29/9/69

I would like to apply for the F.L. Drewry Memorial Scholarship. Please accept my thesis to be considered for the Scholarship. Enclosed is my thesis, "characterization of peptides and proteins in posterior pituitary".

Seon H. Shin

Department of Pharmacology

CHARACTERIZATION OF AMINO ACIDS AND LOW-MOLECULAR-WEIGHT PEPTIDES BOUND TO CYTOPLASMIC GRANULES FROM THE POSTERIOR PITUITARY[1]

Seon Shin, Frank LaBella[2], and Gary Queen

Department of Pharmacology and Therapeutics
University of Manitoba Faculty of Medicine
Winnipeg 3, Manitoba, Canada

Abbreviation used: NSG - neurosecretory granule

Send proofs to: Dr. LaBella at the above address.

Running title: Posterior Pituitary Peptides

FOOTNOTES

1. Supported by United States Public Health Service grant AM05896 and the Medical Research Council of Canada.

2. Medical Research Council Associate.

ABSTRACT

A number of peptides and amino acids, representing 30-40% of the total acid-extractable, ninhydrin-positive material of the tissue were associated with cytoplasmic granules (sedimenting at 3,000,000 g-min after preliminary removal of "nuclei and debris") isolated from bovine posterior pituitary glands. Acetic acid (0.2 N) extracts of a purified neurosecretory granule fraction showed only slight differences in the pattern of peptides and amino acids from extracts of the total cell particulate fraction. Gel filtration of extracts on Sephadex G-25 yielded three major fractions: Fraction I consisting of peptide material of molecular weights > 4,000; Fraction II of molecular weights averaging about 3,000; and Fraction III of molecular weights < 2,000. Fraction III was further resolved by anion-exchange chromatography into 12 subfractions. Vasopressin and oxytocin were contained in subfractions 2 and 3, respectively. Each of these subfractions was in turn chromatographed on a cation-exchange resin and resolved into a total for Fraction III of 22 major components: lysine, arginine, phenylalanine, ammonia, and 18 peptides. Three of the peptides contained only aspartic and glutamic acids in the ratios 8:1, 5:1, and 4:1. The sequences of 4 dipeptides were ascertained. Another peptide was not retarded by Dowex 50 and yielded glutamic acid upon acid hydrolysis. Still another peptide yielded tyrosine plus an unknown ninhydrin-positive component after hydrolysis. The amino acid compositions were determined for nine other peptides containing 3 - 9 residues. Additional peptides in Fraction III were detected in lesser or trace amounts. Isolated granule fractions from both bovine posterior pituitary and rat liver were dialyzed against isotonic sucrose or distilled water. The decrease of ninhydrin-positive material

from the sample dialyzed against water indicated that a large proportion of the "free" amino acids and peptides of these tissues were contained within intracellular organelles.

The posterior pituitary gland is known to contain a remarkably high concentration of peptides relative to concentrations in other tissues (Lande, Lerner and Upton, 1965; Upton, Lerner and Lande, 1966; Winnick, Winnick, Archer and Fromageot, 1955; Saffran, Caplan, Mishkin and Muhlstock, 1962). Winnick et al. (1955) estimated that peptides comprise 3-4% of the dry wt of porcine posterior pituitary. Two peptide hormones, vasopressin and oxytocin, are thought to be the major physiological products of the mammalian neurohypophysis. Several other peptides have been characterized chemically by Preddie (1965), Preddie and Saffran (1965), Penders and Arens (1966) and Schally and Barrett (1968).

The posterior pituitary is composed primarily of terminals of neurons, the perikarya of which are situated in the supraoptic and paraventricular nuclei of the anterior hypothalamus. These terminals store oxytocin and vasopressin in vesicles of 100 - 300 $m\mu$ in diameter, the so-called neurosecretory granules (NSG) (Palay, 1957). NSG can be sedimented from homogenates of the posterior pituitary by centrifugation and contain both oxytocin and vasopressin (Schriebler, 1952; LaBella, Reiffenstein and Beaulieu, 1962, 1963). It has been suggested that NSG are packets of neurophysin to which the hormones are bound, since the van Dyke protein and the NSG protein are apparently identical (LaBella, Vivian and Bindler, 1967). The peptide hormones are easily dissociated from and reassociated with the protein (Frankland, Hollenberg, Hope and Schacter, 1966; Ginsburg and Ireland, 1964). Because of the large number of peptides present in the

gland, it seemed likely that peptides other than oxytocin and vasopressin might be bound to the NSG and perhaps to neurophysin. In the present study the chemical composition of several isolated posterior pituitary peptides was determined as a prelude to investigation of possible physiological roles of additional granule-bound peptides. A low molecular weight (< 2,000) peptide fraction obtained by gel filtration of acetic acid extracts of bovine posterior pituitaries was examined. The chromatographic behavior and amino acid composition was determined for several of the peptides. In addition, it was found that the so-called "free" amino acids and peptides of tissues in general are to a large extent associated with, and apparently contained within, intracellular organelles.

MATERIALS AND METHODS

Bovine posterior pituitary glands. Glands were obtained from the slaughterhouse 30-40 min after death, placed in vessels surrounded by ice, and transported to the laboratory. The time elapsing between death of the animals and tissue homogenization was 2 hr. About 100 posterior lobes (25 g) were used as starting material for a given extraction.

