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Fractionation of Neurophysin by Molecular-Sieve and Ion-Exchange Chromatography

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Neurophysin has been separated into seven distinct protein fractions. One of these components had no hormone-binding activity. The fractions that had hormone-binding activity were similar in amino acid composition: their cystine content was in the range 11·5–14·5%. The major component, neurophysin-M, was distinguished from the protein isolated by van Dyke by the presence of methionine and the absence of histidine. Neurophysin-M binds both oxytocin and vasopressin with similar affinities.

Some time ago a protein having oxytocic, pressor and antidiuretic activities was isolated from the fresh tissue of bovine pituitary glands. The protein appeared to be homogeneous in the ultracentrifuge and by other physical criteria. The molecular weight was about 30000 (van Dyke, Chow, Greep & Rothen, 1942). It was recognized that the protein had an unusually high sulphur content (4·9%) and subsequent work led to the conclusion that all the sulphur was present as cystine disulphide bonds (Block & van Dyke, 1950, 1952).

Later Acher and his co-workers isolated the same protein from acetone-dried powders of bovine pituitary glands (Acher, Chauvet & Olivry, 1956; Chauvet, Lenci & Acher, 1960). They demonstrated that the hormones, oxytocin and vasopressin, could be dissociated from the protein by mild procedures. The possibility that the association involved covalent bonds was thus excluded.

Recent evidence has shown that oxytocin and vasopressin may be released independently, both in man and in the cat (Bisset, Hilton & Poisner, 1963; Gaitan, Cobo & Mizrachi, 1964), which suggests that the hormones are stored in separate neurons. This raised the possibility that two proteins might exist, one binding oxytocin and the other binding vasopressin.

In preliminary work it was shown that neurophysin consists of a number of components (Frankland, Hollenberg, Hope & Schacter, 1966). The present paper concerns the isolation of several fractions from neurophysin; the major fraction was found to contain methionine and to bind both oxytocin and arginine vasopressin. A preliminary account of this work has been published (Hope & Hollenberg, 1966). It has recently been confirmed that neurophysin is a heterogeneous protein and that some constituents contain small amounts of methionine (Brelow & Abrash, 1966).

METHODS

Biological materials. The protein–hormone complex was prepared from acetone-dried posterior lobes of bovine pituitary glands (Chauvet *et al.* 1960). Before fractionation the protein was freed from the polypeptide hormones by gel filtration in 0·1 N-formic acid on Sephadex G-25 (Frankland *et al.* 1966). Synthetic oxytocin used for the binding experiments was obtained by courtesy of Dr B. Berde, Sandoz Products Ltd. (Basle, Switzerland), as a solution containing 450 i.u./ml. The arginine vasopressin was obtained by courtesy of Dr R. O. Studer, F. Hoffman–La Roche and Co. (Basle, Switzerland), as a freeze-dried powder (Studer, 1963).

Bioassay procedures. Oxytocic activity was assayed by the method of Holton (1948) with a magnesium-free solution suggested by Munsick (1960). The rats were injected subcutaneously 17 hr. before bioassay with 40 μg. of diethylstilboestrol as a suspension in liquid paraffin. Pressor activity was assayed by the method of Dekanski (1952) with male albino rats anaesthetized with urethane and treated with phenoxybenzamine. Biological activities were assayed against the International Standard (Bangham & Mussett, 1958).

Column chromatography. For gel filtration two kinds of Sephadex from Pharmacia Ltd. (Uppsala, Sweden) were used: (1) bead-type Sephadex G-25 (fine grade, 20–80 μ); (2) bead-type Sephadex G-75 (fine grade, 20–80 μ). The Sephadex powders were suspended in 50% (v/v) acetic acid and deaerated *in vacuo*. The gels were suspended in 0·1 N-formic acid and poured into Pyrex columns (2·0 cm. internal diam. × 150 cm. long). The proteins were eluted at room temperature with a constant flow rate of 25 ml./hr. The extinction at 257 mμ of the effluent was monitored and 6 ml. fractions were collected.

