[5] A. G. OGSTON AND J. E. STANIER, *Discussions Faraday Soc.*, 13 (1953) 275.
[6] J. H. HIGHBERGER, J. GROSS AND F. O. SCHMITT, *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 286.
[7] S. S. COHEN, *J. Biol. Chem.*, 144 (1942) 353.
[8] B. SYLVÉN AND H. MALMGREN, *Lab. Investig.*, 1 (1952) 413.
[9] B. SYLVÉN AND E. J. AMBROSE, *Biochim. Biophys. Acta*, 18 (1955) 587.
[10] F. A. BETTELHEIM, *Nature*, 182 (1958) 1301.
[11] J. GROSS, *Ann. N.Y. Acad. Sci.*, 52 (1950) 964.
[12] C. E. JENSEN AND F. CARLSEN, *Acta Chem. Scand.*, 8 (1954) 1357.
[13] J. W. ROWEN, R. BRUNISH AND F. W. BISHOP, *Biochim. Biophys. Acta*, 19 (1956) 481.
[14] D. E. PHILPOTT AND A. G. SZENT-GYÖRGYI, *Biochim. Biophys. Acta*, 15 (1954) 165.
[15] R. W. JEANLOZ AND E. FORCHIELLI, *J. Biol. Chem.*, 186 (1950) 495.
[16] L. ANDERSON, *Acta Chem. Scand.*, 7 (1953) 689.
[17] Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189.
[18] J. P. JOHNSTON, A. G. OGSTON AND J. E. STANIER, *Analyst*, 76 (1951) 88.
[19] C. C. CURTAIN, *Biochem. J.*, 61 (1955) 688.
[20] A. K. PARPART, Princeton Univ., private communication.
[21] F. A. BETTELHEIM, unpublished data.
[22] B. S. BLUMBERG AND A. G. OGSTON, *Ciba Foundation Symposium on the Chem. and Biol. of Mucopolysaccharides*, 1958, pp. 22–37.
[23] W. PIGMAN, E. GRAMLING, D. PLATT AND H. L. HOLLEY, *Biochem. J.*, 11 (1959) 201.

BRADYKININ FROM TOTAL BOVINE PLASMA

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SUMMARY

1. The biological homogeneity of bradykinin prepared from whole bovine plasma and purified on an aluminium oxide column was studied. The ratio of uterus activity/guinea pig activity of a total plasma bradykinin and of a standard bradykinin prepared from a precipitated plasma globulins was determined. Results showed that in the case of total plasma bradykinin this ratio was 2–3:1, whereas in the case of standard bradykinin it was 1:1.

2. The effect upon the fall in the cat's arterial blood pressure was approximately equal to that of the standard.

3. The purified bradykinin appeared as a single substance on paper chromatography and paper electrophoresis when localized by the effect upon the rat's uterus or guinea-pig ileum.

4. The plasma bradykinin may be associated with a phospholipid when eluted from aluminium oxide at 50% methanol concentration.

INTRODUCTION

The biological homogeneity of bradykinin prepared on a large scale from whole bovine plasma[1] and purified on an aluminium oxide column has been studied. As indicated

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previously in preliminary small-scale experiments, bradykinin can be adsorbed on aluminium oxide from the crude plasma material and eluted to yield a preparation of considerably increased specific activity. In the present work this method has been used to recover large amounts of crude bradykinin from the crude plasma material and to transform it into a homogeneous preparation that can be more readily used in further purification work. Under these conditions, 20-g portions of crude bradykinin were transformed into a largely lipid-free material with a specific activity (units per mg) 10–30 times that of the original. Though considerably purer than the crude material, it is nevertheless very impure chemically. The homogeneity as studied here applies to the biological properties of bradykinin as determined by its typical effects upon smooth muscle and arterial blood pressure. An agreement was found between the effects on the isolated guinea pig ileum and the cat's blood pressure when comparing the total plasma bradykinin with a standard bradykinin prepared from the precipitated globulins[2]. A discrepancy was observed in the assay on the isolated rat's uterus.

MATERIALS AND METHODS

The crude bradykinin used in this work was a pool of two large-scale preparations (II–III) carried out by Abbott Laboratories, North Chicago, and mentioned in Table I in the previous work[1]. The 535 g of material originated from 40 l of commercial whole bovine plasma and has an approximate activity of 0.3 units of bradykinin per mg as determined on the guinea-pig ileum. It was prepared with low concentrations of partially heat-denatured *Bothrops jararaca* venom[3] in the presence of cysteine during incubation, followed by an extraction with boiling 70% ethanol and subsequent drying. It was previously ascertained to be histamine-free.