Isolation of subcellular granules. The tissue was minced with scissors and homogenized in 10% (w/v) sucrose in a glass tube with a motor-driven teflon pestle (LaBella et al., 1963). An initial centrifugation at 7,000 g-min (R_{max}) to remove nuclei and debris, was done in a Servall centrifuge. Subsequent centrifugation of the supernatant was done at 3,000,000 g-min (R_{maz}) in an International Preparative Ultracentrifuge B-60 in order to prepare a total cytoplasmic granule fraction. This granule fraction would be expected to contain the subcellular components of the neurosecretory cell terminals, which make up the bulk of the posterior pituitary, together with a minority

population derived from pituicytes, other supporting cells, and endothelial cells (Bindler, LaBella and Sanwal, 1967). Where indicated, a purified NSG fraction (LaBella et al., 1967) was used as starting material for acid extraction.

Extraction of peptides. The isolated granules were stirred for 24 hr at 4° in 0.2 N acetic acid. The precipitate was discarded and the supernatant was concentrated in a rotary evaporator at 40° under reduced pressure from a water aspirator.

Gel filtration. Columns of Sephadex G-10, G-25 fine, and G-50 fine (Pharmacia-Uppsala, Sweden) were prepared essentially according to the manufacturer's recommendations. "Fines" were removed from 100 g of Sephadex by decantation and the sediment was suspended in 2 l of distilled water. After 12 hr the water was replaced with an equal amount of 0.2 N acetic acid, and 1 hr later with fresh 0.2 N acetic acid. A small amount of 0.2 N acetic acid was poured into the column, the outlet was closed, and the column was filled with the slurry. Column dimensions were 2.2 x 103 cm for G-10; 5.4 x 60 cm for G-25; and 2.8 x 59 cm for G-50. The samples were dissolved in a minimum amount of 0.2 N acetic acid, applied to the Sephadex column without disturbing the gel, and eluted with 0.2 N acetic acid. Flow rates for G-10 and G-50 were 40 ml/hr and for G-25, 75 ml/hr. Vitamin B_{12} and NaCl were used as markers on the G-25 column.

Ion-exchange chromatography. Dowex 1-X2 Cl^- (200-400 mesh) and Dowex 50W-X2 H^+ (200-400 mesh) were obtained from Bio-Rad Laboratories (Richmond, California). Packing and regeneration of the column were done according to the method of Schroeder, Jones, Cormick and McCalla (1962). The 2.3 x 57 column for 1-X2 and the 1 x 57 cm column for 50W-X2 were maintained at 50°. The sample to

be chromatographed on 1-X2 was dissolved in 2 ml of collidine-pyridine-acetic acid-water buffer (40:40:1.5:3918.5 by vol) pH 8.3 (Schroeder et al., 1962) and adjusted to pH 8.5; the samples to be chromatographed on 50W-X2 were dissolved in 2 ml of 0.2 M sodium acetate, pH 3.1, and adjusted to pH 3.0.

The sample, dissolved in buffer solution, was washed into the column by gravity flow with a small portion of the same buffer. Elution from the 1-X2 column was carried out stepwise with the collidine-pyridine-acetic acid buffer followed by 0.1 N, 0.5 N, 1.0 N, and 2.0 N acetic acid (Table 1). Elution from the 50W-X2 column was carried out stepwise with 0.2 M sodium acetate, (pH 3.1), followed by 2 M sodium acetate (pH 5.1), and 0.2 M sodium bicarbonate (pH 8.3) (Table I). A Technicon Autograd with 9 chambers was used as a gradient device for both anion and cation ion-exchange chromatography. The flow rate for each column was 40 ml/hr. Fractions were collected and portions were reacted with ninhydrin (Cadavid and Paladini, 1964) or the standard protein reagent (Lowry, Rosebrough, Farr and Randall, 1951) in a Technicon Autoanalyzer.

Bioassay. Oxytocic activity of chromatographic fractions was determined on the isolated rat uterus (Holton, 1948) and vasopressor activity was determined by a modification (Bindler et al., 1967) of the method of Landgrebe, Macaulay and Waring (1946).

Amino acid analysis. Purified peptides were hydrolyzed for 24 hr in 6 N HCl at 110° under nitrogen in sealed glass vials. Analyses of amino acids were carried out on a Technicon Instrument as previously described (LaBella et al., 1967). The values for amino acid were not corrected for destruction during hydrolysis. For estimations of tryptophan, samples were hydrolyzed in 1 ml of 5 N NaOH for 24 hr at 110° in Corning No. 7280, alkali-resistant glass tubes; the cooled hydrolysates were acidified with 1.5 ml of 5 N H_2SO_4,

an aliquot was rendered alkaline with 1 M $NaHCO_3$, and fluorescence of tryptophan was determined with an Aminco-Bowman fluorometer.

Thin layer chromatography and electrophoresis. To determine homogeneity, each purified peptide or amino acid fraction was examined by ascending TLC on MN-Polygram cel 300/UV_{254} (Macherey-Nagel & Co., Dueren, Germany) in n-butanol-acetic acid-water (4:1:5 by vol.), and by electrophoresis in pyridine-acetic acid-water (1:10:289 by vol.), pH 3.7 (Katz, Dreyer and Anfinsen, 1959), at 3,000 V/10 min. Ninhydrin aerosol spray NIN-3 (Sigma Chemical Co., St. Louis, Mo.) was used to visualize the spots. Silica gel TLC on plastic sheet, MN-Polygram Sil S-HR/UV_{254} (5 x 20 cm) (Macherey-Nagel & Co., Dueren, Germany) was used for detecting DNP-amino acids using chloroform-benzyl alcohol-acetic acid (70:30:3 by vol.) as developing solvent. DNP-amino acids (Mann Research Laboratories, New York; Kit No. 418) were used as standards.

