chest film, but no evidence of hemorrhages, exudates, or papilledema on funduscopic examination and no nitrogen retention. Thirteen of the patients with essential hypertension fulfilled these criteria. The group with severe hypertensive cardiovascular disease all had evidence of cardiomegaly and funduscopic changes, grade 3 or 4 (hemorrhage and exudates with or without papilledema). Eight of the

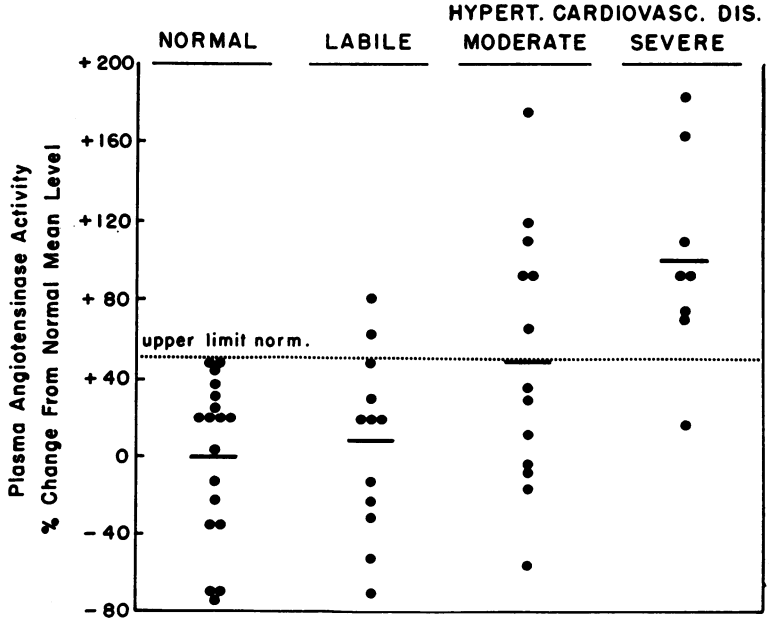

FIG. 5. PLASMA ANGIOTENSINASE ACTIVITY IN TERMS OF PERCENTAGE OF CHANGE FROM THE NORMAL MEAN LEVEL FOR THE INDIVIDUAL SUBJECTS IN THE NORMAL GROUP AND THE GROUPS WITH ESSENTIAL HYPERTENSION OF INCREASING SEVERITY.

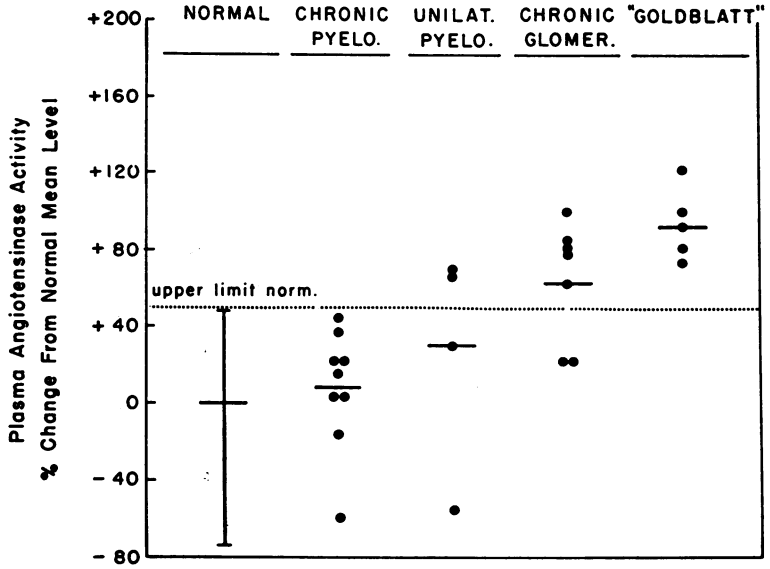

FIG. 6. PLASMA ANGIOTENSINASE ACTIVITY IN TERMS OF PERCENTAGE OF CHANGE FROM THE NORMAL MEAN LEVEL FOR THE INDIVIDUAL PATIENTS WITH THE DIFFERENT FORMS OF RENAL HYPERTENSION. The normal range and mean are shown to the left.

patients with essential hypertension fulfilled these criteria; none was in obvious congestive heart failure and 6 had nitrogen retention by blood urea nitrogen determination.