For ion-exchange chromatography a column (3 cm. × 42 cm.) of bead-type CM-Sephadex C-50 was used. The pro-

teins were eluted with acetate buffers in a pH gradient increasing linearly from pH4·5 (I0·1) to pH5·0 (I0·1) and stepwise from pH5·0 (I0·1) to pH5·6 (I0·2). The Sephadex C-50 was equilibrated in acetate buffer, pH4·5 and I0·1, and the fine particles were removed by decantation. The suspension was deaerated before pouring the columns in five portions. After each experiment the ion-exchange groups were regenerated by the procedure recommended by Pharmacia Ltd.

Protein was recovered from the fractions taken from the peak tubes. The fractions were pooled, concentrated to half the volume in a 'flash' evaporator, dialysed against distilled water and freeze-dried.

Equilibrium dialysis. The hormone-binding ability of the protein fractions was assessed by thin-film dialysis in the 'alternate' cell described by Craig & Konigsberg (1961). A solution (0·5ml.) containing between 2·5 and 10mg. of protein was introduced on the inside of the 18/32 Visking membrane. This solution was dialysed against sodium acetate buffer, pH5·6 and I0·1, with three changes of 8ml. for 24hr. The extinctions of the diffusate at 280 and 260mμ were measured to ensure that no protein had escaped. A solution (8·0ml.) containing oxytocin (50i.u. of oxytocic activity) and vasopressin (50i.u. of pressor activity) in the acetate buffer was then placed in the outer compartment of the dialysis cell. The system was allowed to equilibrate at 4° for 24hr.; the volume of fluid in each compartment was measured and the solution assayed for oxytocic and pressor activities.

Zone electrophoresis of protein. Solutions containing protein (5mg./ml.) were submitted to zone electrophoresis in starch gels (Smithies, 1959) with the discontinuous buffer system described by Poulik (1957). Protein was detected by staining with Nigrosine.

Ultracentrifugation of protein. The ultracentrifugal analysis was performed with a Spinco model E apparatus. All experiments were done at a rotor temperature of 20°, and the changes in concentration were recorded by a schlieren optical system.

The protein, dissolved in sodium acetate buffer, pH4·8 and I0·1, was first dialysed overnight against a large volume of solvent with 18/32 Visking cellulose tubing. This protein solution (7mg./ml.) was then used for the subsequent analyses. All dilutions were made with the buffer against which the protein solution had been dialysed.

Sedimentation analysis was performed by using standard cells with aluminium centre-pieces. A rotor speed of 59780 rev./min. was used and six photographs were taken at 16min. intervals. The x value corresponding to the maximum of the schlieren peak was used in the calculations of the sedimentation coefficient. All measurements for these and subsequent experiments were made directly from the photographs by using a model II Precision Grinding Projectorscope with a ×25 objective (Precision Grinding Ltd., Mitcham Junction, Surrey). Measurements were accurate to ±0·001cm. The sedimentation coefficient was measured at four different protein concentrations (7, 5·6, 4·2 and 2·8mg./ml.).

Measurements of the diffusion coefficient were made with the rubber-valve-type synthetic-boundary cell according to the maximum ordinate–area method reported by Ehrenberg (1957) and outlined by Elias (1961). The solution of protein was prepared as described above and the solvent outside the dialysis sac was used as the upper phase. The rotor speed was 10589rev./min. and six photographs were taken at 16min. intervals. The area bounded by the base line and the schlieren peak was integrated by rectangulation. Values of $D_{app.}$ were calculated from the equation:

$$(A/H_{max.})^2 = 4\pi D_{app.} t$$

where both A, the area under the schlieren peak, and $H_{max.}$, the maximum height of the peak, were corrected for the magnification factor of the optical system (×2·129). In the equation, t is the time from the calculated zero time at which the observations of A and $H_{max.}$ were made. The apparent diffusion coefficient at zero time was determined by a linear regression of at least five values of $D_{app.}$. The diffusion coefficient was measured at two protein concentrations (7 and 4·2mg./ml.).

