PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 374–379 (1975)

Serum Renin and Renin Substrate Levels in Scleroderma[1] (39039)

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Scleroderma is a disease of mesenchymal tissues characterized by fibrosis and vascular changes which eventually lead to organ insufficiency. Two clinical forms have been recognized, localized and systemic scleroderma. The localized form only affects the skin and the lesions have a tendency to resolve spontaneously. Systemic scleroderma is a progressive, usually irreversible process and the organs most frequently involved are the skin, gastrointestinal tract, lungs and kidneys. The following findings strongly suggest that involvement of small blood vessels (capillaries, arterioles and small size arteries) plays an important role in the pathogenesis of systemic scleroderma: (a) the frequent presence of Raynaud's phenomenon, (b) marked reduction in the number of dermal capillaries, (c) increase in thickness of the basement membrane of muscle capillaries (16), (d) thickening and hyalinization of arterioles (e) intimal proliferation of small size arteries, and (f) eventual ischemic necrosis (2, 3, 17). Small blood vessel changes have been noted in the lungs, heart, gastrointestinal tract, kidneys and striated muscle (19). Further evidence of small blood vessel disease in systemic scleroderma is supported by the high incidence of systemic hypertension, which in some patients develops into the malignant phase (2). The renal vascular lesions in scleroderma may closely resemble those found in essential malignant hypertension (2, 5). The purpose of this study was to measure the serum renin and renin substrate levels in normotensive and hypertensive patients with systemic scleroderma.

Material and methods. Thirty seven subjects were included in this study which were divided into four groups, (a) systemic scleroderma, normotensives, 10 patients, (b) systemic scleroderma, hypertensives, 10 patients, (c) localized scleroderma, 7 patients and, (d) normal controls, 10 volunteers.

In the systemic scleroderma groups, there were 17 females and three males, with ages ranging from 16 to 74 years old. There were 16 caucasian and four black patients. The duration of the disease ranged from 2 to 17 years. The localized scleroderma group included five females and two males, all caucasian with ages ranging from 11 to 57 years old. The duration of the disease ranged from 18 months to 9 years. Although patients with localized scleroderma do not develop hypertension related to the disease, they were included in this study as an additional control group since they may have significant small blood vessel pathology (7). The normal control group consisted of five females and five males with ages ranging from 25 to 46 years old. Gastrointestinal involvement was estimated by clinical symptoms and upper gastrointestinal radiologic evidence of reduced mobility of the distal esophagus, dilatation of the duodenum or delayed transit of the opaque mixture. Lung involvement was studied by radiologic examination and pulmonary function studies to determine the presence of restrictive disease and/or impairment in pulmonary diffusion. The three markers suggested by Cannon *et al.* (2) were used to assess for renal involvement, (a) proteinuria, (b) hypertension (systolic 140 or above; diastolic 90 or above), (c) and blood urea nitrogen (abnormal: 25 > mg/100 ml). In addition, serum creatinine was also performed. (abnormal: >1.0 mg/100 ml). Four patients in the hypertensive group had kidney biopsies performed 8 years prior to the present determinations of serum renin levels. The hypertension was present at the time of the kidney biopsy.

[1] Supported by N.I.H. Research Grants Nos. AM 15590 and AM 17918.

Histology. Skin biopsies were performed by surgical excision from indurated areas and were deep enough to include the subcutaneous tissue. The site of the skin biopsy was the dorsum of the hand or forearm in the systemic scleroderma groups and the arm or thigh in the localized group. The specimens were fixed in 10% buffered formalin and subjected to the following stains (a) hematoxylin and eosin, (b) period-acid Schiff (PAS), (c) Masson's trichrome, (d) Van-Gieson (e) Verhoeff's elastic tissue (f) aldehyde fucsin and (g) Alcian blue, pH 2.4.

Renin and substrate determination. Serum renin and its substrate levels were determined in out-patients. The patients were kept on their regular diets but were instructed to avoid salt. Laragh *et al.* (11) have shown that plasma renin levels in outpatients were quite similar to those determinations performed under rigid metabolic ward conditions. All oral medications including antihypertensives drugs were discontinued for at least one week prior to the test. None of the patients were on oral contraceptives. Venous blood was drawn in the sitting position. Twenty-four hour urine specimens were collected for the determination of sodium and creatinine excretion.

Assay for serum renin and its substrate. The concentration of either renin or renin substrate in the serum will influence the rate of angiotensin formation. For this reason we have chosen to measure and report both of these values. Renin and renin substrate in dialyzed serum were measured by the method of Gould, Skeggs and Kahn (9). This method for measuring renin is based on the zero order reaction velocity of angiotensin II formation when serum, essentially free of angiotensinase activity is incubated with an excess of hog substrate. The angiotensin II generated is bioassayed in the rat (21) and compared with a known amount of angiotensin II, (Research Standard A/65 which is obtained from the Department of Biological Standards, Mill Hill, London). Human substrate is measured by reaction with an excess of human renin. The angiotensin II formed is measured by the same bioassay used for the renin assay.

