A similar decrease appears to take place during embryonic development, as shown in Figs. 1 and 2. Work on the early embryo has demonstrated neither changes during development nor local differences in the oxygen uptake if this is calculated per unit of nitrogen or dry weight (see Spratt, 1952). In the present study on older embryos, however, the reduction in Q_{O_2} with age or with increasing body weight during embryonic life was definite and characteristic. Such a reduction might conceivably be due to developmental changes in the respiratory-enzyme systems. Stained sections of embryonic arteries, however, suggested that it might be associated with an increase of relatively inert material such as reticulin, collagen and elastic tissue. The attempt to put this on a quantitative basis by means of densitometer readings gave a surprising degree of correlation between the curves for Q_{O_2} and for reciprocals of densitometer readings of Mallory-stained sections. Such reciprocals may be taken to represent the degree of cellularity of the sections. Whatever other influences may be at work, therefore, the accumulation of connective tissue is likely to be a dominant factor in determining the reduction in respiratory activity of the artery with advancing development.

SUMMARY

1. The Q_{O_2} of artery slices from the rat, rabbit, sheep, pig and ox have been determined with the Warburg manometer.

2. Similar observations on arteries derived from chick embryos ranging in age from 6 to 22 days of incubation have been made with a micro-respirometer.

3. The adult arteries were moderately active tissues with Q_{O_2} values ranging from -0.37 to -2.43 . Comparing one species with another, there was a decrease of Q_{O_2} with increase of size of the animal.

4. A regular and considerable reduction in Q_{O_2} of chick arterial tissue occurred as the age and weight of the embryo increased. This reduction was apparently associated with the development of connective tissue.

This work was aided by a personal grant to A.E.H. and a grant for expenses from the Scottish Hospital Endowments Research Trust to whom we express our thanks. We also wish to thank Dr R. P. Cook for his help and advice, Dr C. N. D. Cruickshank for the use of the micro-respirometer, Mr J. H. Johnstone for making the densitometer readings, and Miss A. H. Ogilvie for technical assistance.

REFERENCES

- Briggs, F. N., Chernick, S. & Chaikoff, I. L. (1949). *J. biol. Chem.* **179**, 103.
 Cameron, G. (1950). *Tissue Culture Technique*, p. 53. New York: Academic Press Inc.
 Cruickshank, C. N. D. (1954). *Exp. Cell Res.* **7**, 374.
 Edwards, J. E. (1950). *McClung's Handbook of Microscopical Technique*, 3rd ed., p. 240. Ed. by Jones, R. McC. New York: Hoeber Inc.
 Hamilton, H. L. (1952). *Lillie's Development of the Chick. An Introduction to Embryology*, p. 74. New York: Holt and Co.
 Krebs, H. A. (1950). *Metabolism and Function*, p. 249. Ed. by Nachmansohn, D. London: Elsevier.
 Lazovskaya, L. N. (1943). *Biokhimiya*, **8**, 171.
 Medawar, P. B. (1948). *Quart. J. micr. Sci.* **89**, 187.
 Spratt, N. T. jun. (1952). *Ann. N.Y. Acad. Sci.* **55**, 40.

The Isolation of a Hypertensin

By W. S. PEART

Medical Unit, St Mary's Hospital, London, W. 2, and National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 19 September 1955)

There have been a number of recent reports on the purification of the pressor substance hypertensin, or angiotonin (Bumpus & Page, 1954; Skeggs, Marsh, Kahn & Shumway, 1954a; Kuether & Haney, 1955), in which the pressor activity was associated with peptide material. The pressor activity of these recent preparations was stated to be greater than that reported by Edman (1945), but the amino acid composition of the preparations varied considerably. The isolation is now reported of an apparently homogeneous peptide containing eight

different amino acids, with a pressor activity 1.5–3 times that of (–)-noradrenaline as measured in the anaesthetized rat (Peart, 1955a). A preliminary report of this work has already appeared (Peart, 1955b).

