CCLX. THE OXYTOMIC HORMONE OF THE POSTERIOR LOBE OF THE PITUITARY GLAND. VII.

SECTION A. ULTRAVIOLET ABSORPTION SPECTRA.

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SECTIONS B AND C. ADSORPTION AND ELECTRODIALYSIS.

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SECTION A. ULTRAVIOLET ABSORPTION SPECTRA.

FROM investigations of the absorption of ultraviolet light by commercial extracts of the posterior lobe, Graubner [1928] concluded that the hormone molecule plays an essential (*wesentlich*) part in the absorption, basing his views on a supposed correlation of the oxytomic activities with the amount of absorption. In our opinion, such correlation was unreliable owing to the very small quantity of active material which can have been present in these extracts as compared with the amounts of inactive substances. The question has therefore been studied anew.

Measurements of the ultraviolet absorption spectra of purified hormone solutions have not demonstrated relationships either between the values of extinction coefficients and the solid content of the solutions (Fig. 1) or between the amount of absorption and the number of oxytomic units present (Table I). Moreover, when the spectra of a hormone solution were measured before and after electrodialysis at p_H 11 (p. 2218), the initial solution showed a spectrum with a pronounced peak at $274m\mu$, whereas the hormone solution after treatment exhibited merely a general adsorption.

It seems probable, therefore, that the amount of absorption is chiefly determined by the presence of variable quantities of absorptive, but non-oxytomic, substances in the extracts and that these are also responsible for the peak at about $274m\mu$ which is shown by some hormone solutions.

A consideration of the shapes of the absorption curves now recorded, and to some extent also of those of Graubner, suggests strongly that these concomitant substances may be peptide in character; this conclusion is in harmony with the observation, based on qualitative chemical tests, that posterior lobe extracts contain proteoses and possibly peptones [Abel and Pincoffs, 1917].

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In a comparison of the ultraviolet absorption spectra of the extracts (Figs. 1 and 2) with those of proteoses and peptones (Fig. 3), the following similarities between the two groups are apparent:

(1) The general resemblance of the shapes and wave-lengths of the absorption spectra curves.

(2) The presence in acid solutions of flattened peaks with centres at about $274m\mu$ but sometimes stretching towards shorter wave-length.

Fig. 1. Hormone solutions at p_H 3.5–3.7. L₁ □—□; L₂ ○—○;
B Δ—Δ; K₃ ×—×; K₄ ●—●.

Table I.

Solution	Solid mg. per ml.	Units per ml.	Units per mg.	E 274–5 $m\mu$	Absorption 274–5 $m\mu$
L ₁	7.4	160	21.5	6.1	4.3
L ₂	1.1	270	245.0	45.4	1.0
B	5.6	300	53.5	26.6	14.0
K ₃	3.6	900	250.0	22.2	8.0
K ₄	5.3	1000	188.0	22.7	12.0

(3) The presence in acid solutions of wide troughs with centres at about $250m\mu$ but sometimes extending towards longer wave-lengths.

(4) The filling of this trough as the alkalinity increases.

(5) The disappearance of this trough in alkaline solutions in some cases, so that peak and trough become merged into a wide plateau.

(6) A shift towards longer wave-length and a tendency for the extinction coefficients to increase, both in alkaline solutions.

The spectra of hormone solutions which had been deprived of their oxytocic activity with alkali resembled the spectra of authentic proteoses and peptones in alkaline media.

It was therefore concluded on these grounds also that the ultraviolet absorption spectra of the hormone solutions at present available are not intimately connected with the presence of the hormone molecule but are a property of peptide substances which accompany it.

Fig. 2. Spectra of hormone solution B at different p_H values.
 o-o p_H 3.7; Δ - Δ p_H 7; $\square$ - $\square$ p_H 8.2; $\times$ - $\times$ p_H 9.8.

Fig. 3. Spectra of proteoses and peptones. Witte's peptone p_H 7 $\bullet$ - $\bullet$; p_H 8.9 $\bullet$ --- $\bullet$; Witte's peptone:dialysed p_H 7 $\circ$ - $\circ$; p_H 9.4 $\circ$ --- $\circ$; Savory and Moore's peptone:peptose p_H 4.2 Δ - Δ ; p_H 9.4 Δ --- Δ ; Savory and Moore's peptone:peptone p_H 4.4 $\times$ - $\times$; p_H 9.6 $\times$ --- $\times$; pressor fraction from Kamm separation [Kamm *et al.*, 1928] p_H 5.2 $\square$ - $\square$.

