A New Direct Method for the Assay of Human Renin

The elucidation of the physiological and pathological roles of the renin-angiotensin system has been hindered by the lack of a direct assay for renin. Current analytical techniques depend on the measurement of the rate of angiotensin I and II formation during incubation with renin substrate. These methods have shown that in some cases of renal hypertension, plasma renin activity is high. But, there is not necessarily a straight forward relationship between the biological activity of renin and its concentration[1]. The reasons for this may only be determined if renin concentration as well as activity can be measured.

Methods. We have carried out preliminary work on a direct, quantitative immunochemical method for human plasma renin determination, based on the 'line electrophoresis' technique of KROLL[2]. This employs the electrophoretic movement of proteins from an agarose sample gel into one containing antiserum. As the antigen-antibody complexes form precipitin lines appear. The final distance moved by a line from the sample gel boundary is proportional to the antigen concentration per unit length sample gel boundary (Figure 1). The movement is inversely proportional to the antibody titre (Figure 2). The method has been adapted to use the minimum amounts of standard renin and plasma samples. The separations were carried out on 5 cm square glass slides, in 1% agarose, 0.02 *M* barbitone acetate buffer (pH 8.6) gel containing 1 mg/ml EDTA. The antiserum had a high titre of antirenin (170 angiotensin units ml measured by in vitro depression of angiotensin formation), and only very weak antibodies to other plasma proteins (assessed by plasma-antirenin 2 dimensional immuno-electrophoresis).

The identification of the renin-antirenin precipitin line and the minute quantities of renin being assayed presented the main problems. Impurity antibodies in the antirenin were precipitated by the addition of anephric human serum[3]. This did not affect the antirenin titre. The electrophoretic mobility of human renin correlated with the position of the peak formed when the human renin preparation was run against the partially purified antirenin in 2 dimensional immuno-electrophoretic separations. The minute quantities of protein used necessitated preliminary adjustment of the antirenin concentration to give maximum line migration and definition. Purified agarose further increased line migration and reduced endosmosis to a minimum[4].

To determine the concentration of renin in human plasma, 2 sample gels were used per slide, one containing standard renin (about 5.4 µg) and the other standard renin (about 5.4 µg) plus plasma (about 100 µl). Error due to variation in antiserum concentration was thus avoided. The exact size of a sample gel was assessed by weighing and line migration measured on an enlarged, projected image.

The differences between the line migrations of known amounts of standard renin, with or without added plasma, were used to determine the concentration of renin in the plasma samples in terms of µg/ml of standard renin. The relationship between renin concentration and line migration was shown to be linear in the range used (Figure 1). Renin activity was measured by the production of angiotensin II from a semi-purified dog renin substrate[5]. One unit of renin activity was taken to be equivalent to an initial angiotensin production rate of 1.0 ng angiotensin II/ml/h.

Results. Using the technique described, preliminary results (Table) show that the mean plasma renin concentration in 3 normal samples taken from subjects with no known history of renal disease, was 5.8 µg standard renin per ml. This is equivalent to a theoretical plasma renin activity of 18.2 units assessed by the bioassay system[3]. The actual plasma renin activity was found to be 1.15 units. Thus the theoretical and actual renin activities differed markedly. However, a closer correlation was obtained between the theoretical and actual activities of 3 plasma samples from renal hypertensive patients. These had a

[1] J. LAZAR, J. C. ROMERO and S. W. HOOBLER, Am. J. Physiol. *220*, 191 (1971).
[2] J. KROLL, Scand. J. clin. Lab. Invest. *22*, 112 (1968).
[3] M. A. WINCH, A. R. NOBLE and K. A. MUNDAY, in preparation.
[4] B. G. JOHANNSON and J. STENFLO, Analyt. Biochem. *40*, 232 (1971).
[5] S. L. SKINNER, Circulation Res. *20*, 391 (1967).

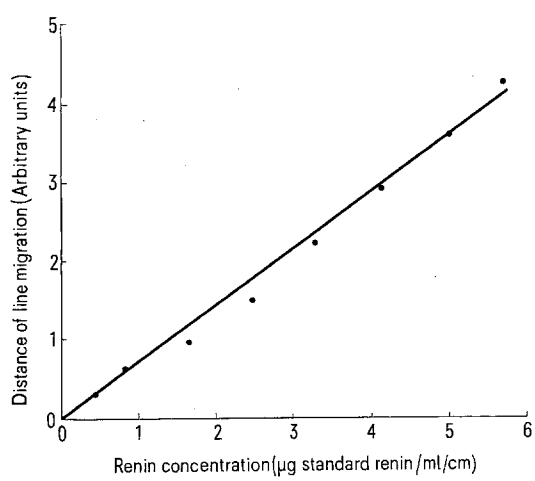

Fig. 1. Relationship between distance of line migration in line electrophoresis and antigen concentration for renin-antirenin interaction.

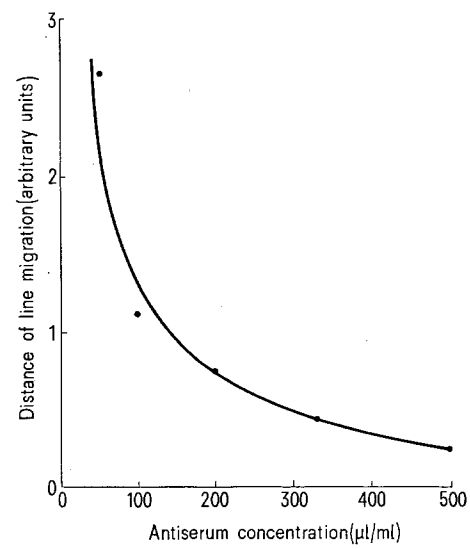

Fig. 2. Relationship between distance of line migration in line electrophoresis and the antiserum concentration for renin-antirenin interaction.