METHODS

Materials. All solvents were redistilled and had a low peroxide content. Hyflo Supercel was obtained from Johns Manville Co., Ltd., Artillery Row, London, S.W. 1. It was suspended in 2N-HCl overnight, washed with H_2O until

nearly neutral, and dried at 105°. Silane-treated Hyflo was prepared as described by Howard & Martin (1950).

Preparation of hypertensin. This was prepared by incubation of renin, made from rabbit kidney, with ox serum, in the presence of charcoal, from which the hypertensin was subsequently eluted with glacial acetic acid (Peart, 1955a).

Assay of hypertensin. Direct measurement was made of the pressor response in the anaesthetized rat given pentapyrrolidinium tartrate (Peart, 1955a). Increase in the activity/weight quotient at each stage was measured by comparison with (-)-noradrenaline.

Partition chromatography. Columns were packed with Hyflo or silane-treated Hyflo, 1 ml. of stationary phase/2 g. of support being used (Martin, 1949).

Amino acid identification. Standard paper-chromatographic techniques were used (Consden, Gordon & Martin, 1944) with phenol-3% NH_3 and *n*-butanol-acetic acid-water (63:10:27 by vol.) (cf. Partridge, 1948) as a solvent pair for two-way chromatograms. *n*-Butanol-3% NH_3 was used to distinguish leucine from isoleucine.

Quantitative amino acid analysis. Paper chromatography on Whatman no. 3 paper by descent for 24 hr. (phase mixture butanol-acetic acid-water, as above) was followed by colour development at 100° for 15 min. after spraying with ninhydrin (1%) in *n*-butanol saturated with phosphate buffer (0.05M) pH 7.0. The spots were eluted with 70% (v/v) acetone and the optical density was read in a Unicam spectrophotometer SP. 500 at 580 m μ . (cf. Pereira & Serra, 1951). Serial dilutions of an equimolar mixture of the eight amino acids present in the hypertensin were run in parallel with the hydrolysate. A curve relating quantity to optical density was made for each amino acid and the values for the hypertensin hydrolysate were obtained from it. The accuracy for duplicate runs of known amounts was $\pm 10\%$, and the lower limit was 0.025–0.05 μmole for all except proline, which was 0.05 to 0.1 μmole . The temperature of colour development was important for proline, since at 65° the usual yellow colour with ninhydrin developed, but at 100° a blue-brown colour was produced, with a maximum optical density at 580 m μ .

N-Terminal group. This was determined by the FDNB method of Sanger & Thompson (1953). The DNP amino acid was identified by paper chromatography, the *tert*. pentanol-phthalate buffer (pH 6.0) system of Blackburn & Lowther (1951) being used.

Paper electrophoresis. Durrum's technique (1950) was used. Whatman no. 1 paper was irrigated for 48 hr. with 30% (v/v) acetic acid before use. The potential drop along the paper was usually 5–10 v/cm. and the buffer solutions employed were as follows: pH 2.0, 5% (v/v) formic acid; pH 2.5, 30% (v/v) acetic acid; pH 3.0, 4.0 and 5.0, acetic acid-sodium acetate (0.05M); pH 6.0 and 7.0, KOH/ KH_2PO_4 (0.1M); pH 8.0, boric acid-sodium borate (0.1M); pH 9.5, glycine/NaOH (0.1M); pH 10.5, proline/NaOH (0.1M); pH 12.0, NaOH (0.01M). There was no rigid control of ionic strength. In the tests for homogeneity a potential drop of 20 v/cm. with 50% (v/v) acetic acid was also used. At the end of a run, successive 1 cm. sections of the paper away from the origin were eluted with 0.3% (w/w) NH_3 and the eluates assayed to discover the site of pressor activity. Electroendosmosis was followed by using glucose as the neutral marker, the glucose being stained with aniline phthalate (Partridge, 1949). A 1 cm. strip at the

edge of the paper was stained for glucose and the remainder of the paper was examined for the pressor activity.

Spectrophotometry. This was carried out by the logarithmic-cam moving-plate method (Holiday, 1937).