EXPERIMENTAL.

Comparison of the absorption spectra of hormone solutions.

A Hilger spectrograph (Type E3) and sector photometer were used to measure the absorptions. The solutions were "Kamm" solutions (for definition, see Gulland and Newton [1932]) prepared as described by Kamm *et al.* [1928].

The measurements, made under comparable conditions at p_H 3.5–3.7, are summarised in Table I. In Figs. 1, 2 and 3 and Table I, the extinction coefficients are calculated on the basis of 1% solutions of the proteoses, peptones or the residues of hormone solutions, dried at 110°, and at the customary thickness of 1 cm.

SECTIONS B AND C.

The aim of these experiments, purification of the hormone, has not been achieved, since preparations assaying about 260 oxytomic units per mg. of solid represent the limit of purification which we have reached. Nevertheless, this publication is made because a variety of novel points have been brought to light and also because it records a fully investigated scheme of attack, based on the observation in Section A that the substances which accompany the hormone in purified extracts are peptide compounds of relatively low molecular weight.

B. ADSORPTION EXPERIMENTS.

1. *Various adsorbents.* The results of a series of experiments on the adsorption of the hormone from a partially purified solution are summarised in Table II. They offer little prospect of extensive purification of the hormone by

Table II. *Effect of adsorbents.*

Hormone solution K_s (900 units per ml., associated with 3.6 mg. of solid).
Total vols. of liquid, 10–15 ml.

Adsorbent	Wt. of adsorbent mg.	p_H	Duration of adsorption hours	Units used	Units in filtrate	Units in eluate
Silica gel	100	2.8	16 at 0°	20	19	—
		6.5	"	20	20	—
		11.0	"	20	20	—
Aluminium hydroxide Cy	30	3.5	16 at 0°	20	20	—
		6.5	"	20	20	—
		10.5	"	20	20	—
Fuller's earth (Surrey yellow)	100	3.3	1.5 at 20°	20	0	20*
		7.0	"	20	0	20*
		11.0	"	20	16	—
Talc (acid-treated)	50	6.7	17 at 20°	18	1.5	4.5†
Talc (ignited)	50	6.7	17 at 20°	18	3.2	2.7‡
Asbestos	100	6.1	18 at 20°	18	18	—
	100	11.0	"	18	18	—

* The hormone was not eluted by 95% acetic acid but was set free by $N/5$ ammonia acting for 1 hour at 0°.

† Eluted with glacial acetic acid.

‡ Eluted with $N/10$ ammonia at 0°.

means of these adsorbents acting alone, because in general those reagents which did not adsorb the active material failed to remove appreciable amounts of solid, whereas elution of the fuller's earth adsorbate with ammonia liberated relatively large quantities of inorganic material in addition to the active substance.

(2) *Adsorption on norite.* The use of norite as an adsorbent was studied by Gulland and Newton [1932], but it was desirable to repeat some of their observations in different circumstances and to attempt to apply the adsorption to the purification of hormone solutions.

The norite was extracted repeatedly with hot 20 % hydrochloric acid, washed with distilled water, dried, extracted twice with silica-distilled glacial acetic acid and washed with silica-distilled water. 100 mg. of the final sample yielded 0.08 mg. of solid in a blank experiment (see below).

In all experiments throughout this paper where determinations of weight were made the acetic acid and water used were distilled and collected in silica apparatus and contained no weighable residue when considerable volumes were evaporated. In alkaline experiments, where ammonia had been used during the adsorption, the norite was freed from ammonia, before being eluted, by being heated at 40° for 30 min. under reduced pressure. This does not diminish the activity. In the other experiments, ordinary reagents were used. Solids were estimated by evaporation of the solutions and drying of the residues to constant weight at 100° in a current of dust-free air.

Adsorption was effected by shaking frequently mixtures of norite, hormone solutions and buffer or diluting liquid (6 ml.) at 0°. The norite was collected, washed with water (10 ml.) which removed no activity, dried and eluted at room temperature. The resulting eluates were concentrated under reduced pressure below 40°.