The bradykinin used for comparison was a standard laboratory preparation* from the precipitated globulins of bovine plasma[2], prepared with *Bothrops jararaca* venom, alcohol-extracted and precipitated with ether from a solution of glacial acetic acid[1]. The activity of this material was 3 units/mg.

The bioassays were based upon the responses of the guinea-pig ileum, the rat's uterus and the fall in cat's blood pressure. An isolated piece of the ileum of adult guinea pigs was suspended from a frontal writing lever in a 3-ml bath of aerated Tyrode solution at 37–38°. Determinations were carried out in the usual way in a Dale's apparatus. Benadryl was occasionally applied (2 μg in 3 ml) as an antihistamine agent. The ileum was sensitive to 0.05–0.1 units of the standard bradykinin (per 3 ml).

Single horns of uteri from adult (140–170 g body weight) virgin rats were suspended in 10- to 14-ml baths of aerated Jalon solution at 31–35°. The uterus was sensitive to 0.005–0.1 units of bradykinin (per 10–14 ml).

The effect of Mg^{++} on the contraction of the uterus with bradykinin was studied by addition of various concentrations of $MgCl_2 \cdot 6\ H_2O$ (Merck, p.a.) to the Jalon solution.

The 4-point assay as described by ROCHA E SILVA[5] for the guinea-pig ileum was applied for determinations of the activity ratios. This 4-point assay was also applied to the rat's uterus and the blood pressure of the cat. As described in more detail else-

* I am indebted to Dr. SYLVIA O. ANDRADE, Biological Institute of São Paulo, Brazil, for this bradykinin standard.

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where[6], the fall in arterial blood pressure of the cat responded to the doses of bradykinin with a standard deviation of 5 %. The difference between the doses was highly significant ($P < 0.001$) and the slope $b = 82.8$; the λ of the assay was small enough to indicate the high sensitivity and accuracy of the test.

The fall in arterial blood pressure of the cat was recorded from the carotid artery, and the injections of bradykinin were given via a cannula inserted into the femoral vein. The cannula was washed with 1–2 ml of 0.15 *M* NaCl after each injection. The cat was anaesthetized with nembutal 30 mg kg and the vagus nerve cut.

For standardization of activity a simpler assay was employed in which a larger or smaller dose of the unknown was compared with the standard and the doses were kept below that required to bring about the maximum contraction of the organ. The activity throughout this work is given in units of bradykinin and, when not otherwise stated, refers to the activity as assayed on the guinea-pig ileum in relation to the standard.

Paper chromatograms were made with Whatman No. 1 filter paper which had previously been washed with a 0.001 *M* solution of disodium versenate followed by bi-distilled water and dried at 60°. The solvent used was the organic phase of *n*-butanol–glacial acetic acid–water (40:10.50) (v/v) saturated for 4–5 h at 20–22°.

For paper electrophoresis* the LKB equipment and Whatman No. 1 filter paper (2-cm wide strips), pre-washed as described above, were used. Runs were made at 220 V and 4 mA in a 0.05 *N* acetate buffer pH 4.1, in a cold room (10°).

Determinations of total phosphorus were carried out by the colorimetric method of FISKE AND SUBBAROW[7] after digestion with 5 *N* sulfuric acid and oxidation with 30 % hydrogen peroxide of aliquots from the column fractions and the original material.

Bradykinin obtained from the aluminium oxide column

Reagents. Aluminium oxide was suspended in concentrated hydrochloric acid p.a. and brought to boiling while stirring. After being cooled the acid was decanted and washing with distilled water was repeated until a pH of 3–4.5 was reached; thereafter the aluminium oxide was washed several times with ethanol. The ethanol used was a common commercial 95 % alcohol, glass-distilled in a fractionation column. The aluminium oxide was left to dry in air and put into the column suspended in 90 % ethanol.