Results. The clinical data are reported in Table I. In the systemic scleroderma group, all patients had skin involvement of the acrosclerotic type, accompanied by Raynaud's phenomenon, except for case No. 6. In most patients more than one organ system was involved. None of the patients in the hypertensive group had malignant hypertension. Four cases with hypertension had kidney biopsies which were reported as follows:

Case 12: Arteriosclerosis with thickening of wall by fibrosis and hypertrophy of media of small size arteries. Case 13: Focal gomerulosclerosis. Thickening of basement membrane and hyalinization of wall of afferent arterioles. Case 14: Focal intraglomerular areas of intercapillary fibrosis and thickening of arteriole walls. Case 19: Thickening of glomerular basement membrane. Mild thickening of arteriole wall and intimal proliferation of small size arteries. None of the patients with kidney involvement had an abnormal blood urea nitrogen or creatinine concentrations.

The skin pathology in the systemic scleroderma group revealed two prominent features: fibrosis and blood vessel changes. The dermis revealed compact connective tissue due to reduction of interfascicular collagenous spaces. There was also considerable replacement of the subcutaneous tissue by hyalinized connective tissue. Blood vessel involvement consisted of (a) devascularization due to reduction of capillaries in the papillary dermis; (b) narrowing of lumen and thickening of the arterial wall of arterioles by increase in perivascular cells, with or without hyalinization and (c) intimal thickening in small size arteries by mesenchymal cells and collagen deposition. In the localized scleroderma group there was marked dermal and subcutaneous tissue fibrosis and four patients revealed blood vessel involvement of capillaries and arterioles, while small size arteries were usually normal. In addition, all patients revealed a panniculitis consisting of plasma cells, round cells and mesenchymal cells, either fibroblasts or histocytic types (6). A detailed report of the skin pathology in systemic and localized scleroderma will be published elsewhere (7).

The results of serum renin and renin sub-

TABLE I. CLINICAL PICTURE AND SKIN PATHOLOGY.

					Clinical picture				Skin Pathology	
	Age	Sex	Race	Duration	Skin	Raynaud's	Lungs	GI	Fibrosis	Blood vessel involvement
Systemic scleroderma										
(a) Normotensives										
Case 1	51	F	C	14 yr	+	+	+	+	+	+
Case 2	38	F	C	2 yr	+	+	+	0	+	+
Case 3	40	F	C	10 yr	+	+	0	+	+	+
Case 4	64	F	C	3 yr	+	+	+	+	+	+
Case 5	16	F	C	10 yr	+	+	0	0	+	0
Case 6	42	F	C	10 yr	+	+	0	+	+	+
Case 7	47	F	C	3 yr	+	+	+	0	Not performed	
Case 8	28	F	C	7 yr	+	+	0	0	+	+
Case 9	63	F	N	6 yr	+	+	+	0	+	+
Case 10	74	F	C	10 yr	+	+	0	+	Not performed	
(b) Hypertensives										
Case 11	48	F	C	2 yr	+	+	+	0	+	+
Case 12	46	F	N	6 yr	+	+	+	+	+	+
Case 13	52	M	C	8 yr	+	+	+	+	+	+
Case 14	53	F	C	8 yr	+	+	+	+	+	+
Case 15	42	F	N	6 yr	+	+	+	+	+	+
Case 16	68	F	C	4 mo	+	0	+	0	Not performed	
Case 17	59	F	C	17 yr	+	+	+	+	+	+
Case 18	41	M	N	5 mo	+	+	+	+	+	+
Case 19	68	F	C	9 yr	+	+	+	+	+	0
Case 20	54	M	C	13 yr	+	+	0	+	+	+
Localized Scleroderma										
Case 21	21	M	C	3 yr	+	0	0	0	+	+
Case 22	11	F	C	3 yr	+	0	0	0	+	0
Case 23	57	F	C	1.5 yr	+	0	0	0	+	+
Case 24	21	F	C	9 yr	+	0	0	0	Not performed	
Case 25	32	F	C	2 yr	+	0	0	0	+	0
Case 26	16	M	C	6 yr	+	0	0	0	+	+
Case 27	45	F	C	2 yr	+	0	0	0	+	+

strate levels are reported in Table II. Renin levels ranged from zero to 0.88 $\times$ 10^4 Goldblatt units/ml, which is within the normal range (9). The serum levels of renin substrate were normal except for cases 7, 16, 20 and 27 where they were elevated (10). There were no significant differences in the mean and standard error of mean in the four groups studied (see Table III). Urinary sodium excretion was low in seven cases. However, five of these patients (cases 3, 7, 9, 12, 15) were prescribed salt restricted diets (Fig. 1).