RESULTS

Paper electrophoresis

Using paper electrophoresis, it was possible to obtain reproducible results at pH values of 2–13 with the pressor activity present in a confined area and readily eluted from the paper. Material (5 mg.) from the butanol-extract stage was applied to the paper. Fig. 1 illustrates such an experiment at pH 6.0, where the pressor activity had moved towards the cathode and was present in segments 7 and 8. In Fig. 2, the movement of hypertensin

Fig. 1. Paper electrophoresis of hypertensin in 0.1M phosphate buffer pH 6.0, run for 3 hr. at 5v/cm. The pressor activity of equal dilutions of eluates from successive cm. strips of paper is shown. Nearly all the activity is in strips 7 and 8. 5 mg. of material from the butanol-extract stage was applied.

Fig. 2. Paper electrophoresis, showing the movement of hypertensin relative to glucose at various pH's. Run for 3 hr. at 5v/cm.

relative to glucose was plotted at various pH values, showing that hypertensin was amphoteric with an isoelectric range of pH 7-8.5. This supported the view that hypertensin was a peptide, and the rather high isoelectric range suggested the possibility that there was a preponderance of basic groups. The value of this procedure was great, since the information was gained by the use of small quantities of the impure hypertensin from the butanol-extract stage, and it is doubtful whether any other method could give comparable information at such a stage of purity. It was shown subsequently that the crude material contained three distinct pressor materials, but the electrophoretic results applied to only one of these, the major fraction, which represented 80% of the total activity. The assays of the eluates from the paper were carried out on dilutions in which only this major fraction could have been detected.

Purification

The main steps in the process of purification, starting with 400 l. of blood, are shown in Table 1, and the data in the following description apply to this batch.

Initial extraction. The crude aqueous hypertensin solution obtained from the charcoal eluate (Peart, 1955a) had a volume of 750 ml. and a dry weight of 25 g. It was saturated with NaCl,

brought to pH 1 with HCl and extracted twice with an equal volume of *n*-butanol (Skeggs, Kahn & Shumway, 1952). The butanol was extracted three times with one-third of its volume of water, which was evaporated to dryness on a rotary still (Craig, Gregory & Hausmann, 1950) at 10 mm. and 45°, and unless otherwise stated this drying procedure was always used in the subsequent steps.

Salt removal. In order to remove the NaCl carried over in the previous step, the residue was suspended in the minimum volume of glacial acetic acid, in which hypertensin was freely soluble and NaCl very slightly soluble. Five washes with 10 ml. portions of acetic acid were needed to dissolve all the hypertensin. The solution was filtered and the filtrate dried, leaving a residue which weighed 4.5 g. These extraction procedures gave a five-fold increase in the activity/weight quotient, with a 90% recovery of activity. It was important that hypertensin could now be used in systems of partition chromatography.

Butanol-acetic acid partition column. The material from the previous step was dissolved in 50 ml. of the mobile phase and applied to a Hyflo column (200 g.) holding the lower phase of a mixture of *n*-butanol-acetic acid-water (9:1:10 by vol.), with the top phase mobile. The partition coefficient of hypertensin in this phase mixture was 10:1 in favour of the lower phase. From this type of

Table 1. *Isolation of a hypertensin*

	Dry wt. (g.)	Increase in activity/wt. quotient	Activity recovery of each stage (%)
400 l. of ox blood (5 batches)	—	—	—
↓			
200 l. of serum (incubated with renin)	—	—	—
↓			
Aqueous hypertensin solution from charcoal eluate	25	—	—
↓			
Butanol extract	4.5	5	90
↓			
Partition chromatography 200 g. column of Hyflo (butanol-aqueous acetic acid)	—	—	—
↙ ↘			
minor peak A	0.22	25	80
major peak			
↓			
200 g. column of Hyflo (butanol-aqueous trichloroacetic acid)	—	—	—
↙ ↘			
minor peak B	0.042	5	80
major peak			
↓			
Adsorption chromatography 150 g. column of Hyflo	—	—	—
↓			
one peak	0.006	6	80

column two peaks of pressor material were obtained with R values of 0.65–0.7 and 0.35–0.4 respectively. The first, minor peak, contained only 5–10% of the total activity and was stored for further study. The increase in the activity/weight quotient of the material in the main peak was 25-fold and the average recovery of activity was 80%. A typical result is shown in Fig. 3, where the two peaks of pressor activity in the effluent from the column are shown. The active material in the major peak, contained in 250 ml., was displaced into 1 vol. of water, after the addition of 2 vol. of ether, and the aqueous solution was evaporated to dryness. This avoided losses which occurred if the butanol solution itself was evaporated to dryness. The material weighed 220 mg.

