STRUCTURE OF BRADYKININ

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BRADYKININ is the name given by its discoverers, Rocha e Silva *et al.*, (1949) to the polypeptide formed by the action of trypsin or snake venom upon the pseudoglobulin fraction of plasma proteins. Other substances, such as pain-producing substance (Armstrong *et al.*, 1957) and kallidin (Werle and Berek, 1950), which can also be produced from plasma, resemble bradykinin very closely, but as none of these have yet been obtained in the pure state it cannot be assumed that they are chemically identical with it. The kinin found in urine (see refs. in Gaddum and Horton, 1959) is also very similar to bradykinin, but it has not been chemically identified.

DEFIBRINATED OX BLOOD	90 l.
↓ Centrifuging of red cells	
SERUM	44 l.
| Addition of saturated $(NH_4)_2SO_4$ soln. (22 l.)	
↓ Centrifuging	
SUPERNATANT	60 l.
| Addition of saturated $(NH_4)_2SO_4$ soln. (13 l.)	
↓ Centrifuging	
PRECIPITATE	

FIG. 1.

SOLN. OF PPT.	5 l.
| Dialysis	9 l.
| Heating at37°, pH2, 30 min	
| Digestion with trypsin (150 mg) 37°, pH 7·5, 6 hr	
| Addition to boiling ethanol 30 l.	
↓ Centrifuging	
SUPERNATANT	300 ml.
| Evaporation	
| Counter-current distribution	
↓ Freeze-drying	
CRUDE BRADYKININ P2 yield 5 g (15 mg)	

FIG. 2.

In our laboratory bradykinin was prepared by the action of trypsin on the pseudoglobulin fraction of ox serum which had been previously heated with acid (Horton, 1958) to inactivate the enzyme which destroys bradykinin. The preliminary steps in the purification followed established procedures as shown in Figs. 1 and 2. The main novelty in our ap-

proach to the problem (Elliott, Horton and Lewis, 1960) was the use of carboxymethylcellulose columns in the presence of a volatile buffer and in three successive stages, starting from P2. Fig. 3 shows the results obtained on the final column. The yield of bradykinin in each tube was expressed in terms of the weight of our standard crude preparation, P2, to which the whole tube contents were equivalent. The slight break in the curve is believed to be due to a flaw in our experimental technique and is not due to incipient separation into two peaks. We have since obtained several curves in which this break does not occur. The final yield of pure bradykinin was about 3·5 mg from 90 l. of ox blood, and this amount is about one-quarter of that originally present in the trypsin digest. At an earlier stage of the work, paper chromatography or paper electrophoresis were used instead of the third carboxymethylcellulose column, but these methods were less convenient for preparative purposes.

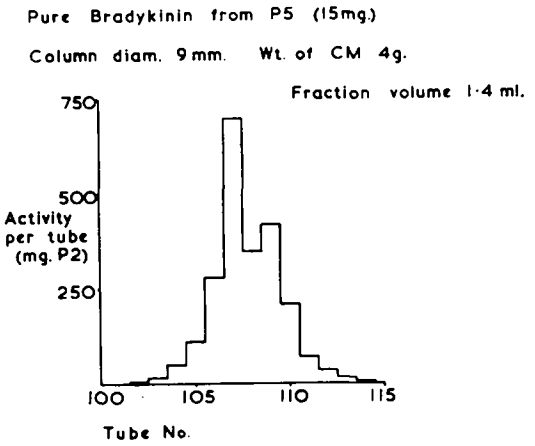

FIG. 3.

Bradykinin formed by the action of snake venom on ox plasma has been isolated by Zuber and Jaques (1960). These workers made use of oxycellulose columns, and high voltage paper electrophoresis.

Hydrolysis of trypsin bradykinin gave only 5 amino acids and Fig. 4 shows a two-dimensional chromatogram of the hydrolysate of the first sample of pure peptide which we obtained. Quantitative estimation gave the following molar ratios for the amino acids:- Arginine 2, Phenylalanine 2, Proline 2, Serine 1 and Glycine 1.

The same result was obtained by Zuber and Jaques on snake venom bradykinin. Unfortunately this analysis contained an error which led us astray in our structural work. These studies led us to the conclusion that the sequence in bradykinin was Arg Pro Pro Gly Phe Ser Phe Arg, the position of each amino acid being positively identified. There was one observation which did not fit in the picture, and that was the presence of

a weak proline spot on a certain chromatogram which could only have been derived from a third proline residue between the serine and phenylalanine residues. Since we had already definitely located two proline residues elsewhere in the molecule we concluded that the proline spot was an artefact. During the course of our work we had received several enquiries about the structure from research groups who were interested