Table II outlines the clinical data for the patients with renal hypertension. After thorough hospital evaluation these patients were grouped as follows: 9 with chronic pyelonephritis, 4 with unilateral pyelonephritis, 7 with chronic glomerulonephritis, and 5 with renal artery stenosis. Surgical exploration and pathologic examination confirmed the diagnosis in 3 of the 4 patients with unilateral pyelonephritis and 4 of the 5 patients with renal artery stenosis. Retrograde pyelography and aortography were diagnostic in the remaining two patients with unilateral renal disease. For purposes of comparing results between the group with renal hypertension and the group with essential hypertension, the renal hypertensive patients are also designated as either Class A, B, or C in severity according to the same criteria outlined for the patients with essential hypertension.

RESULTS

A. *Essential hypertension.* The mean percentage of destruction of angiotensin in 20 minutes for the normal control group of 18 subjects was 27%, with a range of 7 to 40%. Thus, 40% destruction defines the upper limit of normal. The individual values were then calculated in terms of percentage of increase or decrease from this normal mean (Figure 5), which gave a range of − 74 to + 48%, which constitutes a measure of the increase or decrease from the average value in plasma angiotensinase activity for each individual in a normal group. An increase in plasma angiotensinase activity of over 48% from the normal mean, then, may be considered abnormal. The group of 12 patients with labile hypertension had a mean activity that was only 7% above the normal mean, with a range of − 70 to + 82%. Only 2 values were above the upper limit of normal, and there was no significant difference between the mean percentage of destruction in 20 minutes for the normotensive group and the group with labile hypertension ($p > .5$). The group of 13 patients with hypertensive cardiovascular disease of moderate severity had a mean activity that was 48% above the normal mean, with a range of from − 56 to + 175%; 6, or nearly 50%, of the values were above the upper limit of normal, and the mean for the 20-minute percentage of destruction was significantly elevated above the normal ($p < .05 > .025$). The group of 8 patients with severe hypertensive cardiovascular disease had a mean activity that was 100% above the normal mean, with a range of from + 15 to + 185%. All but 1 of the values were above the upper limit of normal, and the difference in the mean from the normal mean for the 20-minute percentage of destruction was

highly significant ($p < .001$). The elevation in plasma angiotensinase activity in essential hypertension appears to be a graded characteristic, becoming progressively elevated as the disease progresses (Figure 5).

B. *Renal hypertension.* Figure 6 shows the results for the 4 groups with renal hypertension in comparison with the normal. The group of 9 patients with chronic pyelonephritis had an elevation of mean activity that was only 7% above the normal mean, with a range of − 59 to + 44%, which is entirely within the normal range. The difference in the mean from the normal mean for the 20-minute percentage of destruction was insignificant ($p > .5$). This could not be attributed to a lack of severity of the hypertensive disease; 5 of the cases were in Class B (comparable to the group with essential hypertension with moderately severe hypertensive cardiovascular disease) and 3 in Class C (comparable to the group with essential hypertension with severe hypertensive cardiovascular disease). The group of 4 patients with unilateral pyelonephritis had a mean activity that was 30% above the normal mean, with a range of from − 56 to + 70%. There was no significant difference between the 20-minute mean percentage of destruction for this group and the normotensive group ($p > 0.2$). However, 2 of the 4 values were in the range above the upper limit of normal, and the implications in terms of a possible renal pressor mechanism in certain instances of pyelonephritis will be considered below. The group of 7 patients with chronic glomerulonephritis had an elevation of mean activity that was 63% above the normal mean, with a range of + 22 to + 100%. The mean for the 20-minute percentage of destruction was significantly elevated above the normal ($p < .01 > .005$). This also represented a significant difference from the group with chronic pyelonephritis ($p < .025 > .01$). Of the 4 patients with chronic glomerulonephritis with severe hypertensive disease (Class C), all fell above the upper limit of the normal range. As seen with the group with essential hypertension, the elevation of serum angiotensinase activity in chronic glomerulonephritis would seem to be a graded characteristic, increasing with the progressive severity of the disease. The group of 5 patients with hypertension related to renal artery stenosis had a mean activity that was 93% above the normal mean, with a range of from + 74 to + 122%. All of the values were above the upper limit of normal,

