touch in this area. The nail of the little finger was discoloured at the base, and was starting to separate (Fig. 3). The nails on the right hand and the toe-nails were unaffected.

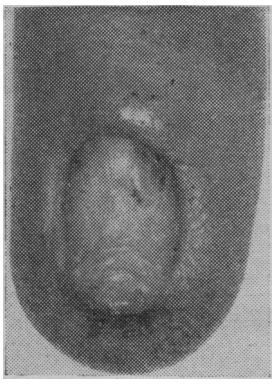


FIG. 2

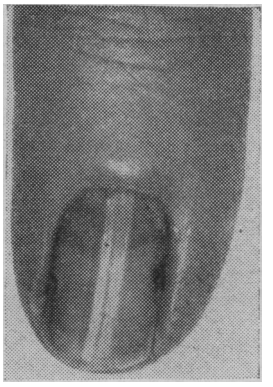


FIG. 3

FIG. 2.—Case 3. Total loss of nail of ring finger. FIG. 3.—Case 3. Discoloration and early loosening at base of nail of little finger.

Comment

All three patients had frequent exposures to the concentrated chemicals, and had not taken special precautions to prevent contamination of the skin. The cause of the nail damage is unknown, but it seems probable that the chemical reaches the nail matrix by entering the nail-fold and stimulates infection and also interferes with formation of the nail from the matrix. The damage is almost certainly due to local effects and is not the result of ingestion, because of the asymmetry of the lesions and the fact that the toenails are unaffected. The curious colour change and the softening of the nail at the base are rather characteristic, and should be a guide to the possible cause when seen. Infection seems to play an important part when the nail is actually lost. It should be stressed that it is the concentrated chemical which causes the damage.

Our thanks are due to the photographic department of the General Hospital, Nottingham, for Fig. 1, and to the photographic department of Westminster Hospital for Figs. 2 and 3. We also thank the doctors who referred the patients, and Dr. A. A. B. Swan for helpful criticism.

REFERENCES

Almog, C., and Tal, E. (1967). *Brit. med. J.*, **3**, 721.
Barnes, J. (1967). In *Third Symposium on Advanced Medicine*, p. 230, edited by A. M. Dawson. London.
Brit. med. J., 1967, **3**, 690.
Bullivant, C. M. (1966). *Brit. med. J.*, **1**, 1272.
Campbell, S. (1968). *Lancet*, **1**, 144.
Cant, J. S., and Lewis, D. R. H. (1968). *Brit. med. J.*, **2**, 224.
Clark, D. G., McElligott, T. F., and Hurst, E. W. (1966). *Brit. J. indust. Med.*, **23**, 126.
Swan, A. A. B. (1967). *Brit. med. J.*, **4**, 551.

Preliminary Communications

Blood Angiotensin II Levels of Normal and Hypertensive Subjects

Brit. med. J., 1969, **1**, 819–821

Summary: A specific radioimmunoassay for angiotensin II has shown that its normal concentration in arterial blood is 2·4±1·2 (S.D.) mμg./100 ml.; the venous level is consistently below this value, being usually 50–75% of it. Definite rises in blood angiotensin II levels were found in some patients with hypertension, both essential and secondary to renal disease. Extremely low levels were observed in two anephric women, and in one patient with Conn's syndrome. This radioimmunoassay offers a valuable alternative to renin bioassay in evaluation of the role of the renal pressor system in clinical disorders associated with hypertension and aldosteronism.

INTRODUCTION

Methods for the radioimmunoassay of angiotensin II in human blood have been described by Vallotton *et al.* (1967), Catt *et al.* (1967), Boyd *et al.* (1967), and Gocke *et al.* (1968). The values reported by these workers have shown some variation, though the ranges of plasma and blood concentration are similar for the latter three assays (Table I). It now seems probable that the normal level of circulating angiotensin II is in the range 1 to 5 mμg./100 ml., a concentration range similar to that previously reported by certain of the bioassay methods used for the measurement of angiotensin II in blood (Mulrow, 1964). Radioimmunoassay of blood angiotensin II has now been performed in a much larger number of normal and hypertensive subjects in order to define more precisely the normal blood levels of the octapeptide and to assess its role in the development of hypertension.