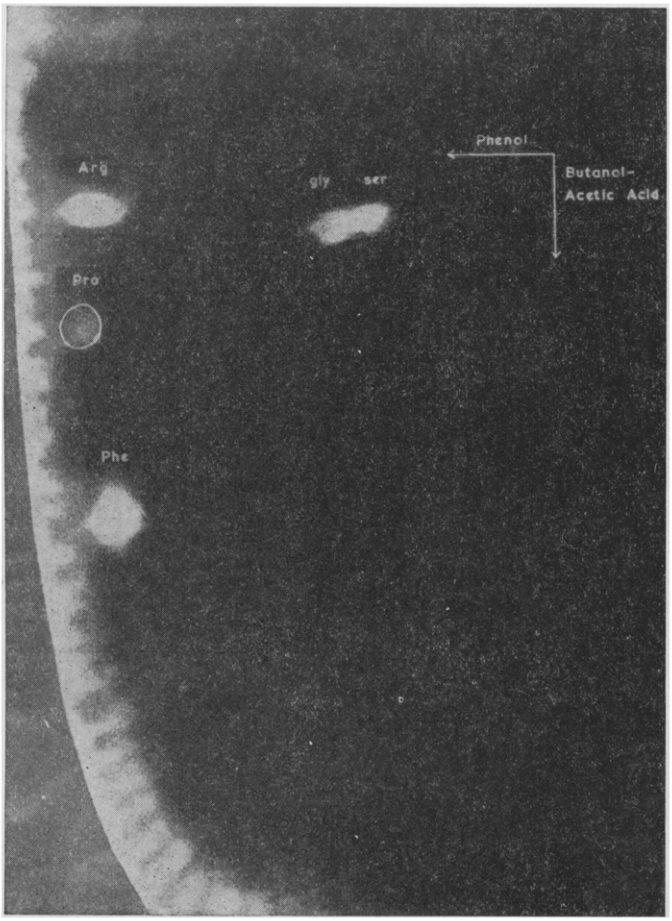

FIG. 4.

in synthesis, but who had been unable to carry out any structural work. Without attempting to confirm the structure by synthesis we immediately informed these research groups of our results and a few weeks later we gave a communication to the Biochemical Society, (Elliott, Lewis and Horton 1960*a*). We very much regret the confusion which must have been caused by the publication of this erroneous structure. Dr. Boissonnas of Sandoz Laboratories wrote to us very shortly afterwards to say that the octapeptide with the structure given was inactive. In the course of the

discussions which followed, I mentioned the possibility of a third proline between the serine and the phenylalanine, but apparently Dr. Boissonnas and his collaborators, Dr. Guttmann and Dr. Jaquenoud, had already put in hand the preparation of the nonapeptide (1960) along with several other variants of the octapeptide structure before our letter reached them.

When we compared samples of the synthetic nonapeptide Arg Pro Pro Gly Phe Ser Pro Phe Arg kindly supplied by Sandoz Laboratories and by Parke Davis and Company (Nicolaides and De Wald 1961) it was found to be equiactive on eleven different biological preparations with the material which we had isolated (Lewis, 1960). The identity of the nonapeptide with natural bradykinin supplied by us was confirmed by two other groups of workers (Collier and Shorley, 1960; Konzett and Stürmer, 1960).

Meanwhile we were able to demonstrate, by further degradative work, the presence and the position of the third proline in natural bradykinin (Elliott, Lewis and Horton, 1960*b*). We are therefore indebted to Dr Boissonnas and his collaborators for pointing out our mistake so quickly.

REFERENCES

D. ARMSTRONG, J. B. JEPSON, C. A. KEELE and J. W. STEWART; *J. Physiol.* **135** 350 (1957).

R. A. BOISSONNAS, ST. GUTTMANN and P.-A. JAQUENOUD; *Helv. Chim. Acta* **43** 1349 (1960).

H. O. J. COLLIER and P. SHORLEY; *Nature, Lond.* **188** 999 (1960).

D. F. ELLIOTT, E. W. HORTON and G. P. LEWIS; *Biochem. J.* **78** 60 (1960).

D. F. ELLIOTT, G. P. LEWIS and E. W. HORTON; *Biochem. J.* **76** 16P (1960 *a*).

D. F. ELLIOTT, G. P. LEWIS and E. W. HORTON; *Biochem. Biophys. Res. Comm.* **3** 87 (1960 b).

J. H. GADDUM and E. W. HORTON; *Brit. J. Pharmacol.* **14** 117 (1959).

E. W. HORTON; *J. Physiol.* **142** 36P (1958).

H. KONZETT and E. STÜRMER; *Nature, Lond.* **188** 998 (1960).

G. P. LEWIS; *Nature, Lond.* **188** 999 (1960).

E. D. NICOLAIDES and H. A. DE WALD; in preparation (1961).

M. ROCHA E SILVA, W. T. BERALDO and G. ROSENFELD; *Amer. J. Physiol.* **156** 261. (1949).

E. WERLE and U. BEREK; *Biochem. Z.* **320** 136 (1950).

H. ZUBER and R. JAQUES; *Helv. Chim. Acta* **43** 1128 (1960).