Dinitrophenylation. For determinations of amino-terminal amino acids peptide dinitrophenylation was done according to Fraenkel-Conrat, Harris and Levy (1955).

Dialysis of cytoplasmic granules in hypotonic solution. 100 ml portions of the total granule fraction isolated from posterior pituitary were placed in cellophane bags (seamless 1-1/8" diameter; Fisher Scientific Co., Pittsburg, Pa.) and dialyzed at 4° (with stirring) against 1 liter of 0.005 M pyridine acetate (pH 6.0) in distilled water or in 0.29 M sucrose. Portions (1 ml) were removed from the exterior volume and reacted with ninhydrin. The portions were diluted with 1 ml of 0.29 M sucrose in the case of the distilled water medium or with 1 ml of water in the case of the sucrose-containing medium.

RESULTS

Three major, ninhydrin-positive fractions were obtained when an acid extract of the total cytoplasmic-granule fraction from 25 g of tissue was subjected to gel filtration on Sephadex G-25 (Figure 1A). Fraction I (40 mg of nitrogen) was contained in the excluded volume and included components of estimated molecular weights $> 4,000$. There were several components in this fraction as judged by chromatography on G-50 and anion-exchanger. Fraction II (10 mg of nitrogen) included components of estimated molecular weight $< 4,000$, and Fraction III (13 mg of nitrogen) contained components of molecular weight $< 2,000$. Fraction II was rechromatographed on Sephadex G-25 and the principal component was concentrated and chromatographed by anion-exchange to yield at least 9 ninhydrin-positive fractions. No further characterization of Fractions I and II was carried out.

This report deals with more extensive fractionation and characterization of the low-molecular weight components of Fraction III only (Figure 1, Table 2). Fraction III obtained from chromatography on Sephadex G-25 yielded upon anion-exchange chromatography 12 ninhydrin-positive subfractions (Figure 1B). Four of the principal subfractions were subjected to cation-exchange chromatography and resolved into several additional components (Figures 1C-1F). Each of these 12 subfractions was desalted by gel filtration on Sephadex G-10, a process which, in some instances, promoted further fractionation. For example, Fraction A4-B2 gave rise to 3 components during passage through G-10. Homogeneity was established for 20 of the components on the basis of (a) ion-exchange chromatography, (b) gel filtration on Sephadex, (c) TLC, and (d) high voltage electrophoresis.

From anion-exchange chromatography, the first fraction (A1) was

resolved by cation-exchange chromatography into ammonia, free lysine and arginine, a dipeptide (alanyl-arginine) and a fifth minor component (Fig. 1C). Vasopressin was identified in Fraction A2 (19-21 ml) and oxytocin in A3 (35-37 ml) by means of bioassays for their biological activities. A2 and A3 each consisted of mixtures of several peptides, as shown by subsequent cation-exchange chromatography (Figs. 1D and 1E).

Subfraction A4 also consisted of a complex mixture of peptides (Figure 1F, Table 2). Free phenylalanine was identified in this fraction. A4-B1 yielded residues of eight identifiable amino acids after acid hydrolysis plus two unidentified ninhydrin-positive components. One of the unknown substances was not retarded by the Dowex 50 column and the other emerged in the basic region of the chromatogram. A4-B2c was strongly retarded when subjected to Sephadex G-10, probably because of its high tyrosine content; acid hydrolysis of this peptide yielded 4 residues of tyrosine, 2 of lysine, and apparently 1 residue of a component whose position on elution corresponded to hydroxylysine. No further attempts were made to identify the latter substance.

Subfraction A5 (Fig. 1B; Table 2) was resolved into ten ninhydrin-positive components by TLC. No further characterization of A5 was carried out. Subfraction A6 consisted of a single component according to 4 criteria mentioned above and the compound was not retarded by the Dowex 50 column used for separation of amino acids, neither by Dowex 50W-X2 column chromatography. After hydrolysis the amount of ninhydrin-positive material was decreased and only glutamic acid was detected in the chromatogram. Dinitrophenylation and subsequent hydrolysis of the peptide yielded DNP-glutamic acid. Subfractions A7, A8, A9, A10 and A12 were found to be relatively pure

preparations of peptides composed exclusively of aspartate and glutamate in the ratios 1:4, 1:1, 8:1, and 5:1, respectively (Fig. 1B; Table 2). A8 was characterized as aspartyl-glutamate and A10 as glutamyl-aspartate. A-11 appeared to be a tripeptide consisting of 2 aspartic residues and 1 leucine residue.

The elution profiles shown in Figures 1-3 were reproduced almost identically with several different batches of posterior pituitary glands. An estimate was made of the proportion of acid-extractable, ninhydrin-positive material associated with the total granule fraction. In the case of the pituitary and, for comparison, in liver, approximately 40% of the total was sedimentable. In both tissues, no differences were noted in the distribution of ninhydrin-positive material between granule and supernatant fractions, when prepared from homogenates at either pH 7.4 or pH 6.0. This observation suggested that the binding of amino acids and of peptides to the granule was relatively insensitive to changes of pH or that they were contained within the granules (Figs. 2 and 3). In order to obtain information on the nature of the association between cytoplasmic granules and amino acids and peptides, rupture of these intracellular organelles by hypotonicity was resorted to. Isolated granules were suspended and in water or in 0.29 M sucrose. Loss of ninhydrin-positive material was greater from granules suspended in water (Figure 4), as would be expected if a significant proportion of the amino acids and peptides were contained within the granules.