TABLE III

Adrenal disease and clinical states associated with hyperaldosteronism

Patient	Age	Sex	Diagnosis		Destruction of angiotensin	Change in angiotensinase activity from normal mean
					% in 20 min	%
			Secondary hyperaldosteronism, refractory edema			
H.R.	64	M	Nephrotic syndrome		90	+233
E.D.	60	F	Nephrotic syndrome		71	+160
N.H.	64	F	Nephrotic syndrome		52	+ 93
J.S.	70	F	Cirrhosis with ascites		44	+ 65
E.Z.	80	F	Congestive heart failure with refractory anasarca		50	+ 85
			Secondary hyperaldosteronism, pregnancy			
A.M.	25	F	Normal pregnancy, trimester[3]		67	+148
B.P.	25	F	Normal pregnancy, trimester[3]		78	+188
A.E.	16	F	Hypertension in pregnancy		58	+115
C.L.	20	F	Toxemia of pregnancy		83	+207
O.L.	23	F	Toxemia of pregnancy		48	+ 78
A.L.	63	F	Cushing's syndrome		23	− 15
C.S.	60	M	Cushing's syndrome		27	0
M.N.	52	F	Addison's disease		47	+ 74
L.M.	22	M	Pheochromocytoma	preop.	65	+140
				postop.	88	+224
L.E.	65	F	Pheochromocytoma	preop.	48	+ 78
				postop.	52	+ 92

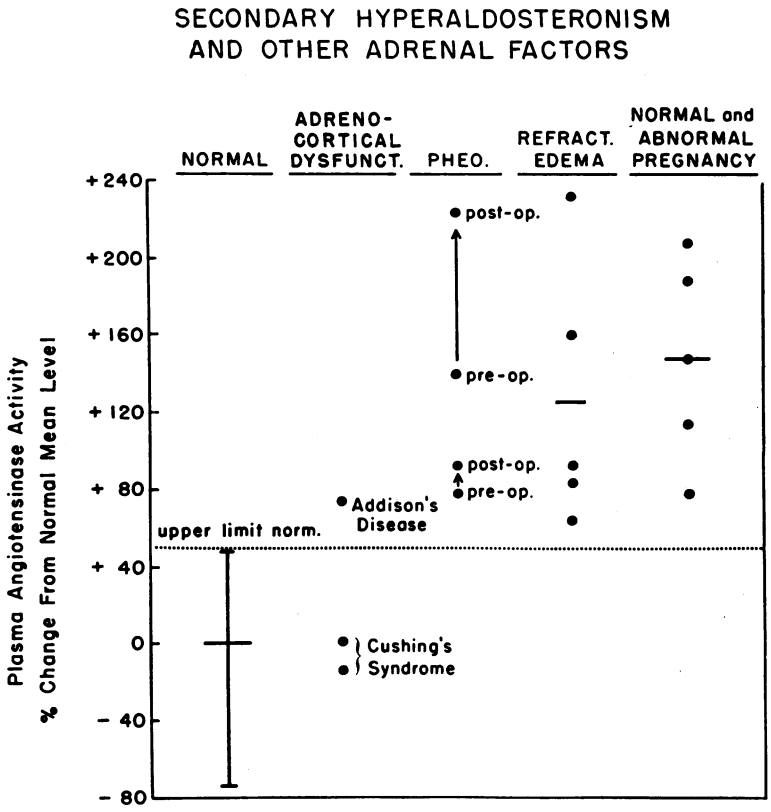

FIG. 7. PLASMA ANGIOTENSINASE ACTIVITY IN TERMS OF PERCENTAGE OF CHANGE FROM THE NORMAL MEAN LEVEL FOR THE INDIVIDUAL PATIENTS WITH ADRENAL DISORDERS AND STATES OF SECONDARY HYPERALDOSTERONISM (REFRACTORY EDEMA AND PREGNANCY). The normal range and mean are shown to the left.

and the difference in the mean 20-minute percentage of destruction from the normal was highly significant ($p < .001$).