TABLE I.—*Radioimmunoassay Values for Blood Angiotensin II Concentration in Normal Human Subjects*

Authors	Specimen	Angiotensin II Concentration (mμg./100 ml.)	
		Range	Mean
Catt *et al.* (1967) ..	Arterial blood	0·5–4·7	2·1
Boyd *et al.* (1967) ..	Venous plasma	0·8–5·6	Not given
Gocke *et al.* (1968) ..	Venous plasma	1·8–11·0	5·4

METHODS

The previously described method for radioimmunoassay of blood angiotensin (Catt *et al.*, 1967) was used to measure circulating angiotensin levels in normal subjects and in those suffering from various forms of hypertension and vascular disease. All blood samples were taken after subjects had rested for at least 15 minutes in the recumbent position. Minor

modifications to the original assay procedure included the collection of blood into a dimercaprol/edetic-acid mixture (Boyd *et al.*, 1967), followed by the immediate addition to ethanol, and the use of 0·05 M NaOH to elute the extract from SE-Sephadex before neutralization and direct assay of the eluate. A Sephadex G-15 column was used to further purify the radioiodinated peptide used for recovery and assay tracers. ^{125}I-tracer angiotensin II (2,000 counts per minute) was again added to each blood sample before extraction in order to correct for losses occurring during the extraction procedure. In this way the value obtained by radioimmunoassay of each extract could be corrected to give the original blood level of angiotensin II. The recovery of angiotensin II, added to samples and extracted and assayed in this way, was 100%. The between-assay reproducibility of the method as given by the coefficient of variation was ±12%.

The subjects studied were subdivided as follows:

(1) Normal subjects. Arterial blood samples were obtained from normotensive volunteers and ward patients free of renal or cardiovascular disease, from whom informed consent was obtained for brachial artery puncture. The venous blood samples were obtained mainly from blood donors, after removal of 500 ml. of blood, together with a number of normotensive volunteers and ward patients.

(2) Patients undergoing angiography for neurological disturbances and peripheral vascular disease. Blood samples were obtained at the beginning of the procedure from patients with normal blood pressure and blood urea.

(3) Severe aortic vascular disease. Patients with aneurysm or occlusion of the aorta as shown by aortography.

(4) Essential hypertension. Patients with essential hypertension were classified as benign or malignant according to the usual criteria of diastolic pressure, neuroretinopathy, and encephalopathy. The benign group was further subdivided according to the height of the blood pressure, those whose diastolic blood pressure exceeded 120 mm. Hg being regarded as suffering from severe essential hypertension and those below 120 mm. Hg being regarded as manifesting a milder form of the disorder.

(5) Renal hypertension. Patients in whom hypertension was thought to be due to an underlying renal lesion. A variety of disorders was included in this group, ranging from glomerulonephritis to renal artery stenosis.

(6) Cirrhosis with ascites (two patients) and severe congestive cardiac failure (two patients).

(7) Bilateral nephrectomy: two nephrectomized female patients awaiting renal transplantation. Conn's syndrome: one patient with clinical features of primary hyperaldosteronism, markedly raised blood levels of aldosterone (50–58 mμg./100 ml.), which were not suppressed by saline infusion, and low plasma renin activity.

Results and Discussion

The mean level of angiotensin II in arterial blood of normal subjects (Table II) was found to be 2·4 mμg./100 ml., while those patients studied during angiography showed a slightly higher mean level of 3·9 mμg./100 ml. Venous levels in a separate group of normal subjects were 40% lower than the arterial values, the mean level being 1·4 mμg./100 ml. This finding is consistent with earlier observations that venous levels of angiotensin II were extremely low (Boucher *et al.*, 1964; Boyd *et al.*, 1967) and with the lower level of radioactivity detected in venous blood by Doyle *et al.* (1968) after infusion of a radioactive derivative of angiotensin II.

Nevertheless, the estimations of angiotensin II performed on arterial and venous blood samples taken from the same subjects (Table III) do not show such a marked difference as these group data suggest. In seven patients with normal arterial levels of angiotensin II the simultaneous venous levels were only 20% lower than the arterial values. The levels in arterial and venous blood of patients with increased circulating angiotensin II were similar, though in all cases studied the arterial level was higher than the venous concentration. In addition, the extremely low levels present in one of the anephric patients and Conn's syndrome also showed an arteriovenous difference. Further studies will be performed to verify these findings, but it appears that venous levels of angiotensin II may be used to give a reasonable approximation to the arterial concentration, and that venous blood samples may be satisfactory for estimation of circulating angiotensin II levels in clinical studies. It is of interest that the venous concentration of angiotensin II was not raised after venesection of 500 ml. of blood, the mean level of the 42 blood donors (1·2±0·8 (S.D.) mμg./100 ml.) being slightly lower than that obtained in the 13 ward patients and volunteers (1·8±0·9 (S.D.) mμg./100 ml.).