DISCUSSION

The number and quantity of peptides found in the bovine posterior pituitary confirms previous observations for this gland (Winnick et al., 1955). The present investigation has emphasized the association of a large

proportion of the total glandular pool of peptides and amino acids with the sedimentable fraction of the tissue homogenate. Approximately the same proportion was associated with the particulate fraction of rat liver. The enhanced release of diffusible material from osmotically shocked granules was a further indication that the amino acids and peptides were contained within cytoplasmic structures. Preparation of a highly purified fraction of neurosecretory granules (LaBella et al., 1967) and its subsequent extraction resulted in a pattern of peptides which differed only slightly from that found in extracts of the total granule fraction. Although some peptides in the NSG fraction may be derived from contaminating organelles, the latter should be present in only small amounts. Since the pattern of peptides from purified NSG is almost identical to that from the total granule fraction, it appears that the bulk of the sedimentable peptides and amino acids were contained in the neurosecretory granules. We have additional support for this postulate, namely, the association of several peptides, besides vasopressin and oxytocin, with the purified carrier protein known to be present in the NSG. It had been generally assumed that acid-extractable peptides and amino acids in tissues are "free" in the cellular cytoplasm, whereas in fact, these tissue components are in part associated with cellular organelles and membranes.

In this study our original aim was to examine the possibility that peptides other than vasopressin and oxytocin were bound to the carrier protein contained within the neurosecretory granules of the neurohypophysis. In view of the large number of peptides existing in the posterior pituitary, it appeared likely to us that other peptides would be found in association with the carrier protein for the established hormones. Previously we showed that vasopressin was located in a denser and oxytocin in a lighter neurosecretory granule fraction (LaBella et al., 1962; LaBella et al., 1963), and

subsequently in denser and lighter nerve ending particles (Bindler et al., 1967). Recently, Dean, Hope and Kazic (1968) have reported that the dense neurosecretory granules contain vasopressin in association with one species of carrier protein, "neurophysin II", and oxytocin with another, "neurophysin I". Witter, Vliegenthart and Arens (1964) reported that the van Dyke protein (a complex of protein and vasopressin plus oxytocin) contained other peptides and amino acids. The extraction procedure for isolating the van Dyke protein is relatively prolonged and involves several steps; one could argue that these procedures could promote artifactual binding to the granule-derived protein of substances, which are ordinarily restricted to an extragranular compartment. Our observations on the binding of peptides and amino acids to subcellular particles isolated by centrifugation of sucrose homogenates would seem to be less subject to this criticism, but it must be acknowledged that the 2 hr delay between the death of animals at the slaughterhouse and the subsequent extraction of tissue may augment the peptide pool by autolytic processes. We are ascertaining the extent of possible autolysis by examining quick-frozen glands from freshly-killed animals in order to establish whether or not these multiple components are granule-bound in situ. An argument against a significant role of autolysis as a source of posterior pituitary peptides is the fact that the presence of an abundant and diverse population of peptides in this tissue has been confirmed by many workers who have worked with tissues removed from various species at varying intervals after death. Also, rat liver which was homogenized in the cold immediately after sacrifice gave a complex pattern of peptides. It seems quite possible that additional peptides (of presently unknown hormonal functions) are synthesized, stored, and secreted in a manner similar to that of the two established peptide

hormones. Such hypothetical peptides could be contained within the same neurons as vasopressin or oxytocin or in separate neurons.

Neurosecretory granules, composed of the peptide hormones bound to carrier protein constitute the major portion of protein in the posterior pituitary. The abundance of amino acids and peptides in the gland may result from enzymatic degradation of the carrier protein. Ingestion of neurosecretory granules by lysosomes within the nerve endings can be observed frequently electronmicroscopically (submitted for publication). Autophagy of cytoplasmic granules has been observed in a number of tissues and is probably one of the continuous, dynamic cellular processes concerned with maintenance of a constant granule population. In addition to the proteolysis of granule protein by lysosomal enzymes in the nerve terminals, pituicytes and other connective tissue cells may also degrade proteins that possibly could be released from the neurons. There is no morphological evidence to indicate that the NSG are secreted in toto from the cell, as is apparently the case for other glandular cells that contain and secrete products from proteinaceous storage granules. Therefore, it is generally believed that vasopressin and oxytocin diffuse away from the NSG and are secreted. Disposal of the residual carrier protein by enzymatic degradation may be the reason for the large peptide pool characteristic of the posterior pituitary gland.

Ramachandran and Winnick (1957) have determined the amino acid composition of several peptides isolated from porcine posterior pituitaries. Their initial peptide fraction was further fractionated by precipitation in organic solvents, adsorption on charcoal, and dinitrophenylation with subsequent chromatography. The fraction they characterized represented only about one-third of the total peptides, and the members ranged in size from dipeptides to one containing possibly as many as 87 residues. The data on

compositions of amino acids indicated very little similarity of their isolated low-molecular-weight components to those in our fraction which contained nonapeptides as the largest members.