The results are summarised in Tables III and IV. Confirmation has been obtained of the observation of Gulland and Newton [1932] that norite adsorbs

Table III. *Norite adsorptions. Activity experiments.*

Solution K₃, 19 units; norite, 50 mg.; adsorption for 5 hours at 0°.

p_H of adsorption	Units in eluate	% units recovered	Units in filtrate	Remarks
Eluted by glacial acetic acid.				
11	15	80	0	—
2	15	80	0.5	—
7	13	68	0	—
11	9	47	0	Electrodialysed hormone solution
Eluted by absolute alcohol.				
11	0	—	0	—
Eluted by mixtures of glacial acetic acid and absolute alcohol.				
11	12	63	—	10 % acetic acid
11	12-13	63-68	—	20 % acetic acid
11	13	68	—	50 % acetic acid

Table IV. *Norite adsorptions. Activity—weight experiments.*

Norite, 100 mg.; p_H 11, N/10 ammonia; elution by glacial acetic acid.

Duration of adsorption hours	Units and solution	Units recovered	% units recovered	Units in filtrate	Solid in sol. used mg.	Solid recovered mg.	% solid recovered
5	135 K ₃	105	78	1.2	0.54	0.57	105
1	250 K ₄ *	200	80	0	1.32	1.19	90

* Solution K₄ contained 1000 units per ml. associated with 5.3 mg. of solid.

all the active material from alkaline Kamm solutions and at p_H 2, and that most of the activity may be eluted by acetic acid, but these facts cannot be used to effect further purification than that already recorded because the percentage recovery of solid is slightly higher than the percentage recovery of activity.

(3) *Use of norite and fuller's earth in purification.* Attempts were made to purify the hormone in Kamm solutions or in whole extracts of posterior lobe

a) by successive adsorptions with small quantities of norite at p_H 11; (b) by successive adsorptions with norite at p_H 6 and 11; (c) by adsorption on fuller's earth, elution and re-adsorption on norite. The purifications effected, however, were not sufficiently marked to warrant detailed descriptions, and in the case of whole extracts of posterior lobe the concentration factors ranged from 10 to 20.

The following is a description of an experiment in which adsorption of the hormone on norite, preceded by treatment of the solution with fuller's earth, replaced the customary concentration of posterior lobe extracts under reduced pressure and adsorption of the active material as a protein-ammonium sulphate precipitate. This concentration is troublesome and time-consuming owing to frothing of the solution and to the relatively low temperature at which the distillation must be made (below 40°), and in our hands extraction of the protein-ammonium sulphate precipitate with acetic acid has frequently failed to remove all the active material from the precipitate. The procedure now outlined is therefore of value.

Dried powdered posterior lobe powder (10 g., 15,000 units) was extracted with 0.25% acetic acid (1 litre) for 30 min. on a boiling water-bath, the solid was collected and re-extracted with 0.25% acetic acid (100 ml.). The combined extracts were cooled to 3° , brought to p_H 11 with sodium hydroxide and treated with (Surrey yellow) fuller's earth (5 g.). 15 min. later, the solid was removed by filtration and washed with water (B), and norite (6 g.) was added to the filtrate at 5° . 45 min. later, the norite was collected and washed with water (600 ml.), and the filtrate was treated with norite (A) (2 g.) for 45 min. at 5° . The activity remaining in the filtrate from this adsorption was assayed (C).

The norite fractions were eluted with acetic acid and the following account applies to the first norite fraction. It was eluted twice with glacial acetic acid (150 ml. for the first elution, 50 ml. for the second) and washed with water (50 ml.) and the combined filtrate and washings were evaporated to dryness at $35-40^\circ$ under reduced pressure. The residual solid was dissolved in glacial acetic acid (20 ml.) at 40° and precipitated with dry ether (50 ml.). The precipitate was re-extracted and re-precipitated in the same way, and the resulting solid (D) was washed with ether and dried over phosphoric oxide. The united ether-acetic acid solutions were filtered and mixed in succession with water (0.8 ml.) and with light petroleum (280 ml.).