C. *Adrenal diseases and clinical states associated with hyperaldosteronism.* Table III shows the clinical data and assay results in the group of patients with disorders of the adrenal gland and clinical states commonly associated with secondary hyperaldosteronism. The angiotensinase activity for the individual patients is plotted in Figure 7 for the various subgroups studied and contrasted with the normal control group. The 2 patients with Cushing's syndrome had an angiotensinase activity in the normal range, whereas the 1 Addisonian patient who was undertreated at the time of study showed an activity above the upper limit of normal. The 2 patients with pheochromocytoma had an elevation of activity above the upper limit of normal, which was shown to persist in the one instance at 10 days and in the other at 2 months after surgical cure. The 2 groups of patients with clinical states that have been shown to be associated with an increase in aldosterone secretory rate are divided into the group with refractory edema and the group with normal and abnormal pregnancy. The group with refractory edema consisted of 3 patients with nephrosis, 1 with cirrhosis and ascites, and 1 with congestive heart failure and refractory anasarca. All 5 values for plasma angiotensinase activity were considerably above the normal range (+ 56 to + 233%) with a mean activity that was 126% above the normal mean. The difference in the mean from the normal mean for the 20-minute percentage of destruction was highly significant ($p < .001$). The group of pregnant patients, all in their third trimester, consisted of 2 normal pregnant females, 2 with toxemia, and 1 with hypertension starting

TABLE IV

Summary of plasma angiotensinase activity in hypertension

Patients	Diagnosis	Mean destruction of angiotensin	SD	SEM*	p factor†	Mean increase in angiotensinase activity above normal mean
no.		*% in 20 min*				%
	Essential hypertension					
18	Normal	27	11	2.6		
12	Labile hypertension	29	12	3.5	>.5	7
13	Hyperten. cardiovasc. Disease—moderate	40	19	5.5	<.05>.025	48
8	Hyperten. cardiovasc. Disease—severe	54	15	5.3	<.001	100
	Renal hypertension					
9	Chronic pyelonephritis	29	8	2.7	>.5	7
4	Unilateral pyelonephritis	35	14	7.0	>.2<.4	30
7	Chronic glomerulonephritis	44	8	3.0	<.01>.005	63
5	Renal artery stenosis	52	5	2.0	<.001	93
	Secondary hyperaldosteronism					
5	Refractory edema	61	19	8.5	<.001	126
5	Pregnancy, normal and abnormal	64	11	5.5	<.001	148

* Standard error of the mean.
† Significance of the difference between the patient groups and the normal group.

during the first trimester. All 5 values for plasma angiotensinase activity were considerably above the normal range (+ 78 to + 148%), with a mean activity that was 148% above the normal mean. The difference in the mean from the normal mean for the 20-minute percentage of destruction was highly significant ($p < .001$).

The angiotensinase activity data for all groups studied are summarized in Table IV.

DISCUSSION

Our working hypothesis has been that states which may have in common an underlying increased rate of angiotensin elaboration, with or without hypertension, may develop an increased plasma capacity to inactivate angiotensin through a process of substrate induction, that is, through a specific enzymatic adaptation at the cellular level. Knox, Auerbach, and Lin (10) have reviewed the subject of enzymatic adaptation, citing animal work in which amino acid oxidase activity increased in response to the administration of protein and specific amino acids. Whether the angiotensinase activity in plasma is represented by a single specific protease or several has not been established as yet. Since a variety of highly purified proteolytic enzymes have the capacity of inactivating angiotensin (5), the degree of specificity of plasma angiotensinase activity will have to await the isolation of such activity from the plasma proteins. Other possibilities exist, such as the elaboration of specific angiotensinase inhibitors or activators to account for the observed alterations in plasma angiotensinase activity.

The 4 groups of patients with hypertension that demonstrated a significant elevation in plasma angiotensinase activity were *1*) those with essential hypertension that had progressed to a stage of hypertensive cardiovascular disease of a moderate to severe degree, *2*) those with hypertension related to chronic glomerulonephritis, particularly in its advanced stages, *3*) those with hypertension related to renal artery stenosis, and *4*) the 2 patients with pheochromocytoma. No significant change was found in the group with hypertension associated with chronic pyelonephritis and unilateral pyelonephritis, irrespective of the severity of the hypertensive disease. Thus, the group with chronic bilateral pyelonephritis serves as a sick control group, where no elevation of angiotensinase activity was found, despite the presence of uremia in 7 of the 9 patients, 3 of whom had the changes of malignant hypertension and were studied shortly before their death. One uremic pa-

tient with an elevated angiotensinase activity associated with chronic glomerulonephritis and hypertension had no change in his angiotensinase activity after hemodialysis, despite a marked reduction in his blood urea nitrogen concentration.