Molecular-weight measurements were made by the short-column equilibrium method described by Yphantis (1960) with the 8-channel cell. A rotor speed of 15220rev./min. was used, and photographs were taken at 16min. intervals to follow the approach of the system to equilibrium. Equilibrium was attained after 1hr., and measurements were made directly from the photographs taken at 80 or 96min. The values of the concentration gradients at the midpoints of each sample cell were given in arbitrary refractive-index units by the difference between solution and solvent traces. The measurements of the initial protein concentrations were made by using the rubber-valve-type synthetic-boundary cell at a rotor speed of 10589rev./min. with the solvent outside the dialysis sac (18/32 Visking cellulose tubing) as the upper phase. Photographs were taken shortly after the boundary had formed and measurements were made directly from the negatives. With the standard schlieren optics the initial concentration was determined in terms of arbitrary refractive-index units by integration of the synthetic-boundary trace. The apparent molecular weight was calculated from the equation:

$$M = \frac{1}{rc_0}\left(\frac{dc}{dr}\right)_{r=\bar{r}} \frac{RT}{\omega^2(1-\bar{v}\rho)}$$

where r is the distance from the midpoint of the sample cell to the centre of rotation (corrected for rotor expansion), c_0 is the original protein concentration in refractive-index units, $(dc/dr)_{r=\bar{r}}$ is the concentration gradient at the midpoint of the sample cell in the same refractive-index units, R is the gas constant, T is the absolute temperature and ω is the angular velocity. The density of the solution, ρ, was assumed to be unity. The partial specific volume, $\bar{v}$, of neurophysin-M was calculated to be 0·71 on the basis of the amino acid composition: this value was calculated according to the method described by Cohn & Edsall (1943) except that 0·63 was used as the partial specific volume of the cystine residue (Edsall, 1953). The value of the apparent molecular weight resulting from each experiment was the average of the values yielded by four independent sets of measurements on each of the four pairs of cells. Measurements of the molecular weight of neurophysin-M were made at two protein concentrations (7 and 4·2mg./ml.).

Analytical procedures. The concentration of protein in fractions of eluate from Sephadex columns was estimated from the extinctions at 280 and 260mμ by the method of Layne (1957).

Amino acid analyses were performed in an automatic amino acid analyser (Evans Electroselenium Ltd.) by the

method of Spackman, Stein & Moore (1958). Samples of protein (2–6mg.) were hydrolysed in constant-boiling HCl for 17hr. at 110° as described by Crestfield, Moore & Stein (1963). For the analysis of tryptophan, protein samples (6–8mg.) were hydrolysed in 4N-$Ba(OH)_2$ *in vacuo* at 110° for 64hr. Barium was precipitated from the hydrolysate by a stream of CO_2.

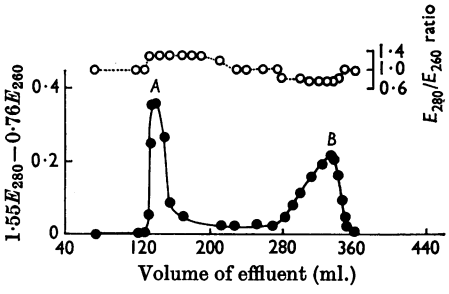

Fig. 1. Column chromatography of bovine neurophysin on Sephadex G-75. Hormone-free protein (90mg.) dissolved in 5ml. of 0·1N-formic acid was applied to a column (2cm.× 150cm.) equilibrated with the same solvent. The flow rate was 25ml./hr.; 3ml. fractions were collected. Peak *A*, protein (mol.wt. ⩾50000) lacking hormone-binding capacity; peak *B*, protein (mol.wt. approx. 20000) which binds both oxytocin and arginine vasopressin. ●, $1{\cdot}55E_{280}-0{\cdot}76E_{260}$; ○, E_{280}/E_{260} ratio.