Procedure. Twenty g of crude bradykinin was suspended in 100 ml of 90 % ethanol, brought to boiling in a water bath and decanted after setting. The procedure was subsequently repeated three times with 50 ml ethanol. The extract was filtered hot and the final precipitate pressed. This extract was used directly or after storing overnight in the icebox. Precipitate formed during storage was removed by filtration through cotton wool. By this procedure 75–85 % of the original activity or 4000–5000 units of bradykinin were extracted. The concentration of the extract was kept at about 0.1 g/ml. A volume of approximately 200 ml was passed through the column under moderate carbon dioxide or nitrogen pressure. Bradykinin was retained by the aluminium oxide and the column was washed with about 1.5 volume of 90 % ethanol.

* The author is indebted to Dr. SERGIO DOHI, Department of Anatomy, for valuable help in performing the electrophoresis.

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Elution was carried out with 50% methanol, and bradykinin was quantitatively recovered as assayed on the guinea-pig ileum.

It was found that the dimensions (2.1 × 35 cm) of the column were critical. If more material was put on the column or a smaller column was used bradykinin was not always retained.

The active methanol fractions were pooled after the assay of activity. Samples of 500–1000 units of bradykinin were dried under reduced pressure in ampoules, which were kept in a desiccator or sealed. The activity was stable over a month. This material, redissolved in 0.15 *M* NaCl, was used in the bioassays for specific activity and activity ratios.

RESULTS

The aluminium oxide column

Fig. 1 shows the results obtained in runs of four ethanol extracts of crude plasma bradykinin on aluminium oxide columns. The curves are plotted from the values

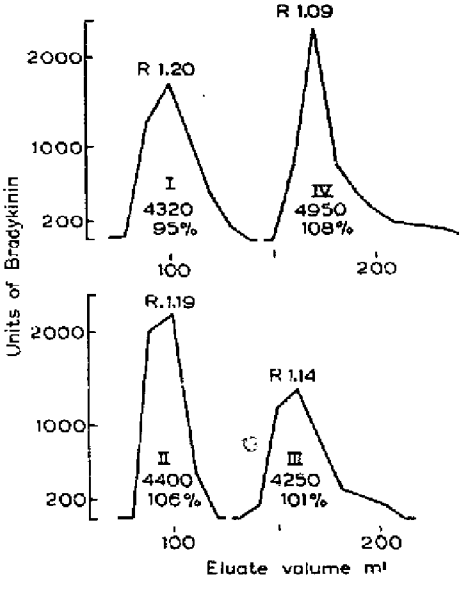

Fig. 1. Elution of bradykinin with 50% methanol from four aluminium oxide columns (I–IV). Units of bradykinin and per cent of recovery are mentioned below column. "*R*"-values of the active peaks are calculated from the following figures: column volume/eluate volume. The respective column dimensions were: I, 2.1 × 35; II, 2.1 × 34.4; III, 2.6 × 33; IV, 2.6 × 36.5 cm.

obtained in bioassay on the guinea-pig ileum of aliquots from the 10-ml eluates. Samples of 0.5 ml from each fraction were dried under reduced pressure in a boiling water bath and redissolved in 5 ml of 0.15 *M* NaCl. The total unitage put into the column was determined by a similar assay of the ethanol extract. The effluent and washings were strongly yellow in colour. The washing was continued until the colour disappeared, which occurred after passing about an equal volume of ethanol through the column. The eluates with methanol were colourless in the beginning but later a faint yellow colour seemed to accompany the active peak.

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No other active (guinea-pig ileum) peak was observed in these experiments. The effluent and washings were inactive or contained only traces of activity (less than 4.4 % of the original) when tested in corresponding doses. Elution of columns I and II was continued until 250 ml of ethanol had been used. All the fractions beyond bradykinin were inactive.

Material retained by the column appeared in three fixed coloured zones in the top region. A yellow zone further down moved with the washing liquid. A turbidity was observed near the active eluates but could be removed by centrifugation and was not connected with the activity. When a larger column was used this turbidity appeared just before the activity was eluated, overlapping the active fractions in the beginning.

The determined "R" values for bradykinin under these conditions allowed a simplification in the experiment. From a column of 2.6 × 38.5 cm (not recorded in Fig. 1) the first 150 ml of eluate were collected together and discarded after a test for absence of activity. Elution was continued in 10 ml fractions until all the activity was collected. The "R" value of the peak was 1.13. With this longer column (V) the turbidity did not overlap the active fractions but was removed before reaching them.

When the larger columns were applied a tailing of the activity occurred on elution. This was estimated to amount to 9.8 % of the activity in column III, 17 % of that in column IV, and 16 % of that in column V. The pool of active fractions from column V had a maximum light absorption of 270–280 mμ in the ultraviolet region.