In the low-molecular-weight population in the present study several unusual peptides were identified which contained only glutamic and aspartic acids in ratios of 1:1, 1:5, 1:8 and 4:1, respectively. Glutamic acid was found in 10 of the 19 peptides and comprised 4 of the 8 residues of peptide A5 and was linked with an unidentified component in A6. Aspartic acid was present in 11 of the peptides. Aspartic and glutamic acids are among the most abundant of the amino acids found in neurophysin, the carrier protein (LaBella et al., 1967). Histidine constituted 5 of the 8 residues in A3-B3 and tyrosine 4 of the 7 residues of A4-B2C.

REFERENCES

Bindler E., LaBella F.S. and Sanwal M. (1967) J. cell Biol. 34, 185.

Cadavid N.G. and Paladini A.C. (1964) Anal. Biochem. 9, 170.

Dean C.R., Hope D.B. and Kazic T. (1968) Brit. J. Pharmacol. Chemother. 34, 192P.

Fraenkel-Conrat H., Harris J.I. and Levy A.L. (1955) Methods biochem. Anal. 2, 359.

Frankland B.T.B., Hollenberg M.D., Hope D.B. and Schacter B.A. (1966) Brit. J. Pharmacol. Chemother. 26, 502.

Ginsburg M. and Ireland M. (1964) J. Endocrinology 30, 131.

Holton P. (1948) Brit. J. Pharmacol. Chemother. 3, 328.

Katz A.M., Dreyer W.J. and Anfinsen C.B. (1959) J. biol. Chem. 234, 2897.

LaBella F.S., Reiffenstein R.J. and Beaulieu G. (1962) Nature (London) 193, 173.

LaBella F.S., Reiffenstein R.J. and Beaulieu G. (1963) Arch. Biochem. Biophys. 100, 399.

LaBella F.S., Vivian S. and Bindler E. (1967) Biochem. Pharmacol. 16, 1126.

Lande S., Lerner A.B. and Upton G.V. (1965) J. biol. Chem. 240, 4259.

Landgrebe F.W., Macaulay M.H. and Waring H. (1946) Proc. Roy. Soc. Edinburgh. B 62, 202.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951) J. biol. Chem. 193, 265.

Palay S.L. (1957) Progr. in Neurobiol. 2, 31.

Penders Th. J. and Arens J.F. (1966) Experientia 22, 722.

Preddie E.C. (1965) J. biol. Chem. 240, 4194.

Preddie E.C. and Saffran M. (1965) J. biol. Chem. 240, 4189.

Ramachandran L.K. and Winnick T. (1957) Biochim. biophys. Acta 23, 533.

Saffran M., Caplan B.U., Mishkin S. and Muhlstock B. (1962) Endocrinology 70, 43.

Schally A.V. and Barrett J.F. (1968) Biochim. biophys. Acta 154, 595.

Schiebler T.H. (1952) Z. Zellforsch. 36, 563.

Schroeder W.A., Jones R.T., Cormick J. and McCalla K. (1962) Anal. Chem. 34, 1570.

Upton G.V., Lerner A.B. and Lande S. (1966) J. biol. Chem. 241, 5585.

Winnick T., Winnick R.E., Acher R. and Fromageot C. (1955) Biochim. biophys. Acta 18, 488.

Witter A., Vliegenthart J.F.G. and Arens J.F. (1964) Proc. Kgl. ned. Akad. Wetensch. B 67, 45.

TABLE I

GRADIENT SYSTEMS FOR ION-EXCHANGE CHROMATOGRAPHY

AG 1-X2 column					AG 50W-X2 column			
collidine:pyridine: acetic acid:water (40:40:1.5:3918) pH 8.3	acetic acid (M)				0.2M NaAc	2M NaAc	0.2M $NaHCO_3$	H_2O
	0.1	0.5	1	2	pH 3.10	pH 5.10	pH 8.30	
100	0	0	0	0	90	0	0	0
0	100	0	0	0	90	0	0	0
0	100	0	0	0	90	0	0	0
0	0	100	0	0	90	0	0	0
0	0	100	0	0	55	15	0	20
0	0	0	100	0	5	45	0	38
0	0	0	100	0	0	87	0	0
0	0	0	0	100	0	65	22	0
0	0	0	0	100	0	0	87	0

Figures represent number of ml in each chamber of the Technicon Autograd.

For details, see text under Methods.

LEGEND TO TABLE 2

The fractions noted by an asterisk are assumed to be homogeneous on the basis of ion-exchange chromatography, gel filtration, TLC, and high voltage electrophoresis.

TABLE 2

AMINO ACID COMPOSITION OF ISOLATED PEPTIDES

Peptide fraction	Major components after acid hydrolysis
A1-B1*	NH_3
A1-B2*	lysine (free)
A1-B3*	arginine (free)
A1-B4*	Alanyl-arginine
A2-B1	arg_2, gly_1, leu_1, val_1
A2-B2*	leu_4, his_2, gly_1
A3-B2*	NH_3
A3-B3	his_5, gly_2, ser_1
A3-B4	lys_2, ala_1, arg_1, asp_1, glu_1, gly_1, phe_1, ser_1
A3-B5*	lys_3, phe_3, asp_1, pro_1
A4-B1*	glu_2, ala_1, asp_1, gly_1, pro_1, ser_1, thr_1, X_1, Y_1
A4-B2a*	ala_2, met_2, asp_1, ser_1
A4-B2b*	phenylalanine (free)
A4-B2c*	tyr_4, lys_2, $hylys(?)_1$
A4-B4a*	phe_3, ala_1, gly_1, tyr_1
A4-B4b*	phe_2, ala_1, gly_1, tyr_1
A4-B5*	lysyl-glutamic
A5	ten spots on TLC
A6*	glu, X, (free NH_2 of glu)
A7	glu_4, asp_1
A8*	aspartyl-glutamic
A9*	asp_8, glu_1
A10*	glutamyl-aspartic
A11*	asp_2, leu_1
A12*	asp_5, glu_1

LEGENDS

Figure 1. (A) Elution profile from Sephadex G-25 (at 25°) of a 0.2 N acetic-acid extract of the total granule fraction from the posterior pituitary. Flow rate was 75 ml/hr and 14.4 ml fractions were collected and measured after reaction with ninhydrin.