RESULTS

Starch-gel electrophoresis had already suggested that neurophysin consisted of several proteins (Frankland *et al.* 1966); the first step was to separate these into a high-molecular-weight fraction and a low-molecular-weight fraction by gel filtration.

Gel filtration on Sephadex G-75. The neurophysin was submitted to gel filtration in 0·1g. portions on a column (2cm.× 150cm.) of Sephadex G-75. The proteins were eluted with 0·1N-formic acid and were recovered in two peaks (Fig. 1). The first peak (*A*) emerged with the void volume of the column (120ml.), which indicated a molecular weight above 50000. The second peak (*B*) was eluted after a further 150ml. of eluent. Although peak *B* was unsymmetrical the E_{280}/E_{260} ratio was constant across the peak. Further, material isolated from the leading or tailing portion of the peak behaved similarly on rechromatography. Of the protein recovered, 15% by weight was found in peak *A* and 85% in peak *B*.

The material from protein peaks *A* and *B* were submitted to starch-gel electrophoresis. The usual practice was to dissolve a protein sample in a little of the buffer used to prepare the gel, but material from peak *A* was insoluble in the alkaline buffer: *A* solution was prepared in dilute acetic acid. The

Table 1. *Amino acid composition of protein fractions of neurophysin*

The values obtained for protein from peaks *A* and *B* and for fraction 3 represent the mean of three analyses. The values for other fractions, because of the small amount of material available, represent a single analysis only. The analyses were performed on hydrolysates prepared by heating protein samples in 6N-HCl *in vacuo* at 110° for 17hr. Tryptophan was measured separately on alkaline hydrolysates.

	Amino acid composition of neurophysin fractions (%, by wt.)						
Amino acid	Fractions from Sephadex G-75		Fractions from CM-Sephadex C-50				
	A	*B*	1	2	3	4	5
Lysine	4·18	3·14	2·85	2·94	3·01	3·29	3·01
Histidine	3·22	0·38	1·45	0·84	0·00	1·64	0·00
Arginine	7·32	10·87	8·30	9·57	12·50	7·64	10·50
Aspartic acid	10·43	5·91	6·15	6·56	5·87	7·32	5·12
Threonine	5·02	2·26	2·37	2·27	1·93	2·48	2·15
Serine	4·88	4·72	5·01	4·62	4·50	4·84	4·43
Glutamic acid	13·83	16·88	16·51	16·59	17·25	14·00	18·07
Proline	6·46	8·36	9·71	8·33	7·87	10·78	8·22
Glycine	4·81	8·84	9·93	9·57	9·21	9·60	9·81
Alanine	5·09	4·15	5·67	5·25	3·91	5·53	3·87
Cystine	4·04	14·25	12·47	11·77	13·40	14·29	14·46
Valine	5·67	3·29	2·42	3·61	3·73	2·52	3·96
Methionine	1·10	0·35	0·00	0·40	0·48	0·00	0·00
Isoleucine	3·01	2·30	2·37	2·54	2·08	2·52	2·28
Leucine	8·39	6·54	6·85	6·79	6·68	5·66	5·55
Tyrosine	4·84	2·16	1·93	2·01	2·02	2·13	1·89
Phenylalanine	6·04	4·44	4·35	4·42	4·47	4·50	4·43
Tryptophan	0·90	0·00	—	—	—	—	—
Ammonia	1·88	1·23	1·67	1·94	1·34	1·27	2·24