Specific activity

Aliquots from the pool of active eluates corresponding to 600–1250 units (guinea-pig ileum) were dried under reduced pressure in ampoules of known weight. After drying overnight the weight of the material was deduced by difference. The total bradykinin activity of this material was determined in several bioassays on the guinea-pig ileum, after redissolving the contents of the ampoule in 0.15 M NaCl. The following results in units per mg were obtained from various column materials: 2.5–3.0 (column II); 8.8–9.4 (column III); 6.0 (column V). The activity was stable when kept in frozen solutions of 100–200 units per ml.

The eluate from column III was found to be remarkably stable: when kept in the icebox, bradykinin activity was conserved for a month, as assayed on the guinea-pig ileum and the rat's uterus. The activity ratio uterus/ileum remained unchanged at 2.5 ± 0.

Activity ratios

Table I shows the activity ratios relative to the standard obtained in bioassays with various preparations of the total plasma bradykinin. When solutions with equal effect on the guinea-pig ileum were assayed on the rat's uterus, the activity of the new bradykinin was 2–3 times higher than the standard. This difference did not occur in the case of the arterial blood pressure of the cat.

A similar comparison was made between two standard preparations[2] of crude bradykinin, one of them (3 units/mg) precipitated from glacial acetic acid with ether[4], the other (1 unit/mg) not. Both standards differed from whole plasma bradykinin in that they originated from the plasma globulins precipitated with ammonium sulfate and dialysed. The second standard was therefore comparable to the total plasma bradykinin as far as the preparation technique is concerned (see MATERIALS AND

METHODS) while it was made from a different substrate. The ratio uterus/ileum activity, however, was 0.93.

TABLE I

ACTIVITY RATIOS U/S OF BRADYKININ PREPARATIONS (U) IN RELATION TO A STANDARD (S) (3 UNITS/mg)

Based upon U/S = 1 in assay on the guinea-pig ileum.

Preparation	*Rat's uterus*			*Cat's BP*	
Original crude	2.0	2.0	2.0	1.0	0.80*
Column II	2.38*	2.54	3.0*	0.85	1.16
Column III	2.2	2.3	3.0*	1.22*	1.40
Column V	2.38	3.04	2.5	1.19	0.80*
Standard (1 unit/mg)		0.03		1.0	

Each figure represents a separate bioassay.
* A 4-point assay.

It seemed reasonable that some factor present in the total plasma but absent from the dialysed globulins might account for this difference. A possible contaminator might be oxytocin[8], which would stimulate the uterine contractions but not the intestinal ones. As shown by LOCKETT AND OWEN[9] a sensitization of the dose-response to oxytocin on the rat's uterus can be obtained at a certain concentration of Mg^{++}, while higher concentrations would depress the response. This effect was studied with the bradykinin obtained from the aluminium oxide column. The unknown solution was first diluted to equipotent concentration into the standard as tested on the guinea-pig ileum. The bio-assay on the rat's uterus was started with no Mg^{++} in the Jalon solution, and a 4-point assay was carried out (Table II, No. 1). Two equal doses of standard and two equal doses of a preparation of unknown activity were selected, one of each pair being twice as concentrated as the other. The doses to the uterus were repeated at random in four groups of the 4-point assay. After completing this assay 100 mg/l of $MgCl_2 \cdot 6H_2O$ were added to the Jalon solution and the 4-point assay was repeated without interruption. The concentration of $MgCl_2 \cdot 6H_2O$ was then increased to 1 g/l and the assay repeated again. In this case the uterus was left for 2 h between the assays. The results are shown in Table II, No. 1. Addition of Mg^{++}

TABLE II

INFLUENCE OF Mg^{++} ON THE ACTIVITY RATIO RAT'S UTERUS/GUINEA PIG ILEUM

Assay of solutions* of equal potency on the guinea-pig ileum.

Rat's uterus	*Jalon***	*Moles of Mg^{++} added to Jalon*		
		0.0005	0.005	0.02
No. 1	3.0	3.21	3.27	—
2	2.7	—	—	1.70
3	2.2	2.0	—	1.67

* Bradykinin from column III.
** Composition of the Jalon solution: 420 mg KCl, 9 g NaCl, 60 mg $CaCl_2$, 500 mg $NaHCO_3$ and 500 mg glucose per liter.