There is a striking correlation between these findings and the degree of juxtaglomerular hypertrophy as determined by juxtaglomerular cell counts, reported by Turgeon and Sommers (11). These workers reported juxtaglomerular hyperplasia in the same 4 hypertensive groups in which a significant increase in plasma angiotensinase activity is reported here (essential hypertension, chronic glomerulonephritis, renal artery stenosis, and pheochromocytoma). Further, they failed to find hyperplasia in the pyelonephritic group with hypertension, corresponding with the lack of a significant increase in plasma angiotensinase activity reported here. The evidence that the renin secretory mechanism is situated in the juxtaglomerular apparatus has been reviewed by Tobian (12) and is substantial; it is assumed that a high rate of renin secretion may be associated with a high rate of angiotensin elaboration (13).

Although a modest elevation of angiotensin blood levels in humans with severe essential hypertension has been reported in the past (14), Genest and co-workers, employing a more specific method (15), found an elevation in only 30% of their determinations (16). There is more uniform agreement on a consistent elevation in the renal vein blood in hypertension due to renal artery stenosis (17, 18). Angiotensin data in hypertension associated with chronic nephritis and pheochromocytoma are not as yet available. The failure to show consistently elevated blood angiotensin levels in essential hypertension in no way precludes the possibility of an elevated rate of elaboration, particularly in the more advanced stages of the disease, in the light of the finding of a progressive elevation in plasma angiotensinase activity as the disease advances, which would tend to mask such a phenomenon. It does suggest, however, that angiotensinemia is not the underlying etiological factor in essential hypertension, but more likely a secondary manifestation of the progressive intrarenal vascular disease attending its more advanced stages. A stronger argument can be made for angiotensinemia as the etiological factor in hypertension relating to renal artery stenosis, where the angiotensin-angiotensinase levels may be elevated in even the early stages of the disease.

It is possible that in the very late stages of hypertensive disease associated with progressive renal failure the renin mechanism is finally obliterated, as suggested by the experimental work in rats of Omae, Masson, and Page (19), where "morphological examination showed a relationship between the presence of nephrosclerotic lesions and absence of, or decrease of, renin-like pressor substance released." This may account for the only instance of a normal angiotensinase level in the group with severe hypertensive cardiovascular disease, Case J.R., who was admitted with grade 4 eyegrounds, hypertensive encephalopathy, and progressive azotemia.

This leads to the question of the mechanism of the hypertension in the group with chronic pyelonephritis, where juxtaglomerular hypertrophy and elevated plasma angiotensinase activity are strikingly absent. Assuming a predominantly renal medullary destruction in this disease, one may speculate that this represents a human manifestation of renoprival hypertension as suggested by several studies (20, 21), where a protecting, antihypertensive medullary factor, as identified by Muirhead, Jones, and Stirman (22), may have been eliminated by the disease. Although a figure is hard to arrive at, a significant reduction of pressure following extirpation of a unilaterally pyelonephritic kidney may be expected in "less than half of such cases" (23), whereas a significant improvement in the range of 75% has been reported (24) following surgery for occlusive renal arterial disease. However, one must account for those isolated reports of definite improvement following excision of a unilaterally pyelonephritic kidney (25). In this regard it is of interest that, although the mean angiotensinase level was not significantly elevated above the normal mean, 2 of the 4 cases of unilateral pyelonephritis had a level above the normal range. A renal pressor mechanism may be contributing to the hypertension in certain instances of pyelonephritis through a process of proliferative endarteritis, as emphasized by Smythe (26). Saphir (27) proposes the existence of 2 types of pyelonephritis, one being clinically evident with the predominant feature of medullary destruction, and the other, "pyelonephritis lenta," being clinically silent with a prominent feature of

vascular intimal fibrosis and hyalinization leading to hypertension (and often misdiagnosed as essential hypertension). Sommers (21) on the basis of juxtaglomerular cell counts, states that "pyelonephritis that causes an intrarenal Goldblatt-type phenomenon does occur but it is relatively rare in the material analyzed so far." If the plasma angiotensinase level proves to be differentiating, a greater degree of sophistication may be obtained in the surgical selection of those patients with hypertension associated with unilateral renal disease. The degree of surgical success may be proportional to the degree to which the renal pressor mechanism is responsible for the hypertension.

The common denominator for those instances of an elevated plasma angiotensinase activity in the various hypertensive groups may prove to be a renal pressor mechanism resulting from extrinsic or intrinsic renovascular disease. This may be either a cause or a consequence of the elevated arterial pressure. In pheochromocytoma, persistent renal vasoconstriction may be sufficient to account for the mechanism, even in the absence of nephrosclerosis.