Discussion. The substrate for renin is a circulating alpha-2 globulin known as angio-

TABLE II. SERUM RENIN AND ITS SUBSTRATE, 24 HOUR SODIUM URINARY EXCRETION AND KIDNEY MARKERS INVOLVEMENT.

	Renin G. units/ml serum $\times 10^{-4}$	Renin substrate p moles/ml	Urinary Na mEq/24 hr	Kidney markers			
				BP	BUN	Serum creatinine	Protein-uria
Systemic sclero-derma							
(a) Normoten-sives							
Case 1	0.52×10^{-4}	818	298.0	120/65	13.0	0.70	0
Case 2	0.55×10^{-4}	857	165.0	110/70	15.0	0.80	0
Case 3	0.04×10^{-4}	730	103.0	110/70	17.0	0.40	2+
Case 4	0.04×10^{-4}	1.200	109.0	130/70	11.0	1.10	0
Case 5	0.23×10^{-4}	947	83.0	90/60	10.0	0.60	0
Case 6	0.14×10^{-4}	857	47.0	120/74	6.0	0.60	0
Case 7	0.36×10^{-4}	1.636	33.0	90/60	19.0	0.70	0
Case 8	0.08×10^{-4}	924	77.0	128/82	7.0	0.22	0
Case 9	0.88×10^{-4}	1.010	22.0	110/90	19.0	0.80	0
Case 10	0.09×10^{-4}	928	23.0	120/80	11.0	0.80	0
(b) Hyperten-sives							
Case 11	0.10×10^{-4}	888	75.0	160/100	10.0	0.80	+
Case 12	0.08×10^{-4}	800	26.0	170/100	5.0	0.70	+
Case 13	0.21×10^{-4}	914	75.0	160/100	15.0	1.00	0
Case 14	0.32×10^{-4}	692	144.6	170/90	12.0	0.50	0
Case 15	0.61×10^{-4}	1.133	13.5	130/100	11.0	1.00	2+
Case 16	0.79×10^{-4}	2.186	79.0	220/116	29.0	0.80	0
Case 17	0.30×10^{-4}	837	99.0	170/80	15.0	1.00	0
Case 18	0.00	738	129.0	140/100	12.0	1.00	0
Case 19	0.19×10^{-4}	818	77.0	140/85	20.0	0.90	0
Case 20	0.26×10^{-4}	1.440	113.0	170/115	12.0	1.00	4+
Localized Sclero-derma							
Case 21	0.78×10^{-4}	760	64.0	90/50	13.0	0.70	0
Case 22	0.04×10^{-4}	821	92.0	110/80	10.0	0.50	0
Case 23	0.15×10^{-4}	1.074	138.0	167/100	17.0	0.90	0
Case 24	0.11×10^{-4}	783	131.0	105/70	10.0	0.70	0
Case 25	0.33×10^{-4}	1.286	78.0	95/60	12.0	0.80	0
Case 26	0.14×10^{-4}	930	119.0	105/60	15.0	0.70	0
Case 27	0.32×10^{-4}	2.668	109.0	140/88	18.0	1.00	0

tensinogen. Renin acts on angiotensinogen to yield angiotensin-I, a decapeptide, which is converted into angiotensin-II, an octopeptide by the action of the converting enzyme, which splits off two C-terminal amino acids from angiotensin I. Angiotensin II, is the most powerful vasopressor substance known and acts directly on peripheral arterioles. Angiotensinases (groups of peptidases) can be found in the wall of blood vessels, in various organ tissues and in the plasma, and their function is to degrade angiotensin II to metabolites which are largely inactive.

Serum renin and its substrate levels are increased in patients with malignant hypertensin but are normal in patients with benign hypertension (9). According to Laragh and associates (12, 13) serum renin levels in essential hypertension, when correlated with urinary sodium excretion, fall into three categories: low (26%), normal (54%) and high renin levels (18%). This classification appears to have clinical significance since Brunner *et al.* (1) reported that patients with low plasma renin levels are less prone to develop vascular disease (myocardial infarc-

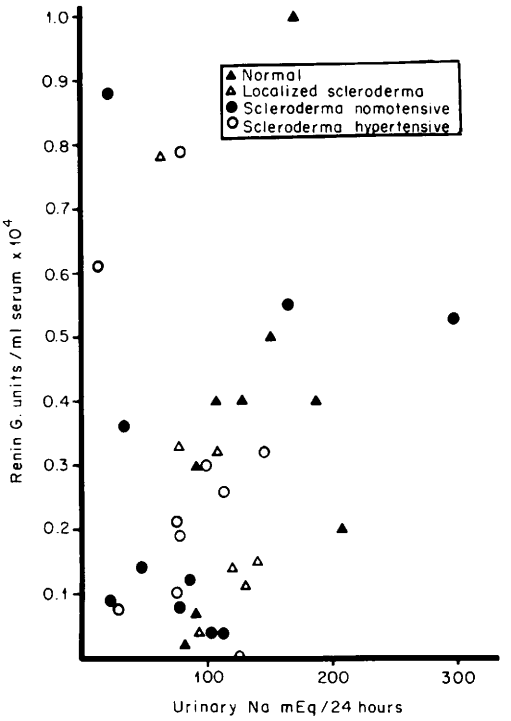

FIG. 1. Correlation of 24 hr urinary Na excretion and serum renin levels, of normal and scleroderma patients.

tions and strokes) than those with normal or high renin activity.