(B) Elution profile of fraction III of (A) on Dowex 1-X2) (at 50°), developed with collidine:pyridine: acetic acid (X). The flow rate was 40 ml/hr and 6.3 ml fractions were collected and measured after reaction with ninhydrin (C, D, E, F). Sub-fractionation of fractions A1, A2, A3, and A4, respectively, on Dowex 50 W-X2 (at (50°) developed with sodium acetate: sodium bicarbonate buffer (X). The flow rate was 40 ml/h4 and 9.5, 11.1, 12.5, and 10.6 ml fractions, respectively, were collected and measured after reaction with ninhydrin.

Figure 2. (A) Elution profile from Sephadex G-25 (at 25°) of a 0.2 N acetic-acid extract of 5 g (wet wt) of a homogenate of bovine posterior pituitary. The flow rate was 75 ml/hr and the 17 ml fractions were assayed for protein by the procedure of Lowry et al. (1951).

(B) The toal granule fraction was isolated in buffered 0.29 M sucrose (pH 7.4) the granules were extracted with 0.2 N acetic acid, and the extract treated and assayed as in (A).

(C) Granules were isolated in buffered 0.29 M sucrose (pH 6.0) and continued as in (B).

Figure 3. Elution profiles as in Figure 3, except that rat liver was used as the source of homogenate and granules.

Figure 4. Portions of the total granule fraction from posterior pituitary were dialyzed against 0.005 M pyridine-acetate buffer (pH 6.0) in (a) distilled water and (b) 0.29 M sucrose. At intervals, portions were removed from the solution surrounding the dialysis bag and reacted with ninhydrin.

FIGURE 1

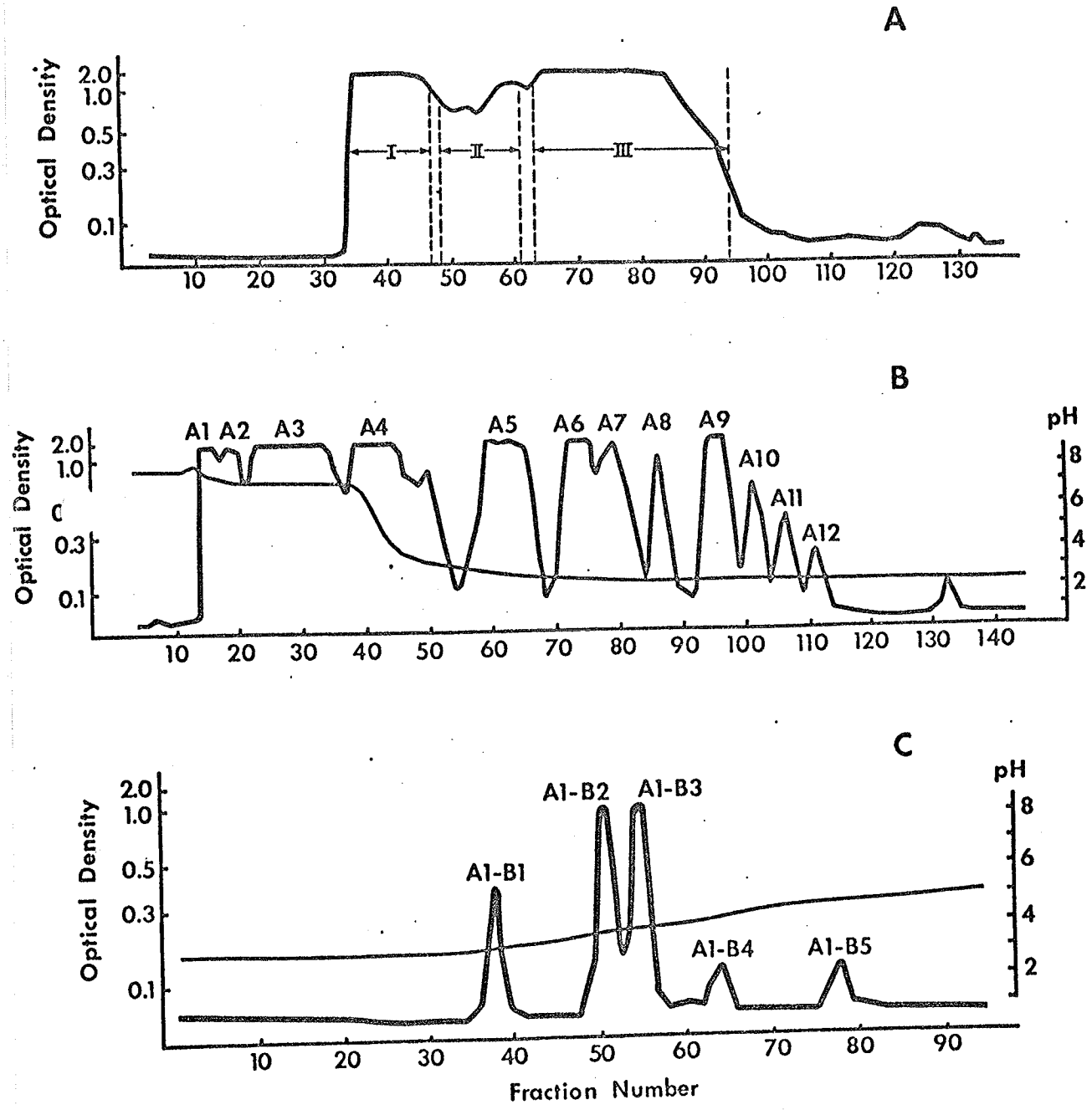
A
Optical Density
2.0
1.0
0.5
0.3
0.1
I
II
III
10 20 30 40 50 60 70 80 90 100 110 120 130
B
Optical Density
2.0
1.0
0.3
0.1
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12
pH
8
6
4
2
10 20 30 40 50 60 70 80 90 100 110 120 130 140
C
Optical Density
2.0
1.0
0.5
0.3
0.1
A1-B1
A1-B2
A1-B3
A1-B4
A1-B5
pH
8
6
4
2
10 20 30 40 50 60 70 80 90
Fraction Number