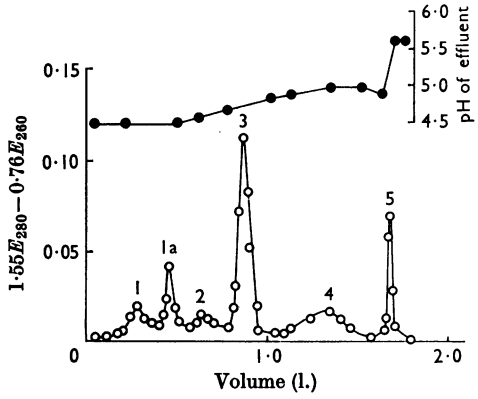

Fig. 2. Column chromatography of hormone-binding protein (peak *B*, Fig. 1). Freeze-dried protein (150mg. of material from peak *B*) was dissolved in dilute acetic acid and applied to a column (3cm. × 40cm.) of CM-Sephadex C-50 equilibrated in sodium acetate buffer, pH4·5 and *I* 0·1. The column was eluted at a flow rate of 12·5ml./hr. with acetate buffer increasing pH and ionic strength to pH5·6; 3ml. fractions were collected. The contents of peak tubes were pooled, dialysed and freeze-dried. ●, pH of effluent; ○, $1·55E_{280}-0·76E_{260}$.

Fig. 3. Diagrammatic representation of starch-gel electrophoretograms of neurophysin fractions eluted from CM-Sephadex. Electrophoresis was performed in vertical starch gels according to the method of Smithies (1959). Gels (starch concn. 10·4g./100ml. of buffer) were in the buffer described by Poulik (1957). Protein samples dissolved in the same buffer (5mg./ml.) were applied and electrophoresis was allowed to proceed for 17hr. with a constant voltage gradient of 6v/cm. Protein was detected by staining the gel with Nigrosine (0·05%, w/v) in water–methanol–acetic acid (5:5:1, by vol.) for 10min. The original material (peak *B*, Fig. 1) is shown on the left. The numbers above each band refer to the peaks in Fig. 2. The material in peaks 1 and 1a were combined and designated fraction 1.

protein from peak *A* did not form distinct bands but appeared as a continuous streak along the path of migration.

The material from peak *B* separated into four distinct bands in the buffer system used.

Amino acid analysis. The amino acid analyses of hydrolysates of material from peaks *A* and *B* are shown in Table 1. Tryptophan was present in *A* but absent from *B*. On the other hand, methionine was present in both. The material from peak *B* contained large amounts of glycine, cystine and glutamic acid. These three amino acids together accounted for about 40% of the weight of the protein.

Hormone-binding properties. The material from peak *A* lacked the ability to form complexes with the polypeptide hormones. This activity was confined to material from peak *B*. In an equilibrium dialysis experiment the ratio of bound to free oxytocin was 102·3 when 9·8mg. of protein was dialysed against a solution of oxytocin (90i.u.). In the absence of hormones this material (5mg./ml.) was soluble in a solution containing sodium acetate buffer, pH3·9 and *I* 0·1, and sodium chloride (60g./l.): a precipitate formed on addition of either oxytocin or vasopressin.

Chromatography on CM-Sephadex C-50. Ion-exchange chromatography of protein (150mg.) from peak *B* on CM-Sephadex C-50 gave six fractions as shown in Fig. 2. These were eluted in the following pH ranges; 1 and 1a (18·8mg.), pH4·50; 2 (3·8mg.), pH4·55–4·70; 3 (51·1mg.), pH4·75–4·90; 4 (14·2mg.), pH4·95–5·00; 5 (8·0mg.), pH5·00–5·60. In some experiments fractions 1 and 1a emerged together. More than 50% of the protein recovered was in fraction 3.

Electrophoretic analysis. The protein fractions were analysed by starch-gel electrophoresis and the results are shown in Fig. 3. Each fraction gave rise to a single band with the exception of fraction 4, which contained two components. There was some correlation between the point of emergence from the column and the electrophoretic mobility: the fractions eluted from the column first had the highest electrophoretic mobility and they were presumably more anionic.

Amino acid analysis. The amino acid compositions of the five protein fractions are shown in Table 1. The five analyses show that they are all extremely similar in composition. The main differences are in their contents of methionine and histidine. Fraction 3, the major component, contained methionine, but was free from histidine. On the other hand, fractions 1 and 4 contained histidine, but were free from methionine. Fraction 2 contained both histidine and methionine, but fraction 5 was free from both of these amino acids. The presence of methionine and absence of histidine distinguishes the major fraction (fraction 3) from the protein described by van Dyke and co-workers (van Dyke *et al.* 1942; Block & van Dyke, 1950, 1952). We have already proposed to call this fraction neurophysin-M (Hope & Hollenberg, 1966). In terms of amino acid residues, the amino acids present in greatest numbers were glycine, cystine, glutamic acid and proline in all five of the fractions.