In a recent review, Davis, Carpenter, and Ayers (28) have summoned up convincing evidence for the role of the renin-angiotensin system in the control of aldosterone secretion. It is of interest that Laragh (29) found elevated aldosterone secretory rates in the advanced and particularly the malignant phase of essential hypertension, but not in the milder stages. This report is quite parallel to the plasma angiotensinase data presented above. If an angiotensinemia is assumed to apply in general to clinical states associated with secondary hyperaldosteronism, it seemed reasonable to look for an elevated angiotensinase activity in patients with refractory edema and pregnancy. As cited above, the highest mean levels observed for plasma angiotensinase activity were found in these conditions. The absence of hypertension in these groups (excepting the toxemic patients), does not preclude the possibility of an increased rate of angiotensin elaboration in the light of Laragh's finding (30) of a refractoriness to the pressor effects of infused angiotensin in cirrhotic patients with ascites. The finding of hypergranulation of the juxtaglomerular cells from kidneys of sodium deficient rabbit (31), of dogs with hyperaldosteronism secondary to thoracic caval construction (32), and of rats with experimental nephrosis (33) is supportive evidence.

If Tobian's view (12) that the juxtaglomerular cells act as stretch receptors is sustained, then the common denominator between hypertension associated with renal vascular disease on the one hand and edematous states on the other for the activation of the renin-angiotensin-aldosterone system would be a decreased renal perfusion pressure, whether through a vascular obstruction at the renal level or through the sequestration of effective circulating volume as edema fluid. Such a contraction of plasma volume has been determined in the nephrotic syndrome and cirrhosis with ascites; in severe congestive failure the redistribution of blood on the venous side of the circulation has the same effect (34). In pregnancy, however, cardiac output and renal blood flow are increased until week 32 except in severe pre-eclampsia (35), and in this situation this line of reasoning cannot be applied. The possibility exists that an unidentified placental factor may be operative in activating the renin mechanism to meet the need for a positive sodium balance during pregnancy. An increase in plasma oxytocinase activity at term is another possibility.

The finding of an elevated angiotensinase activity in an undertreated patient with Addison's disease is probably indicative of an activation of the renal pressor system due to volume depletion, comparable to the observations in hemorrhagic shock (36). Experimental support is offered by the observation of increased juxtaglomerular granularity in the adrenalectomized cat (37). The finding of a normal level in 2 patients with hypertension secondary to Cushing's syndrome suggests that the assay may be helpful in identifying forms of secondary hypertension where the renal pressor system is not involved.

It is hypothesized than angiotensinemia and hyperaldosteronism may only develop in those conditions where an increased rate of angiotensin elaboration exceeds an adaptive increase in the rate of angiotensin destruction.

The authors are aware that there may be other disease states not associated with hypertension or edema which may also prove to have alterations in the plasma angiotensinase activity. Any profound disturbance in the plasma proteins and alteration in circulating proteases such as may at-

tend severe pancreatic or hepatobilary disease carries with it this potential. A systematic appraisal will be a subject for further investigation.

SUMMARY

1) An *in vitro* biological method for the determination of plasma angiotensinase activity is described. Plasma angiotensinase activity is used for convenience to designate the capacity of plasma to inactivate synthetic angiotensin, although a specific plasma enzyme, as such, has not been isolated to date.

2) A significant elevation was found in the following hypertensive disorders: essential hypertension complicated by moderately severe to severe hypertensive cardiovascular disease, chronic glomerulonephritis, occlusive renal arterial disease, and pheochromocytoma.

3) Evidence is cited from the literature for the increased activity of the renin secretory mechanism in each of these diseases, which may have in common an ischemic or obstructive renal vascular disorder.

4) In the two groups with conditions commonly associated with secondary hyperaldosteronism, refractory edema and pregnancy (normal and abnormal), marked elevations of plasma angiotensinase activity were also found.

5) It is suggested that the increased plasma angiotensinase activity in these states may ensue from an increased rate of angiotensin elaboration through a process of enzymatic adaptation.

6) In the light of these findings, reports of the failure to find a consistently elevated blood angiotensin level in even the more severe stages of essential hypertension and in malignant hypertension do not preclude the possibility of an increased rate of angiotensin elaboration.

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