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at these lower levels of concentration did not change the activity ratio but affected the dose-response of the uterus to bradykinin, as shown in Fig. 2.

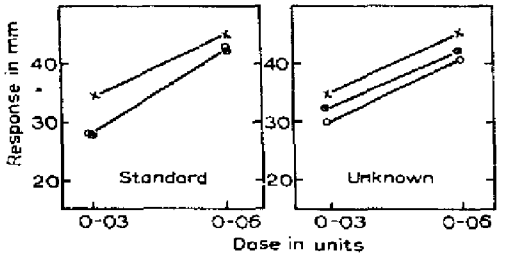

Fig. 2. The dose-response curves given by aluminium oxide (column III) purified (unknown) and standard bradykinin in assay on the rat's uterus. (·) Jalon solution; (○) addition of 0.0005 and (●) 0.005 *M* Mg^{++} (see Table II, No. 1).

The latter inhibitory effect was repeated in the two following experiments recorded in Table II, using the simpler bioassay. Again the activity ratio was not appreciably influenced at the low concentration but decreased to 1.70 and 1.67 with the high concentration of Mg^{++}. In all of these experiments an immediate depression of the dose-response occurred after addition of Mg^{++}. This was especially pronounced in expt. 3, which resulted in an immediate decrease of about 50% following the addition of 0.0005 mole Mg^{++}. With the high Mg^{++} concentration a very strong depression occurred which required an approximately ten-fold increase of the dose. As appears in Fig. 2, Mg^{++} caused changes in the sensitivity of the uterus but did not change significantly the slope of the dose-effect curves.

The influence of cysteine was of interest because of its effect in capturing metal ions from chelates. A possible chelating effect with Co^{++} at a certain concentration has been shown with bradykinin by ROCHA E SILVA (unpublished results), who was able with cysteine to reverse the inactivation of bradykinin by Co^{++}. Cysteine HCl (approximately 1 mg/unit) was added (from a solution of pH 8) to two solutions of standard and unknown bradykinin and left at room temperature for about 30 min. The activity ratio remained at 2 and cysteine did not influence the effect upon the rat's uterus.

Chymotrypsin was found to abolish the activity on the uterus of the bradykinin obtained from an aluminium oxide column. Incubation in parallel of 10 units of this bradykinin and 30 units of the standard with 200 μg chymotrypsin destroyed the activity in both in 3–6 min at pH 8 and 36°. After the activity had been destroyed the unknown solution was boiled to dryness in 0.2 *N* HCl to inactivate chymotrypsin. It was redissolved in distilled water, neutralized, and 10 units of the standard bradykinin were added to a final concentration of 1 unit/ml. In comparison with a standard, no augmentation of the response on the uterus occurred with this solution. This seems to rule out the direct influence of some cation that might sensitize the uterus to bradykinin. On the other hand, chymotrypsin is known to destroy a number of other active polypeptides besides bradykinin.

A mixture of standard and column III bradykinin was tested on the uterus. Equal portions of two solutions with equal activity on guinea-pig ileum but 1:3 (standard/unknown) activity on the uterus were mixed. The mixture was then diluted

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twice to abolish the potentiating effect on the uterus. When compared with a standard the expected potency ratio of 1 was found on the uterus.

The bioassays on the cat's blood pressure were carried out as described by ROCHA E SILVA AND HAMBERG[6]. Solutions of 10–20 units of bradykinin/ml were determined in parallel assays on the guinea-pig ileum. Doses of 5–14 units were injected into the femoral vein alternately with the standard. In these experiments the activity ratios showed agreement with the ileum activity (Table I). A 30–50 mm Hg fall in blood pressure was obtained with these doses. When the dose of bradykinin obtained from an aluminium oxide column was increased to 100–400 units (50–200 units/kg) the response was no longer proportional to the fall in blood pressure but appeared as a prolonged effect in keeping the pressure at low level. With 400 units (2.1 kg cat) of column III bradykinin the blood pressure remained at a level 30 mm Hg lower than normal for 30 min. In the dose-range used in these bioassays the normal blood pressure was recovered in about a minute. Fig. 3 shows the slope and linear dose-response with preparations II and III.