Minimum molecular weights for the proteins were calculated from the amounts of the less abundant

amino acids (lysine and phenylalanine). On the assumption that each protein contains four residues of lysine and six of phenylalanine, the molecular weights of the five protein fractions were calculated to be: fraction 1: 19000; fraction 2: 19000; fraction 3: 19000; fraction 4: 18000; fraction 5: 18500. There appears to be one mole of methionine/mole of protein in fraction 3 and no trace of histidine.

Ultracentrifuge studies of neurophysin-M. One symmetrical peak was observed on sedimenting neurophysin-M at 20° in the ultracentrifuge at 59780rev./min. (Fig. 4*a*). The sedimentation coefficient ($S^0_{20,\,buffer}$) uncorrected for solvent viscosity in sodium acetate buffer, pH 4·8 and I 0·1, was 2·00. The sedimentation coefficient was independent of protein concentration in the range 2–7mg./ml.

The molecular weight determined from sedimentation-equilibrium studies with short columns of solution at 20° was 20700 at a concentration of 7mg./ml. and 19100 at 4mg./ml. The partial specific volume was calculated from the amino acid analysis to be 0·71ml./g. The diffusion coefficient was measured at the same protein concentrations. The values of D_{20} uncorrected for viscosity at 20° were $8{\cdot}64 \times 10^{-7}$cm.^{2}sec.$^{-1}$ at 7mg./ml. and $8{\cdot}47 \times 10^{-7}$cm.^{2}sec.$^{-1}$ at 4mg./ml. The molecular weights calculated from the values of D_{20} and S_{20} were 19200 at 7mg./ml. and 19600 at 4mg./ml.

Equilibrium dialysis. The ability of the protein fractions to bind both oxytocin and vasopressin was measured by equilibrium dialysis. Neurophysin-M (2·5mg.) was dissolved in 0·5ml. of sodium acetate buffer, pH 5·6 and I 0·1. This was dialysed against a solution containing 50units of oxytocic and 50units of pressor activity. At equilibrium the volumes were: inside, 1·0ml.; outside, 7·5ml. The inner compartment contained 26·8units of oxytocic and 27·7units of pressor activity/ml. whereas the outer compartment contained only 1·6units of oxytocic and 0·9unit of pressor activity/ml. Thus the protein had associated with 10·1units of oxytocic activity and 10·7units of pressor activity/mg. A similar experiment was performed with fraction 5; in this instance the protein associated with 1·8units of oxytocic activity and 2·0units of pressor activity/mg. There was not sufficient material to carry out similar experiments with other protein fractions.

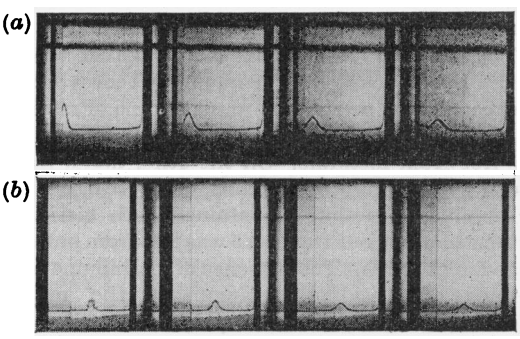

Fig. 4. (*a*) Sedimentation pattern of neurophysin-M. The protein concentration was 7·0mg./ml. in sodium acetate buffer, pH 4·8 and I 0·1. The solution was dialysed overnight against the same buffer at 4°. The solution was centrifuged at 59780rev./min. at 20° in a Beckman–Spinco model E ultracentrifuge. Pictures were taken at 16min. intervals. The phase-plate angle was 80°. (*b*) Diffusion pattern of neurophysin-M. A synthetic-boundary cell was used. Conditions were as above, with the boundary formed at 10589rev./min.