FIGURE 1 (CONT'D)

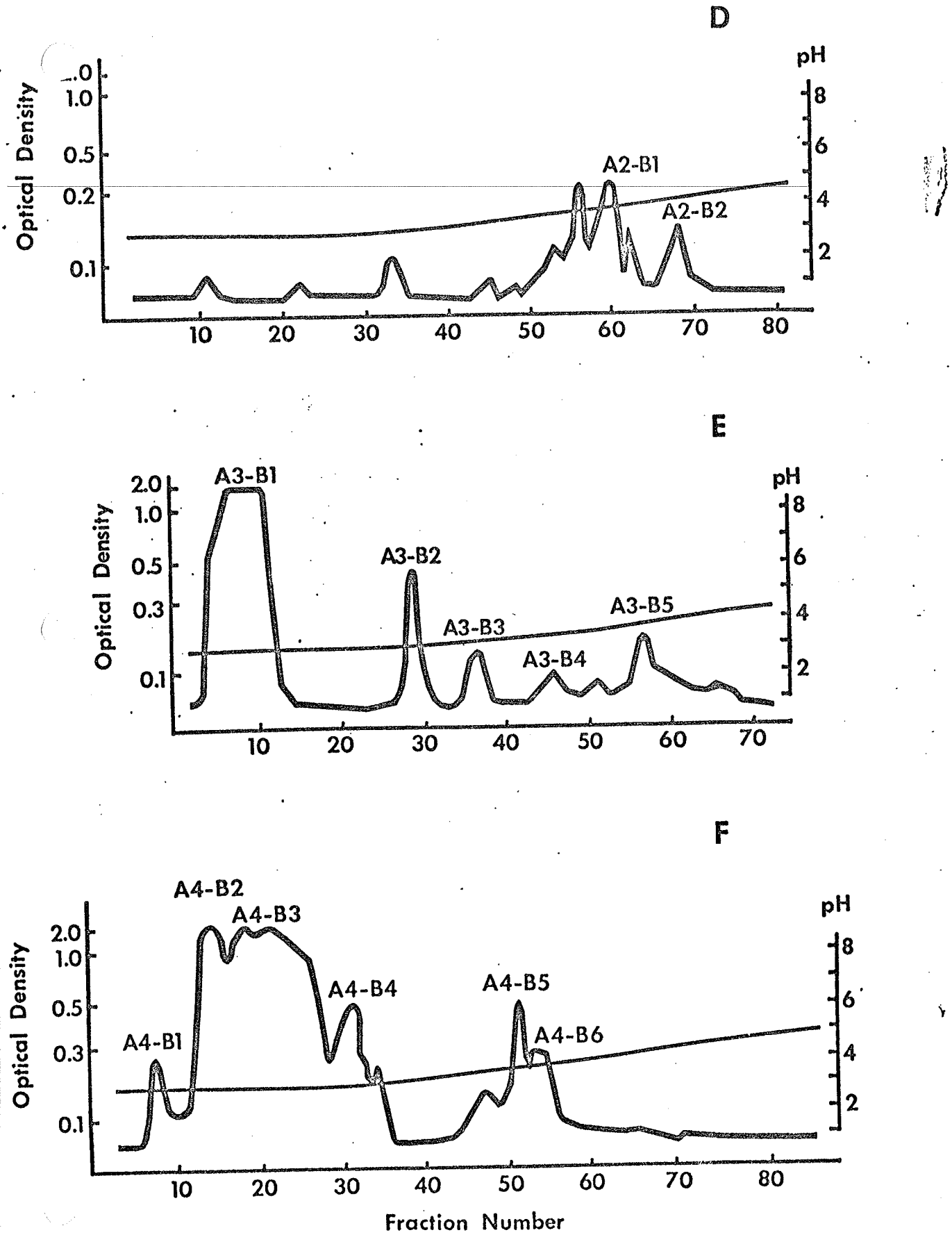

Figure 2

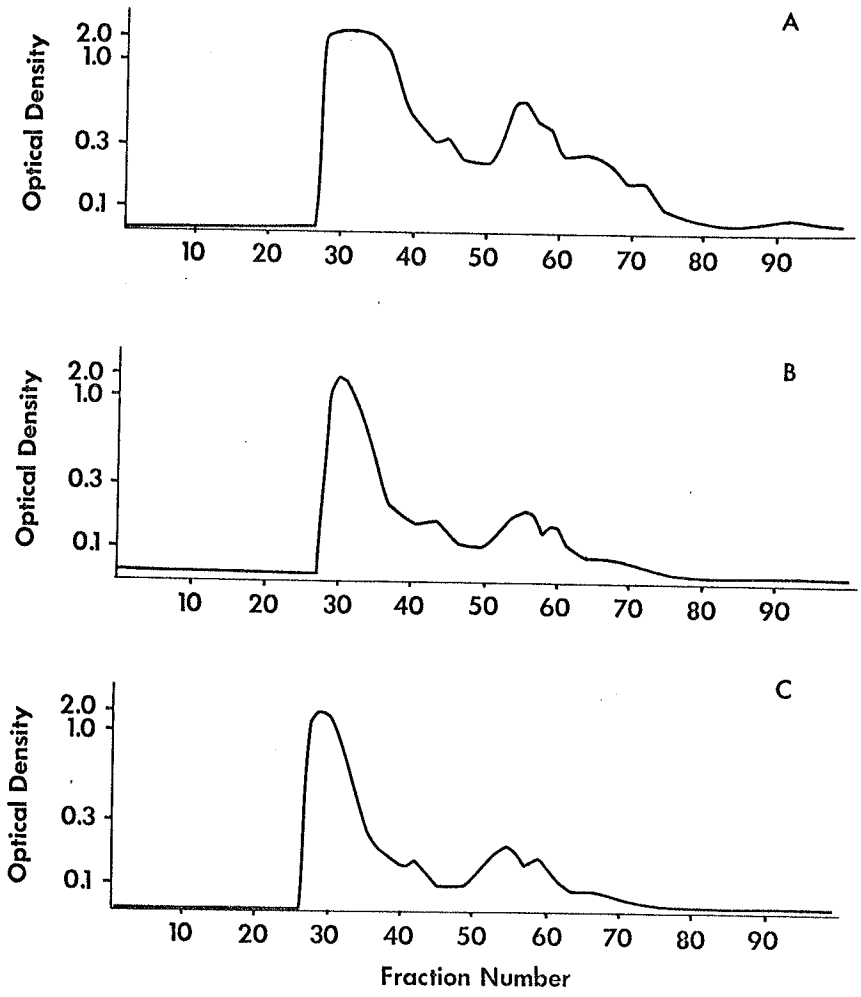
A
B
C
Optical Density
2.0
1.0
0.3
0.1
10
20
30
40
50
60