Fig. 3. Elution of hypertensins from a Hyflo column (200 g. of support; diameter 3.2 cm.). Phase mixture: *n*-butanol-acetic acid-water (9:1:10 by vol.). Upper phase mobile. Fractions, 50 ml. A major and a minor peak are shown. 4.5 g. of material was applied to the column.

Hyflo adsorption column. At this stage the use of a Hyflo adsorption column was not a direct step in the line of purification, but it can be conveniently considered here. A sample (2 mg.) of the active material from the major peak of the butanol-acetic acid column was adsorbed from water on a 2 g. column of Hyflo and eluted with 0.005M ammonium formate (pH 6.5). This gave clear evidence of two pressor materials: a minor peak displaced at the front, representing 5–10% of the total activity, and a slower-moving peak starting at about R 0.15 and trailing slowly off the column. This represented the remainder of the activity, and the trailing elution could be sharpened by increasing the concentration of the eluent. Fig. 4 shows an experiment in which the first peak is shown fully (fractions 2–5) and the first three fractions (7–9) of the main activity, which continued to trail out of the column, in a further nine fractions.

Both peaks when re-run on a similar column ran with the same R values.

Butanol-trichloroacetic acid partition column. This was the next step in the main line of purification. The material (220 mg.) from the major peak of the butanol-acetic acid column was dissolved in 50 ml. of the mobile phase and applied to a reverse-phase column of 200 g. of silane-treated Hyflo holding the upper phase of a mixture of *n*-butanol-0.25% aqueous trichloroacetic acid (1:4 by vol.) with the lower phase mobile. The partition coefficient was 15:1 in favour of the upper phase. It was noted that trichloroacetic acid markedly increased the partition coefficient of hypertensin in favour of certain water-immiscible alcohols. This resembles the effect of dichloroacetic acid on the partition of insulin between water and *sec*-butanol (Harfenist & Craig, 1951). From this type of column two

Fig. 4. Adsorption chromatography of hypertensins on a Hyflo column (2 g. of adsorbent; diameter 1.1 cm.). Eluent, 0.005M ammonium formate. The tracing shows pressor responses to equal dilutions of the 1.8 ml. fractions. The initial minor peak (2–5) and the start of the major peak (7–9) are shown. 2 mg. of material was applied to the column.

peaks of pressor activity were obtained, with R values of 0.5–0.55 and 0.25–0.3 respectively. The first represented 5–10% of the total activity and was stored for further study. There was a five-fold increase in the activity/weight quotient for the material in the major peak, and the recovery of activity was 80%. The weight of material in the peak was obtained by acidifying a portion (5% by volume) of the solution with HCl to pH 1.0, and extracting five times with ether to remove the trichloroacetic acid, before evaporating to dryness. The dry weight of the fraction was 2.1 mg., and the total weight was therefore 42 mg. Fig. 5 shows the position and relative activity of the two peaks in the effluent from a 50 g. column. It will be noted that there was some trailing of the major peak, and this was almost certainly due to adsorption of the hypertensin on the Hyflo. When the products from the two peaks were adsorbed on to a Hyflo column and elution was carried out with 0.005M ammonium

formate they yielded a major and a minor peak respectively which were chromatographically identical with those obtained from the Hyflo adsorption column described in the preceding section. It was at this stage that the adsorption of hypertensin on glass was noted. The adsorption could be reduced by treating the glass with silane, and, if the active solutions of the major peak were brought above pH 8.5 with NH_4OH or NaOH , desorption was complete from untreated glass.