TABLE II.—*Blood Angiotensin Levels in Human Subjects*

Subjects	No.	Blood Samples	Angiotensin II Concentration (mμg./100 ml.) Mean ± S.D. ± S.E.	Individual Subject Values. Angiotensin II (mμg./100 ml.)
1. Normal subjects	23	Arterial	2·4±1·2±0·3	
	55	Venous	1·4±0·9±0·1	
2. Patients undergoing angiography	42	Arterial	3·9±2·3±0·3	
3. Aortic aneurysm, dissecting aneurysm, and aortic occlusion	5	Arterial	8·4±1·6±0·7	
4. Essential hypertension:				
Benign Mild	7	Arterial	5·2±2·6±1·0	
Benign Severe	6		9·0±6·5±2·7	
Malignant	4			10·3, 173·0, 4·1, 2·1
5. Renal hypertension	12	Arterial	18·5±8·7±2·5	
Renal artery stenosis	2			21·8, 39·8
Acute glomerulonephritis	1			24·5
Chronic glomerulonephritis	3			17·2, 18·8, 25·1
Chronic pyelonephritis	1			10·8
Unilateral hydronephrosis	1			10·6
Polyarteritis nodosa	1			9·1
Unilateral renal disease	2			10·6, 14·4
Polycystic kidneys	1			19·1
6. Cirrhosis with ascites	2	Arterial		13·0, 10·5
6. Severe cardiac failure	2			25·7, 12·5
7. Bilateral nephrectomy	2	Arterial		0·19, 0·14
7. Conn's syndrome	1			0·38

The arterial blood angiotensin II levels in a variety of patients with essential hypertension, renal hypertension, and other disorders are listed in Table II and shown in diagrammatic form in the Chart.

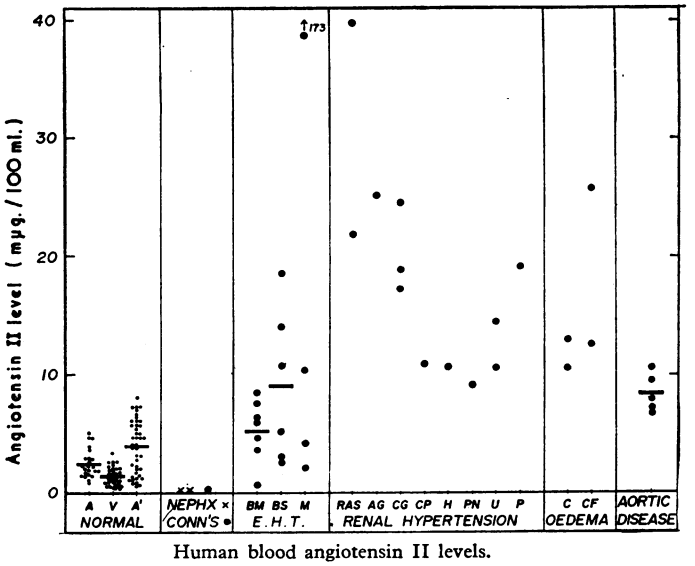
TABLE III.—*Simultaneous Arterial and Venous Angiotensin II Levels*

Subjects with:	Angiotensin II Level (mμg./100 ml.)	
	Arterial	Venous
Normal levels	2·4	2·0
	3·6	2·0
	1·9	1·2
	2·6	2·5
	2·8	2·6
	0·8	0·7
	3·5	3·1
Raised levels		
Cirrhosis with ascites	12·9	11·1
Acute glomerulonephritis	24·5	24·2
Unilateral renal disease	10·6	9·5
Subnormal levels		
Bilateral nephrectomy	0·19	0·12
	0·21	0·10
Conn's syndrome	0·38	0·27

The results obtained in patients with essential hypertension show a wide scatter, with no significant change in the mildly affected patients and a tendency to raised levels in the more severe group. Of the four patients with malignant hypertension, those studied before the institution of long-term hypotensive therapy showed raised levels, while those on treatment had normal arterial levels. The numbers of patients in these groups are not adequate to draw definite conclusions about the frequency of raised angiotensin levels at the various stages of essential hypertension, but there is certainly no significant change in the mildly hypertensive group. A much wider range of blood angiotensin II levels was found in patients with severe

hypertension, an association that must be interpreted with some caution, as most of the patients were on long-term hypotensive therapy at the time of study. However, the frequent observation of normal angiotensin II levels in patients treated with hypotensive agents makes it unlikely that such therapy would be the cause of the raised angiotensin levels seen in some cases in the severe hypertensive group. A further possibility is that those patients in this group with raised angiotensin II levels (three of the six patients studied) may have been suffering from an undetected renal lesion as the basis for their severe hypertension, since aortography and divided renal function tests had not been performed on these subjects at the time of study.

It was notable that the majority of the high angiotensin values observed during the study were found to occur in patients with hypertension due to underlying renal disease. While this finding was not unexpected in the patients with renovascular hypertension, there was also a substantial incidence of



Human blood angiotensin II levels.

raised angiotensin levels in patients with bilateral diffuse renal disease. Whether this association reflects a role of angiotensin II in the development of hypertension secondary to diffuse renal disease cannot be stated with certainty, though the frequent amelioration of hypertension occurring after bilateral nephrectomy in patients with chronic renal failure lends support to this possibility. Furthermore, the high level of 25 mμg./100 ml. observed in the patient with hypertension due to acute glomerulonephritis subsided to the normal level (4 mμg./100 ml.) three weeks later, when the blood pressure had returned to normal. However, it is also possible that the sodium depletion commonly present in patients with chronic renal disease may be responsible for some of the raised angiotensin levels observed in this group, since sodium loss is known to be a potent stimulus to angiotensin II formation (Blair-West *et al.*, 1968).