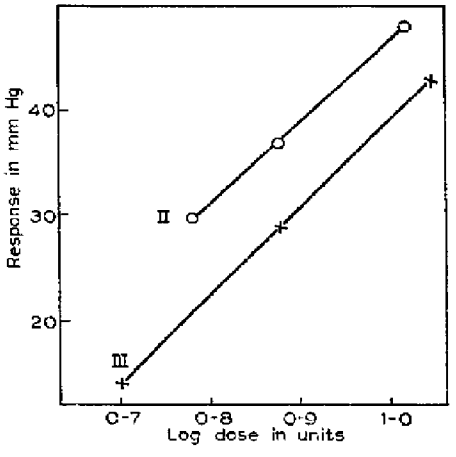

Fig. 3. The dose-response curves for the fall in the cat's (two separate) arterial blood pressure with bradykinin obtained from an aluminium oxide column.

Dialysis

Crude plasma bradykinin was dissolved in bidistilled water to a concentration of approximately 50 units/ml and centrifuged. A 5-ml sample (250 units) was dialysed through cellophane against 100 ml of 0.1 *N* HCl at 5°. After 6 h, 43.5 % of the bradykinin was found in the dialysate by the guinea-pig ileum test. The activity ratio uterus/ileum was determined for both the dialysate and the dialysed sample and was found to be equal and uninfluenced by the dialysis (Table III). For a rapid removal of ions a 1-h dialysis was performed against running tap water. This frees the slower dialysable bradykinin from potassium, which interferes with the oxytocic test on rat's uterus[8]. Again the activity ratio was unchanged when determined in the dialysed (80 % recovered) bradykinin sample.

The activity upon rat's uterus in dialysate and dialysed sample (0.1 *N* HCl) was destroyed by chymotrypsin at pH 8. The bradykinin activity was also destroyed in

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the dialysate by boiling for 15 min at pH 12–13 (approximately 0.1 *N* NaOH). When standard bradykinin was dissolved in the neutralized hydrolysate no potentiation of the standard dose-effect on the uterus occurred. A similar comparison was made after destroying organic matter by heat-drying at pH 1.8 and redissolving in distilled water, with the same negative result.

TABLE III

INFLUENCE OF DIALYSIS UPON THE ACTIVITY RATIO UTERUS/ILEUM OF CRUDE PLASMA BRADYKININ

250 units bradykinin	Units in bioassay		Ratio U/I
	Uterus	Ileum	
Dialysed 6–7 h*	248	130	1.9
Dialysate*	220	109	2.1
Dialysed 1 h**	403	200	2.0

* 0.1 *N* HCl.
** Running tap water.

Paper chromatograms

The pool of active eluates from column III was used for the chromatograms. Two paper strips of 10 × 46 cm were marked down the midline with a pencil in order to divide the paper into 2 equal areas for two parallel runs with 6 and 12 units (guinea-pig ileum) respectively (0.1 and 0.2 ml eluate). At the end of the run the papers were cut into numbered transverse sections of 0.5 × 5 cm and tested for activity by submerging each of them in the chamber containing the guinea-pig ileum. The sections from the second paper (12 units) were eluted for 5 h in 2 ml of 0.15 *N* NaCl at room temperature and assayed on the uterus. The uterus was sensitive to 0.01 units of the standard bradykinin. Only one peak of activity was found corresponding to the R_F obtained with the ileum (Table IV). For further control the opposite sections of 6 units were tested likewise.

TABLE IV

LOCALIZATION OF BRADYKININ FROM PAPER CHROMATOGRAMS BY DIFFERENTS BIOASSAYS

Bioassay	R_F values			
	Preparation III			Mixture
	6 U.	12 U.	24 U.	12 U.–10 U.S
Ileum	0.304	0.291	—	—
Uterus	0.317	0.304	0.282*	0.205*

Chromatograms were run for 17 h at room temp. 20–22°.
* Room temp. 17–22°.

A third chromatogram of equal dimensions was run to compare a mixture of 12 units of preparation III and 10 units of the standard with an equipotent (ileum) amount (24 units) of preparation III alone. Sections 1 cm long were eluted in 5 ml of 0.15 *M* NaCl. The active peaks on each side of the midline were located by testing

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on the uterus (Table IV). The total activity eluted from the 24 units run (preparation III) yielded 33.3 units on the uterus (4-point assay) but only 10 units on the ileum. The activity ratio uterus/ileum after chromatography on paper therefore was 3.3 for preparation III. In an assay of the methanol eluate performed previous to the run the ratio was 2.5–3.0.