Fig. 5. Elution of hypertensins from a silane-treated Hyflo column (50 g. support; diameter 2.2 cm.). Phase mixture *n*-butanol-0.25% aqueous trichloroacetic acid (1:4 by vol.). Lower phase mobile. Fractions 10 ml. Minor and major peaks are shown. 60 mg. of material was applied to the column.

Adsorption on Hyflo. For the final stage of purification, the solution containing the active material of the main peak from the butanol-trichloroacetic acid column was extracted five times with an equal volume of ether, without acidification, to remove most of the trichloroacetic acid, before evaporation to about 100 ml. This solution was applied to a 150 g. column of Hyflo. After washing with two column volumes of water the activity was displaced with 0.02M ammonium formate (pH 6.5) as a single peak at the front. After drying at 0.01 mm. Hg and 30° to remove the formate a small amount of insoluble inorganic matter often remained. The hypertensin was then taken up from the flask in repeated small volumes of water, leaving the insoluble material stuck to the

walls. This did not lead to any loss of activity and, on drying *in vacuo*, the solution yielded a freely soluble white powder which had an activity/weight quotient 1.5-3 times that of (-)-noradrenaline when assayed in the rat. There was a six-fold increase in the activity/weight quotient at this step, and the recovery of activity was 80%. The dry weight was 6 mg. The assay for any one preparation varied within the stated limits when tested on different rats, and Fig. 6 shows an assay where hypertensin was three times as active as noradrenaline (wt./wt.).

Fig. 6. Assay of hypertensin. For method, see text. This shows that 0.02 µg. of hypertensin (H) is equivalent to 0.06 µg. of (-)-noradrenaline (NOR).

Chemical properties of the final product

Ultraviolet absorption spectrum. At pH 8.0 the fine structure of the ultraviolet absorption spectrum of the hypertensin revealed absorption bands due only to phenylalanine and tyrosine. There was no evidence of tryptophan. The fine-structure absorption bands assigned to phenylalanine varied individually up to +5Å compared with those of pure phenylalanine. The similar bands assigned to tyrosine varied individually from +7 to +10Å, compared with those of pure tyrosine. In simple di- and tri-peptides the phenylalanine bands are unchanged, but the individual tyrosine bands may shift up to +5Å. In native proteins the phenylalanine bands shift between +5 and +15Å, whereas those for tyrosine may shift up to +60Å (cf. Beaven & Holiday, 1952).

Amino acid composition. The hypertensin was hydrolysed in 6N-HCl for 14 hr. at 105°, and the hydrolysate studied by paper chromatography. The chromatograms showed the presence of leucine, phenylalanine, valine, tyrosine, proline, aspartic acid, arginine and histidine (Fig. 7). A further chromatogram in *n*-butanol-3% NH_3 showed that isoleucine was absent. Whether the aspartic acid is

present in the peptide as an asparagine residue has not yet been determined.

The results of a quantitative experiment are shown in Table 2. They show that the simplest formula for the molecule would be that of a decapeptide (mol. wt. 1445) containing the following numbers of amino acid residues: leucine (1), phenylalanine (1), tyrosine (1), proline (1), aspartic acid (1), arginine (1), valine (2) and histidine (2).

this was the only DNP amino acid found was consistent with the assumption that the peptide was homogeneous.

Behaviour on paper electrophoresis. An initial test of the homogeneity of this peptide was by paper electrophoresis. At pH 2.0 and 8.6 the pressor activity was associated with the same pattern of amino acids on hydrolysis. This is illustrated (for pH 2) in Fig. 8; Fig. 8 (a) shows the

Fig. 7. Paper chromatogram of a hypertensin hydrolysate. (a) One-dimensional. Butanol-acetic acid-water (63:10:27 by vol.). (b) Two-dimensional: (i) as in (a), (ii) phenol-3% NH_3 . Ninhydrin-developed. Contact photograph. Proline does not show in this process. The other seven amino acids are shown.