The rise in blood angiotensin II levels detected in these various groups of patients by radioimmunoassay are in general agreement with the bioassay results of Massani *et al.* (1966). Although these authors did not find a significant rise in blood angiotensin concentration in patients with chronic renal insufficiency, increased levels were observed in acute glomerulonephritis, severe and malignant hypertension, renal artery stenosis, congestive cardiac failure, and cirrhosis with ascites.

Small quantities of angiotensin II, well below the range found in normal subjects, were detected in the plasma of two anephric subjects. This finding is consistent with the previous report of low levels of angiotensin in anephric ewes (Catt *et al.*, 1967), and with the demonstration by Capelli *et al.* (1968) of a renin-like enzyme in the plasma of anephric female human subjects. It is significant that this finding has so far been reported only in the female, and that uterine tissue has been shown to contain renin-like activity, since the two humans and six sheep we have studied after nephrectomy were all females. The blood angiotensin II level was measured on three occasions over a period of three months in one of these nephrectomized patients, the levels obtained being 0·16, 0·21, and 0·19 mμg./100 ml. In the other anephric patient the blood angiotensin II level rose from 0·14 to 4·1 mμg./100 ml. after renal transplantation.

In contrast to the high values obtained in patients with renal artery stenosis and other forms of renal hypertension, the levels measured in a patient with the features of Conn's syndrome were extremely low, in keeping with the well-known suppression of renin activity in this condition (Conn *et al.*, 1964). These levels were the lowest obtained, apart from those of the anephric subjects, and provide a further indication of the specificity of the assay as well as indicating its potential usefulness during the evaluation of patients with suspected primary hyperaldosteronism.

The use of radioimmunoassay has made the estimation of blood angiotensin concentration a relatively simple, precise, and specific procedure. Since the main applications of renin assay in clinical medicine are concerned with the evaluation of conditions in which abnormal blood levels of angiotensin are suspected, and angiotensin II is responsible for all of the known physiological functions of renin, the direct assay of this peptide in blood offers the most meaningful estimate of the effective activity of the renin–angiotensin system in vivo. The observed rises of immunoreactive angiotensin concentration sometimes present in the blood of patients with severe essential hypertension, and the frequent rises seen in renal hypertension indicate that increased angiotensin II production may contribute to the maintenance of high blood pressure in these conditions.

This work was supported by a grant from the National Heart Foundation of Australia. We are grateful to Dr. J. P. Coghlan and Dr. R. B. Scoggins for plasma aldosterone determinations.

K. J. CATT, M.D., PH.D., M.R.A.C.P.
M. D. CAIN, M.SC.
P. Z. ZIMMET, M.B., B.S.
E. CRAN, B.SC.

Department of Medicine, Monash University, Prince Henry's Hospital, Melbourne, Australia.

REFERENCES

Blair-West, J., *et al.* (1968). *XXIV Int. Congr. Physiol. Sci.*, **6**, 249.
Boucher, R., Veyrat, R., de Champlain, J., and Genest, J. (1964). *Canad. med. Ass. J.*, **90**, 194.
Boyd, G. W., Landon, J., and Peart, W. S. (1967). *Lancet*, **2**, 1002.
Catt, K. J., Cain, M. C., and Coghlan, J. P. (1967). *Lancet*, **2**, 1005.
Capelli, J. P., Wesson, L. G., jun., Aponte, G. E., Faraldo, C., and Jaffe, E. (1968). *J. clin. Endocr.*, **28**, 221.
Conn, J. W., Cohen, E. L., and Rovner, D. R. (1964) *J. Amer. med. Ass.*, **190**, 213.
Doyle, A. E., Louis, W. J., Jerums, G., and Osborn, E. C. (1968). *Amer. J. Physiol.*, **215**, 164.
Gocke, D. J., Sherwood, L. M., Oppenhoff, I., Gerten, J., and Laragh, J. H. (1968). *J clin. Endocr.*, **28**, 1675.
Massani, Z. M., Finkielman, S., Worcel, M., Agrest, A., and Paladini, A. C. (1966). *Clin. Sci.*, **30**, 473.
Mulrow, P. J. (1964). *Canad. med. Ass. J.*, **90**, 277.
Vallotton, M. B., Page, L. B., and Haber, E. (1967). *Nature (Lond.)*, **215**, 714.