Cannon *et al.* (2) recently reported that renal involvement in scleroderma was the leading cause of death in a group of 40 autopsied cases. Renal failure and the cardiac complications of malignant hypertension accounted for 43% of the deaths. Three markers were considered as indicative of renal disease: hypertension, proteinuria and azotemia. In a series of 210 scleroderma patients, 45% had one or more positive kidney markers. Peripheral renin activity was measured in eight patients with systemic scleroderma. One patient with no renal involvement and three with proteinuria, hypertension and normal or mild deviations of blood urea nitrogen concentrations showed normal renin levels, as related to the patients urinary sodium excretion. Four patients with malignant hypertension showed a marked increase in plasma renin levels. Renin substrate levels were not reported in that study. In our series, there were no significant differences in the substrate concentrations between the scleroderma normotensive and hypertensive groups. We recently studied a patient with systemic scleroderma in complete renal failure. This patient had a blood urea nitrogen of 85 mg and a blood creatinine of 5.4 mg. His renin levels were markedly increased (18.9 G units/ml serum $\times 10^{-4}$) while the substrate concentration was within normal limits (1.044 p moles/ml). These results are in agreement with the observations of Cannon *et al.* (2). Cannon *et al.* (2) also raised the question as to whether peripheral plasma renin activity could be used as a useful marker to detect early renovascular disease in scleroderma. This did not appear to be the case since four hypertensive patients in this study, who showed early renal vascular changes, still maintained normal serum renin and substrate concentrations, eight years following abnormal kidney biopsies. On the other hand, since they all had benign hypertension with normal blood urea and creatinine levels, one could postulate from this observation that hypertension with early vascular renal changes in scleroderma does not exclude a benign course of the disease over a prolonged period of time.

It was postulated by Peacock (18) that increased tissue concentration of epinephrine and/or norepinephrine may be responsible for the vasoconstriction in primary Raynaud's phenomenon. However, recently Sapira *et al.* (20) measured venous plasma

TABLE III. MEAN AND STANDARD ERROR OF THE MEAN OF SERUM RENIN AND ITS SUBSTRATE IN ALL GROUPS STUDIED.

	Renin G.Units/ml serum $\times 10^{-4}$ mean and SE	Renin substrate p moles/ml mean and SE
Systemic scleroderma		
1. Normotensive	0.326 $\pm 0.092 \times 10^{-4}$	1101 ± 82
2. Hypertensive	0.289 $\pm 0.086 \times 10^{-4}$	1001 ± 154
Localized scleroderma	0.267 $\pm 0.095 \times 10^{-4}$	1189 ± 256
Normals	0.369 $\pm 0.086 \times 10^{-4}$	876 ± 25

catecholamine concentration and urinary catecholamine excretion and their results failed to support the above hypothesis. LeRoy *et al.* (14) studied skin capillary flow in eight patients with systemic scleroderma by measuring local clearance of radioactive xenon. Capillary flow was found reduced. However, cooling followed by reflex warning revealed normal recovery of xenon clearance while the skin temperature remained low, as compared to normal controls.

The present study failed to establish a relationship between serum renin and renin substrate levels and the vasoconstriction and vascular pathology in normotensive and hypertensive patients with scleroderma. However, according to our current state of knowledge, it is not possible to rule out the possibility that a local vascular increase of renin and/or angiotensin II concentration may be responsible for the vasoconstriction in scleroderma, either by direct effect or by potentiating local catecholamines. In this regard, Gould *et al.* (8) have shown renin activity in extracts from arteries and veins. Eskildsen (4) working with rabbit uterus showed high concentrations of renin in small vessels and in muscle and connective tissue devoided of arterioles and venules. Recently, Malik *et al.* (15) showed that angiotensin I and II, and natural as well as synthetic renin substrates potentiate the vasoconstrictor response to adrenergic stimuli in rat mesenteric arteries. Future studies to attempt to quantitate renin, angiotensin I and II and converting enzyme in sleroderma arterioles, may release useful information as to the mechanism of the vasoconstriction, and possible, blood vessel pathology in scleroderma.

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Received May 6, 1975. P.S.E.B.M. 1975, Vol. 150.