The lower R_F value obtained (Table IV) with the mixture may be due to the lower temperature of this run. Also, bradykinin preparations of different degrees of purity show variations of R_F[10].

Electrophoresis

Electrophorograms of the column V material were made in parallel with standard bradykinin and in a mixture with the standard. Strips (1 cm long) from the anode and cathode side were eluted with 0.15 *M* NaCl and tested on the rat's uterus and guinea pig ileum. All activity was found on the cathode side in a single peak, (the anode side was inactive) localized at 3–4 cm distance from the start after a run of 12 h, for the column V material alone and mixed with the standard. Approximately 20 units (guinea-pig ileum) of bradykinin were applied; the recovery after elution was about 30 %. The activity ratio uterus/ileum decreased to 1.67 after electrophoresis for the column V bradykinin (*cf.* Table I).

Total phosphorus

Determination of the distribution of phosphorus in relation to bradykinin activity was made in fractions from columns I and II. The results indicated that the bradykinin obtained from an aluminium oxide is almost lipid-free (contains approximately 1.5 % of the total phosphorus in the crude material); however, some phosphorus-active material was eluted with methanol together with bradykinin. From both columns the peaks of phosphorus- and bradykinin-active material coincided but the ratio between the two was not constant in all the fractions (Fig. 4). Because of high blank values obtained in these experiments the amounts of phosphorus given are only relative.

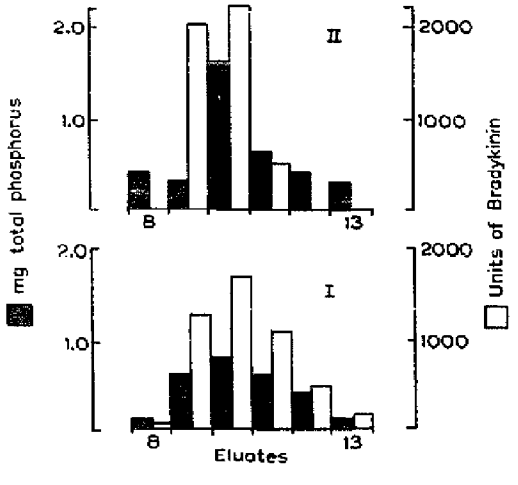

Fig. 4. Distribution of total phosphorus in relation to the elution of bradykinin from columns I and II (10-ml eluates 8–13).

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DISCUSSION

The oxytocic effect of bradykinin has been less well studied, most papers dealing with its biological activity as regards the contraction of the guinea-pig ileum and the fall in rabbit's, cat's or dog's blood pressure. An index of discrimination[11] is frequently applied to distinguish two substances by their respective action upon a certain tissue. As shown in this paper, a different index (2–3) can occur between two preparations of the same substance although they are indistinguishable by other methods and prepared under controlled and comparable conditions[2–3]. This may be attributed to the great difference in the sensitivity of the rat's uterus to bradykinin as compared with the other tissues. The rat's uterus has been estimated to be 25–30 times more sensitive to bradykinin than the guinea-pig ileum and to have an approximately equal sensitivity to oxytocin and the purest bradykinin yet prepared[12].

Bradykinin obtained by passage through an aluminium oxide column is far from pure. ANDRADE AND ROCHA E SILVA[12] found that when bradykinin was passed through an aluminium oxide column peaks for biological activity and ninhydrin colour value (after hydrolysis) and absorption at 270 mμ coincided, but on further purification on an Amberlite column the biologically active material no longer absorbed at 270 mμ and the yellow component also disappeared. From the crude plasma bradykinin used here the biologically active material is also eluted from the aluminium oxide column, with a maximum light absorption of 270–280 mμ and together with a yellow and phosphorus-active material which does not seem to be directly associated with bradykinin activity and may be a lipid component. Rechromatographing the material on an aluminium oxide column showed that a second elution with a solvent of less polarity resulted in bradykinin being extracted at a methanol concentration of approximately 90 %; the recovery was smaller (about 50 %), but the specific activity considerably increased (154 units/mg). It is interesting to note that in these fractions the activity ratio rat's uterus/guinea pig ileum decreased to 1.33 and also that bradykinin activity was less stable after this elution.

It seems apparent that impurities present are likely to upset the discrimination index, although it was not possible in the present experiments to specify a contaminator. Because of the greater sensitivity of the rat's uterus a potentiating effect may perhaps be brought about by dilution and may thus be due to this indirect way of removing a possible inhibitor.