The solution was decanted from the gum (E) which had separated overnight and deposited a second gummy precipitate (F) during the subsequent 24 hours. These precipitates were washed with light petroleum and dissolved in water and the solutions were brought to p_H 3 with acetic acid.

Assays of activity and determinations of weight of solid were made at suitable stages and are recorded below.

Fraction	Weight mg.	Oxytomic units	Units/mg.
First precipitate (E)	49.2	7,500	152
Second precipitate (F)	7.40	830	112
Second norite fraction (A)	—	320	—
Filtrate from second norite adsorption (C)	—	120	—
Washings from first norite adsorption (B)	—	40	—
Solid dried over P_2O_5 (D)	350	2,960	—
		<u>11,770</u>	

We are greatly indebted to Prof. J. H. Burn who estimated the pressor activity of the first precipitate as being 13% of the oxytomic activity.

C. DIALYSIS EXPERIMENTS.

It has frequently been suggested that the hormone is a base, and the acceptance of this view is encouraged by its susceptibility to attack by nitrous acid [Gulland, 1933] and by the present observation that the activity is destroyed when acetylation is effected under mild conditions.

Section A shows that some of the substances which accompany the hormone in whole extracts of posterior lobe or in purified solutions are peptide in character—probably resembling proteoses or peptones.

The numerous records of the passage of the active material through dialysing membranes indicate that the molecular size of the hormone is small, and it has now been shown that the hormone and the accompanying material not only pass through cellophane at the same rate but also leave little undialysable residue. The molecular size of the accompanying material is therefore also not large.

On the assumptions, therefore, that the hormone is predominantly basic in character and that the concomitant substances are ampholytic, it is theoretically possible to effect a separation by electrodialysis, provided that the active material is not adsorbed on the ampholytes under the experimental conditions. A series of experiments was therefore made on the cataphoresis of hormone solutions in a three-compartment cell under conditions which varied only as regards the p_H value of the centre compartment containing the hormone at the beginning of the experiment (Table V). Fig. 4 shows the effect of changes in the p_H value of the centre compartment on the number of units remaining in it after electrodialysis had proceeded for a standard time.

The hormone did not migrate to the anode under any conditions, and as the p_H values investigated ranged from 1.6 to 12.5, it must be concluded either that the hormone is not an ampholyte and does not contain a "free" carboxyl group, or that throughout the alkaline range the hormone is adsorbed on basic, non-ampholytic material. Experiments are in progress to determine this point.

At p_H values more alkaline than p_H 8.5 the hormone did not migrate, whereas it behaved as a cation at reactions more acid than p_H 6.5. Between these values the curve of Fig. 4 may be an approximate ionisation curve having p_K 7.5. Discussion of the theoretical implications of these facts is postponed until further information is available on the question of the adsorption of the hormone by basic substances (see above).

It was then shown by comparative experiments *ad hoc* that the passage of the hormone through the cellophane membrane is essentially due to the passage of the current and is not the consequence of simple diffusion.

Disregarding temporarily, therefore, the theoretical significance of these experiments, the following practical results are evident. First, at p_H values corresponding to the probable isoelectric points of the proteoses in the acid p_H range the hormone migrates into the cathode compartment.

Second, the hormone remains in the centre compartment at alkaline reac-

Fig. 4. Cataphoresis of oxytocic hormone solutions. Solution K_2 ○—○; solution $K_3/10$ ●—●; solution $K_3D/10$ ×—×.

tions, when the proteose molecules, as anions, should migrate into the anode compartment.

The straightforward electro dialysis of hormone solutions at acid reactions is not practicable as a method of fractionation, because the hormone molecule undergoes reduction at the cathode, with consequent diminution of the activity, which cannot be completely or permanently regained [Gulland and Randall, 1935].

A number of electro dialysis experiments at acid reactions, designed to obviate this difficulty, are described in the experimental section, and studies were also made of electro dialyses at alkaline reactions, in which the hormone did not migrate and was subsequently adsorbed on norite. In this latter connection it was shown for comparative purposes that the proteoses and peptones of "Savory and Moore's peptone" migrated into the anode compartment of the same apparatus from a solution maintained at p_H 10.