DISCUSSION

The isolation of six protein fractions with different amino acid compositions confirms the previous finding that bovine neurophysin is heterogeneous (Frankland *et al.* 1966; Hope & Hollenberg, 1966). In addition to the constituents rich in cystine (fractions 1–5) the material from peak *A* was found to contain relatively little cystine and lacked the ability to form complexes with the polypeptide hormones. Its exclusion from Sephadex G-75 suggests a molecular weight above 50000.

According to the work of Block & van Dyke (1950, 1952) the protein–hormone complex isolated from pituitary posterior-lobe tissue was free from methionine and tryptophan. We were surprised some time ago to find small amounts (less than 1%, w/w) of both of these amino acids in the complex prepared by the procedure of Chauvet *et al.* (1960).

Tryptophan was confined to the higher-molecular-weight protein from peak *A*; methionine, on the other hand, was present in protein from both peaks. Material from peak *B*, which had the ability to bind both oxytocin and arginine vasopressin, yielded one major fraction (fraction 3) and small quantities of several other components. Fraction 3 accounted for most of the methionine found in material from peak *B* but it was entirely free from histidine. We have previously described this protein fraction as neurophysin-M. In the analytical ultracentrifuge neurophysin-M appeared to be homogeneous although under certain conditions of electrophoresis it gives rise to two bands (Hollenberg & Hope, 1966). The results obtained by the use of the ultracentrifuge established that the molecular weight of neurophysin-M is close to 20000. The minimum molecular weight was calculated to be 19000 on the assumption of one methionine, four lysine and six phenylalanine residues/molecule. The molecular weight of the protein is somewhat less than that reported by van Dyke *et al.* (1942). This can be attributed partly to the presence in the material of the polypeptide hormones (mol.wt.

1000) and also to the fact that the partial specific volume, $\bar{v}$, used in their calculations was taken as 0·749ml./g. The value of $\bar{v}$ calculated from the amino acid composition of our protein was 0·71 ml./g. and with this value the molecular weight of the protein–hormone complex would be decreased from 30000 to 26000. On removal of the polypeptide hormones a value of 24000 is obtained for the molecular weight of neurophysin, which is in closer agreement with the values obtained in the present work.

The amino acid composition of neurophysin-M was similar to that reported by Block & van Dyke (1952) for the protein–hormone complex but contained more arginine and leucine. The amino acids present in largest amounts were glycine, glutamic acid and cystine. Perhaps the most striking differences are the qualitative differences in methionine and histidine content. The hormone–protein complex obtained by van Dyke and co-workers contained a small amount of histidine, but it appeared to be free of methionine. Neurophysin-M, on the other hand, contained methionine, but no trace of histidine could be detected. Fractions 1 and 4 resembled the hormone–protein complex in that they contained histidine but lacked methionine, although they accounted for only a small proportion of the total protein. Fraction 2 contained small quantities of both methionine and histidine, in contrast with fraction 5, from which both amino acids were absent. Neurophysin-M is relatively rich in arginine and valine compared with the other constituents of neurophysin, but the proline content on the other hand is lower.

Neurophysin-M and fraction 5 bind both oxytocin and arginine vasopressin, and it can reasonably be inferred that the other fractions also bind them since all the protein components are precipitated by salt only in their presence.

The results show that neurophysin as described previously (van Dyke *et al.* 1942; Acher *et al.* 1956; Chauvet *et al.* 1960) is not a homogeneous protein. The chromatographic systems described in the present paper lead to the isolation of six fractions. The physical and chemical properties of these fractions show that they are members of a group of closely related proteins. It is noteworthy that recent work on the soluble proteins of neurosecretory granules isolated from bovine posterior-lobe pituitary tissue has shown them to contain two principal proteins whose electrophoretic mobilities were identical with those of two of the constituents of neurophysin (Dean & Hope, 1966). The functions of more than one neurophysin in the storage and transport of the polypeptide hormones remain to be elucidated.

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