In previous work[3] an equal activity was found when comparing the action of trypsin-liberated bradykinin from the denatured plasma proteins or separated globulins on the rat's uterus with that of bradykinin prepared with snake venom. Since in these cases dialysis of the globulins[2] or washing of the precipitated plasma proteins[13] was performed prior to the release of bradykinin, the potentiating factor may be some constituent of the unprecipitated plasma. The results obtained with chymotrypsin and in dialysis, however, exclude the influence of a potentiating ion.

The lower activity ratios obtained after electrophoresis and in a preliminary experiment after readsorption on aluminium oxide were accompanied by heavy losses in activity.

Oxytocin itself may be excluded on the basis of the experiments with Mg^{++}, which failed to increase the dose-effect on the uterus[9,16]. The addition of Mg^{++} decreased the sensitivity of the uterus to bradykinin and in the higher concentrations

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brought it down to the level of the guinea-pig ileum response. LOCKETT AND OWEN[9] reported a maximum oxytocic sensitivity of the rat's uterus with 50 mg $MgCl_2$/l for oxytocin and with 500 mg for vasopressin. Also, when tested on fowl's blood pressure the bradykinin obtained from an aluminium oxide column was inactive*.

Differences in activity ratios between structurally and biologically similar peptides have been demonstrated in cases where contamination or impurities can be excluded. BERDE, DOEPFNER AND KONZETT[14] found in the case of four synthetic analogues of oxytocin that the substitution of isoleucine by another amino acid caused marked differences in their oxytocic activities without changing the oxytocic characteristics for each derivative. Substitution of an amino acid was shown *in vivo* in hog and beef vasopressin by POPENOE, LAWLER AND DUVIGNEAUD[15]. RESSLER[16] found that the cyclic disulphide ring of oxytocin, deprived of the prolylleucylglycinamide side chain, acted upon the rat's uterus but was (almost) without effect on the chicken's blood pressure. The relative ratio between oxytocic, milk-ejecting and avian vasopressor effect was 3:1:0.03. In the presence of 10 mg% $MgCl_2$ the oxytocic effect of this synthetic peptide increased only 10 times compared with a 63-fold potentiation with the synthetic oxytocin.

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REFERENCES

1 U. HAMBERG AND H. F. DEUTSCH, *Arch. Biochem. Biophys.*, 76 (1958) 262.
2 M. ROCHA E SILVA, W. T. BERALDO AND G. ROSENFELD, *Am. J. Physiol.*, 156 (1949) 261.
3 U. HAMBERG AND M. ROCHA E SILVA, *Arch. intern. pharmacodynamie*, 110 (1957) 2.
4 E. S. PRADO, W. T. BERALDO AND M. ROCHA E SILVA, *Arch. Biochem.*, 27 (1950) 410.
5 M. ROCHA E SILVA, *Acta Physiol. Latinoamer.*, 2 (1952) 238.
6 M. ROCHA E SILVA AND U. HAMBERG, in preparation.
7 G. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
8 G. W. BISSET AND J. M. WALKER, *J. Physiol.*, 126 (1954) 588.
9 M. F. LOCKETT AND G. E. OWEN, *J. Pharm. and Pharmacol.*, 9 (1957) 406.
10 S. ANDRADE, C. R. DINIZ AND M. ROCHA E SILVA, *Arch. int. pharmacodynamie*, 95 (1953) 100.
11 J. H. GADDUM, in *Polypeptides which Stimulate Plain Muscle*, E. & S. Livingstone Ltd., Edinburgh and London, 1955, p. 130.
12 S. ANDRADE AND M. ROCHA E SILVA, *Biochem. J.*, 64 (1956) 701.
13 C. G. VAN ARMAN, in *Polypeptides which Stimulate Plain Muscle*, E. & S. Livingstone Ltd., Edinburgh and London, 1955, p. 103.
14 B. BERDE, W. DOEPFNER AND H. KONZETT, *Brit. J. Pharmacol.*, 12 (1957) 209.
15 E. A. POPENOE, H. C. LAWLER AND V. DUVIGNEAUD, *J. Am. Chem. Soc.*, 74 (1952) 3713.
16 C. RESSLER, *Proc. Soc. Exptl. Biol.*, 92 (1956) 725.

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