The experiments outlined above were unsuccessful in purifying the hormone; in fact, the ratio of activity/solid invariably fell as a result of the dialyses. This was probably due in part to reduction of the hormone molecule at the cathode and in part to destruction of the hormone in the alkaline solutions, for it has been observed that removal of proteose-like material by electro dialysis greatly decreases the stability of the hormone towards alkalis. It is clear that electro dialysis can be used to eliminate inactive substances from hormone solutions, and that the method could be used as a means of purification if the hormone could be protected from simultaneous destruction, but in our opinion the decreased stability of the purified hormone does not strengthen the probability of its isolation in pure state.

Finally, a single attempt was made to separate the oxytomic and pressor substances by electro dialysis at an alkaline reaction. This was unsuccessful because neither hormone migrated to any marked extent. Presumably the pressor hormone is either basic or was adsorbed on basic substances.

EXPERIMENTAL.

Action of acetic anhydride.

A mixture of solution K_3 (0.1 ml.) glacial acetic acid (1.0 ml.) and acetic anhydride (1.0 ml.), added in that order, was diluted to 2.5 ml. with glacial acetic acid and left at room temperature for 2 hours. Water (30 ml.) was added, and the solution was concentrated to 1 ml. at 35–40° under reduced pressure, diluted with water and assayed. The total activity was about 2% of that initially present, and this value remained unaltered after the solution had been kept at p_H 10 for half an hour, showing that hydrolysis did not occur in the testing-bath as a result of its alkalinity.

Dialysis through cellophane.

Bags of No. 400 cellophane were soaked before use in repeated changes of silica-distilled water, and silica vessels were used throughout.

The following experiment shows that the active substance and the solid material present in a Kamm solution pass through the dialysing membrane at approximately the same rate. A mixture of solution K_3 (2 ml.) and 0.1% acetic acid (2 ml. together with some toluene) inside the membrane was dialysed at room temperature against 0.1% acetic acid (4 ml. and toluene). The liquid

outside was changed daily, assays were made, and aliquot portions were dried to constant weight at 110°. The p_H remained constant at 3.5.

	Inside sol. at start	Outside sols.					
		1 day	2 days	3 days	4 days	7 days	Total
No. of units	1800	759	579	245	105	63	1751
Weight (mg.)	7.2	2.86*	2.40	1.04	0.46	0.54†	7.30
Units/mg.	250	260	247	236	228	116	—

* This is a corrected value, solid matter (0.72 mg.) extracted from the cellophane having been present, as shown by a blank determination on a new membrane from the same sample.

† Extraneous solid was seen to be present.

Cataphoresis.

Cell. The cell was of plate glass. The dimensions of each compartment were $4 \times 3 \times 1$ cm.; the dividing membranes consisted of cellophane, No. 400, 0.00126 in. thick, and the electrodes of platinum foil measured 2.5×2.5 cm. Each compartment was cooled by coils containing running water, which maintained the temperature in these experiments at about 18°. The contents of the centre compartment were stirred mechanically.

Solutions used. The preparations used were K_2 (450 units and 24.4 mg. per ml.), a tenfold dilution of K_3 (90 units and 0.36 mg. per ml.), and a solution prepared from $K_3/10$ by simple dialysis through No. 600 cellophane followed by concentration to 90 units per ml. (called $K_3 D/10$).

Procedure. The centre compartment contained the hormone solution (45 units), $M/10$ buffer solution (1.0 ml.) and water, the total volume being 9.0 ml. The anode and cathode compartments contained water (8 ml.) and 0.1 ml. of the basic and acidic constituents of the buffer solution respectively. The current strength was maintained at 50 milliamps. Measurements of the hydrogen ion concentration of the centre and cathode compartments were made colorimetrically. The reaction of the centre compartment was maintained by the addition of sodium hydroxide or sulphuric acid, and carbon dioxide was passed into the cathode solution when necessary. This reduced the alkalinity to p_H 9.5–10 in the experiments in which the centre compartment was at p_H 1.6–8.5 and maintained it at the same p_H value as the centre compartment solution in the experiments at p_H 9.5–12.5. It was not possible to maintain these reactions accurately and the values quoted were at times subject to fluctuations of ± 0.4 p_H unit.