Table 2. *Quantitative amino acid analysis of hypertensin by paper chromatography*

Amino acid	Molar proportions* (Duplicate samples)	
	2.1	2.0
Histidine	2.1	2.0
Arginine	0.98	1.1
Aspartic acid	1.05	1.08
Proline	0.94	1.05
Valine	1.9	1.8
Leucine	1.0	1.0
Phenylalanine	0.98	0.98
Tyrosine	0.9	0.88

* The value 1.0 was arbitrarily assigned to leucine.

N-terminal group. Hydrolysis of the product obtained by allowing the hypertensin to react with FDNB yielded DNP-aspartic acid. The fact that

movement of the pressor activity on the paper after a run in 30% (v/v) acetic acid, and Fig. 8 (b) shows the amino acid content of hydrolysates of eluates from corresponding cm. strips of the paper. Section 9, which contained 95% of the pressor activity, was the only section containing the peptide, which therefore appeared to be homogeneous under these conditions. The one ninhydrin spot common to all sections was a contaminant in the filter paper which was not removed by prolonged acid washing.

In the hypertensin hydrolysate there were faint traces of glutamic acid, threonine and lysine shown by two-way paper chromatography, and the hypertensin could best be freed of this slight contamination by paper electrophoresis in 50% (v/v) acetic acid at a potential drop of 20 v/cm. for 4 hr.

DISCUSSION

There were three main problems in the purification of hypertensin which deserve further mention:

(1) *Association*. In the partition of hypertensin between water and organic solvents, there was a marked tendency for the hypertensin to associate with impurities and so to give a false partition coefficient. The butanol-extraction procedure made possible the succeeding stages of partition chromatography by reducing this effect, presumably by leaving behind many of the impurities responsible.

Comparison of reported hypertensins

Recent reports of the purification of hypertensin have shown varying results. Bumpus & Page (1954), using pig renin and substrate, obtained a product with 14 different amino acids. The *N*-terminal amino acid was stated to be aspartic acid. The product of Kuether & Haney (1955), who used the same species of starting material, contained 15 different amino acids, but on investigation by the FDNB method of Sanger & Thompson (1953) ϵ -DNP-lysine was found. Skeggs *et al.* (1954*a*), using pig renin and horse-serum substrate, obtained

Fig. 8. Paper electrophoresis. Acetic acid 30% (v/v). 3 hr. run. 5v/cm. (a) Pressor activity of equal dilutions of eluates from successive cm. strips of paper. The percentage of the activity in section 9 was 95. (b) Paper chromatography of hydrolysates of the eluates shown in (a). Solvent *n*-butanol-acetic acid-water (63:10:27 by vol.). Ninhydrin-treated. Contact photograph. Proline does not show in this process. The amino acids characteristic of hypertensin are present in 9, corresponding to the pressor activity.

(2) *Solvent destruction*. Losses of activity were considerable in many organic solvents, and it was found essential to use redistilled solvents with a low peroxide content.

(3) *Adsorption*. This was usually troublesome only at the higher stages of purification, but it is characteristic of this particular peptide and occurs readily on glass, cellulose and kieselguhr. Adsorption of hypertensin on glass has previously been noted by Skeggs *et al.* (1954*a*). On all three adsorbents, desorption could be effected by raising the pH above 8.5 or lowering it to 1.0. This property may be related to the basic groups present in the peptide, which could be held by the acidic groups of the adsorbents.

a product with nine different amino acids, but made no statement about the *N*-terminal amino acid. It is possible that not all these materials were homogeneous. A further reason for variation in the amino acid composition may be that different species of renin and substrate have been used for the preparation of the starting material (cf. Bumpus & Page, 1954; Skeggs *et al.* 1954*a*; and the present work). Comparisons will be on a firmer basis when the molar ratios of the amino acids in these reported hypertensins are given.

Apart from species differences in renin and substrate, the preparations of renin used by most workers were impure and probably consisted of mixtures of enzymes. The possibility must be

considered that differences in the renin preparations were responsible for the formation of different end products. The formation of three pressor substances, probably all peptides, in the present work, lends some weight to this suggestion.