Human plasma renin concentration[a], theoretical activity[b] and actual activity[c] in normal, anephric and renal hypertensive patients

	Mean plasma renin concentration[a] (μg standard renin per ml plasma)	Mean theoretical renin activity[b] (ng angiotensin per ml plasma per h)	Mean actual renin activity[c] (ng angiotensin per ml plasma per h)
Normal[3]	5.8	18.2	1.15
Anephric[2]	0.0	0.0	0.0
Renal hypertensive[3]	1.2	3.7	4.45

[a] Measured by line electrophoresis
[b] Calculated from renin concentration and the activity of the standard human renin preparation
[c] Measured by bioassay
Individual assays were done in triplicate

mean renin concentration of 1.2 μg standard renin-ml, equivalent to a theoretical plasma renin activity of 3.7 units. The mean plasma renin activity was estimated to be 4.5 units. Anephric human plasma contained no renin detectable by these techniques.

Discussion. These initial results suggest two things. First that the production of angiotensin II from semi-purified dog substrate by human renin is considerably inhibited in normal plasma. This is shown by comparison with the production of angiotensin II during incubation of highly purified human renin in similar conditions. Second that patients with renal hypertension have a lower concentration of renin than normal, yet, in the few cases studied show a higher renin activity. These observations could be explained in two ways. Either the renin is in a more active form, or there is less inhibition of angiotensin production in the plasma of patients with renal hypertension. The possible existence of an inhibitory system has already been postulated[6].

Résumé. Nous avons institué une nouvelle méthode directe pour l'estimation du rénin par l'immunoélectrophorèse. Les résultats préliminaires obtenus par cette méthode indiquent que des changements importants peuvent se faire dans l'activité enzymatique du rénin dans le cas de patients ayant de l'hypertension rénale.

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[6] R. R. Smeby, S. Sen and F. M. Bumpus, Circulation Res. *21*, Suppl. 2, 129 (1967).
[7] We are indebted to Dr. C. Robinson of the Division of Biological Standards Medical Research Council, Mill Hill, for samples of purified human renin, and to Prof. J. R. Hobbs of the Department of Chemical Pathology, Westminster Hospital Medical School, for his support of this work.
[8] Correspondence to Dr. A. R. Noble, Department of Physiology and Biochemistry, The University, Southampton SO9 5NH, England.

Storage of Streptomyces in Soft Agar and by Other Methods

Long term conservation. There are numerous methods available for the long term preservation of microorganisms[1,2]. We have employed several of them for the maintenance of a rather large collection of streptomycetes and found the following quite useful: 1. Soil culture – a method recommended repeatedly for the conservation of fungi and streptomycetes[3,4]: Most of our 165 soil cultures prepared in 1958[5] could be subcultured after 8 years of storage and even now many of them give a high viable count. 2. Deep freeze: Our observations on the viability of 400 cultures kept at −20 °C for 5 years agree well with those made by other investigators[6,7]. In order to save storage space in the deep freeze unit, the streptomycetes were grown in ampoules which were sealed after the development of a mature aerial mycelium had taken place. 3. Lyophilization – the method most widely employed: The procedure suggested by Hopwood and Ferguson[8] has been used. Our liophils – prepared $1^1/_2$ years ago – give heavy growth. 4. Drying in nutrient gelatin discs[9]: Of 50 streptomycetes dried 2 or 3 years ago 35 yielded a high viable count recently, whereas the others gave only few colonies per disc or had died. 5. Drying on unglazed porcelain beads[10,11]: 60 stains – preserved by this method $1^1/_2$ years ago – are easily revived by placing a bead charged with spores on a nutrient medium. – A similar method suggested by Perkins[12] using anhydrous silica gel as acceptor of the spore suspension proved to be unsuitable for streptomycetes as these organisms could not be recovered after a short time of storage.

With most methods for long term conservation, the organisms have to be revived and propagated before they can be used in the experiments intended; soil cultures and agar cultures stored in deep freeze may be exceptions. Therefore, the inoculum for 'experimental media' usually

[1] C. T. Calam, Progr. ind. Microbiol. *5*, 1 (1964).
[2] S. M. Martin, Ann. Rev. Microbiol. *18*, 1 (1964).
[3] A. Bakerspiegel, Mycologia *45*, 596 (1953).
[4] W. Frommer, Arch. Mikrobiol. *25*, 219 (1956).
[5] W. Flaig and H. J. Kutzner, Arch. Mikrobiol. *35*, 105 (1960).
[6] J. W. Carmichael, Mycologia *48*, 378 (1956).
[7] H. D. Tresner, F. Danga and J. N. Porter, Appl. Microbiol. *8*, 339 (1960).
[8] D. A. Hopwood and H. M. Ferguson, J. appl. Bact. *32*, 434 (1969).
[9] L. Stamp, J. gen. Microbiol. *1*, 251 (1947).
[10] G. A. Hunt, A. Gourevitch and A. Lein, J. Bact. *76*, 453 (1958).
[11] B. J. Lange and W. J. R. Boyd, Phytopathology *58*, 1711 (1968).
[12] D. D. Perkins, Can. J. Microbiol. *8*, 591 (1962).