70
80
90
Fraction Number

Figure 3

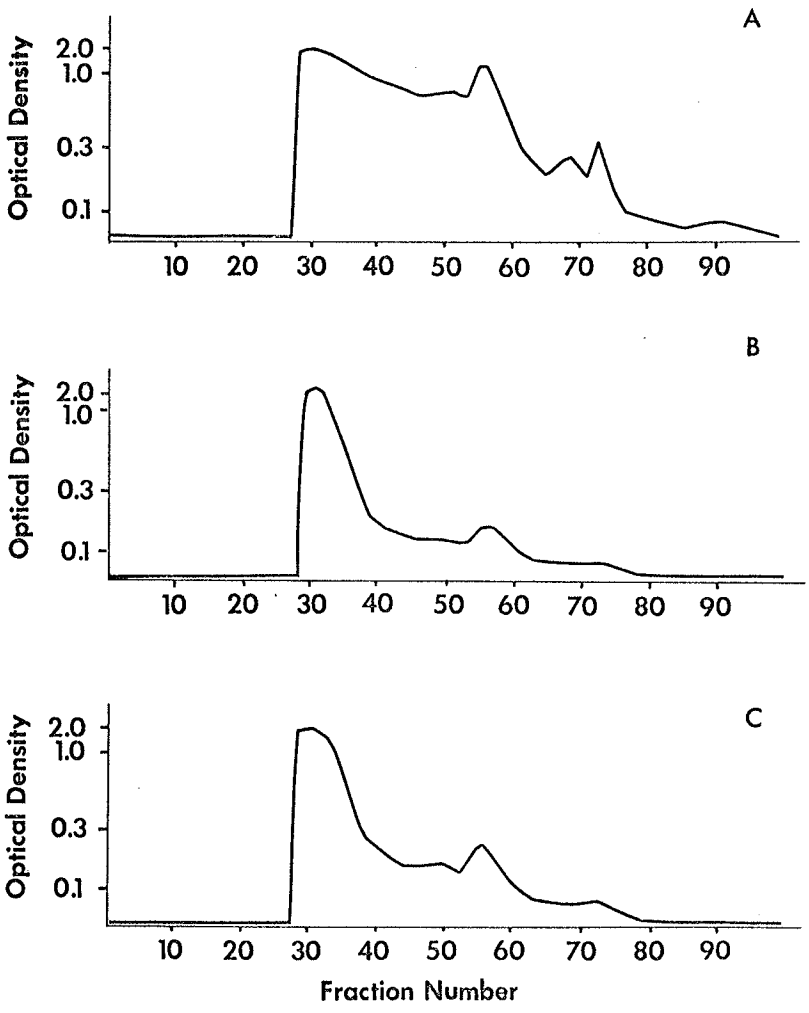
A
B
C
Optical Density
2.0
1.0
0.3
0.1
10
20
30
40
50
60
70
80
90
Fraction Number

FIGURE 4