Pressor activity. Comparison of the pressor activity of the various reported hypertensins is difficult, since many different assay methods have been used. However, Kuether & Haney (1955) have compared directly the products of Bumpus & Page (1954) and Skeggs *et al.* (1954*a*) with their own material and claim to have a much more active preparation. On standing in solution for 24 hr., over 90 % of the activity was lost. There has been no observed loss of activity in the present preparation, and it must be presumed that the preparation of Kuether & Haney contains a different material. The activity/weight quotient of the present peptide is closest to that of the preparation of Skeggs *et al.* (1954*a*), whose preparation was four times as active as (–)-noradrenaline when assayed by the same type of procedure.

Multiplicity of hypertensins. It is not known whether the three pressor substances obtained in the present study are related. Skeggs, Marsh, Kahn & Shumway (1954*b*) claimed to show the existence of two different forms of hypertensin. Hypertensin I was formed from renin and its substrate, but was rapidly converted into hypertensin II by a factor present in the plasma. They further showed that these two hypertensins could be differentiated in a Craig countercurrent distribution apparatus. Whether there is a similar relation between any of the substances now reported has not yet been investigated.

The main conclusion must be that there is evidence for a variety of hypertensins. From the evidence obtained by paper electrophoresis at different pH values, the demonstration of only one *N*-terminal amino acid, and the results of quantitative amino acid analysis, it appeared that one homogeneous hypertensin peptide had been isolated in the present work.

SUMMARY

1. Hypertensin formed by the action of rabbit renin on ox serum has been resolved by partition chromatography into three active components.

2. The amphoteric nature of the major active

component has been shown by electrophoresis on paper.

3. The major active component has been isolated in a form which appears to be homogeneous. It is a peptide containing eight different amino acids which are present as ten residues in its minimum stoichiometric unit. It shows a pressor action up to three times that of (–)-noradrenaline.

4. The hypertensin isolated in this work has been compared with hypertensins previously described.

It is a pleasure to thank Sir Charles Harington, F.R.S., for the generous provision of facilities at the National Institute for Medical Research. I am deeply indebted to Dr A. J. P. Martin, F.R.S., and Dr R. R. Porter for their interest and helpful advice, and to Professor G. W. Pickering for continual encouragement. Dr G. H. Beaven kindly measured ultraviolet absorption spectra. Mr C. O'Neill provided valuable technical assistance.

REFERENCES

- Beaven, G. H. & Holiday, E. R. (1952). *Advanc. Protein Chem.* **7**, 319.
Blackburn, S. & Lowther, A. G. (1951). *Biochem. J.* **48**, 126.
Bumpus, F. M. & Page, I. H. (1954). *Science*, **119**, 849.
Consdon, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **46**, 532.
Craig, L. C., Gregory, J. D. & Hausmann, W. (1950). *Analyt. Chem.* **22**, 1462.
Durrum, E. L. (1950). *J. Amer. chem. Soc.* **72**, 2943.
Edman, P. (1945). *Ark. Kemi Min. Geol. A*, **22**, 3.
Harfenist, E. J. & Craig, L. C. (1951). *J. Amer. chem. Soc.* **73**, 877.
Holiday, E. R. (1937). *J. sci. Instrum.* **14**, 166.
Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
Kuether, C. A. & Haney, M. E. (1955). *Science*, **121**, 65.
Martin, A. J. P. (1949). *Symp. biochem. Soc.* **3**, 4.
Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
Peart, W. S. (1955*a*). *Biochem. J.* **59**, 300.
Peart, W. S. (1955*b*). *Biochem. J.* **60**, vi.
Pereira, A. & Serra, J. A. (1951). *Science*, **113**, 387.
Sanger, F. & Thompson, E. O. P. (1953). *Biochem. J.* **53**, 353.
Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1952). *J. exp. Med.* **95**, 241.
Skeggs, L. T., Marsh, W. H., Kahn, J. R. & Shumway, N. P. (1954*a*). *J. exp. Med.* **100**, 363.
Skeggs, L. T., Marsh, W. H., Kahn, J. R. & Shumway, N. P. (1954*b*). *J. exp. Med.* **99**, 275.