After the experiments had proceeded for 1 hour, the solutions were diluted at p_H 3.5, and were assayed. In the cases of solutions from the centre compartment the contractions were grouped as described by Gulland and Newton [1932], and this procedure was followed whenever possible with the solutions from the cathode compartment. The solutions from the anode compartment never contained more than a trace of activity, too small to be estimated even by single contractions. The results are given in Table V.

In most experiments a few oxytocic units were apparently lost. This discrepancy between experimental and theoretical values may have been due to unavoidable error in the assays, to inactivation by acid or alkali or to electrolytic reduction at the cathode. The loss due to reduction probably became significant in those experiments in which migration to the cathode was extensive.

In an experiment at p_H 3.5–4, similar to those described above but in which 225 units were used, the activity in the centre compartment had fallen to 50 %

Table V. *Cataphoresis of oxytocic hormone.*

45 units used in each experiment. Figures show units present at end.

p_H	Buffer	K_2		$K_3/10$		$K_3 D/10$	
		Centre	Cathode	Centre	Cathode	Centre	Cathode
1.6	Glycine— H_2SO_4	30, 25	5, 5	21	8	—	—
	Citrate— H_2SO_4	—	—	29	7	27	5
3.3	Acetate	23	19	—	—	—	—
5.0	Acetate	19	22	—	—	—	—
6.5	Borate	18	26	—	—	—	—
7.5	Phosphate	35	5	36	Trace	30	2
8.5	Phosphate	42	Trace	—	—	—	—
9.5	Borate	40	1	—	—	—	—
11.5	Glycine—NaOH	44	0.5	40	0.5	46	Trace
12.5	Citrate—NaOH	43	Trace	—	—	—	—

after 1 hour and to 4% after 4 hours, showing that all the active material was capable of passing through the membrane under the conditions of these experiments.

Electrodialysis at acid p_H values.

(i) *In 3-compartment cell using norite in cathode compartment.* Two experiments at acid reactions were carried out in the usual apparatus, the alkaline cathode compartment containing norite; in Exp. 1 the reaction of the liquid in the central compartment was maintained at p_H 6–6.5, the least acid reaction at which migration towards the cathode seemed to occur (Fig. 4), whereas in Exp. 2 the reaction was not controlled and fell from p_H 4 to 3.6.

The anode compartment A contained water (9 ml.) and *N* acetic acid (0.1 ml.). The centre compartment B contained water (9 ml.) and solution K_4 (1 ml., 1000 units, 189 units per mg.), previously evaporated to dryness to remove acetic acid. The cathode compartment C contained water (9 ml.), *N* NaOH (0.1 ml.) and purified norite (100 mg.). The cathode compartment was maintained at p_H 10 by the addition of acetic acid and the temperature was 7–10°. The filtrate from the norite and the anode and centre liquids were adjusted to p_H 3.5; the norite was dried and eluted with 98% acetic acid, the eluate being concentrated and diluted with water for assay. The results, summarised in Table VI, show a fall in the ratio activity/solid weight.

Table VI.

Exp.	Duration of dialysis hours	Current milliamps	Units in				Units per mg. in norite eluate
			Centre	Filtrate from norite	Anode	Norite eluate	
1	2.5	100	625	Nil	Nil	80	178
2	2	44–80	Trace	Nil	Nil	205	105

(ii) *Using additional compartments.* The object of these experiments was to interpose between the cathode and the hormone cations a compartment of which the reaction was maintained at an alkalinity such that the ionisation of the hormone cations of the original acidic solution would be depressed. The molecules would therefore not migrate further to the cathode but would remain in the additional compartment, into which the peptide molecules would not have penetrated.

(a) *A 4-compartment cell* similar to that already described, was constructed with the anode and cathode in the outside compartments. The compartments

were filled as follows: A (anode) and C, *N*/100 sodium acetate (10 ml.); B, solution K_4 (0.5 ml., 500 units) and water (9.5 ml.); D, *N*/100 NaOH (10 ml.). Dialysis proceeded at 50 milliamps for 2 hours, B being maintained at p_H 6–7, C at p_H 9–11. The solutions were assayed as follows: B, 412 units; C, 51 units. Evidently little migration occurred, probably owing to the p_H value of 6–7 being insufficiently acidic (compare previous experiment immediately above).

(b) *8-compartment cell*. The compartments were filled as follows: A (anode) and B, *N* acetic acid (1 drop) and water (10 ml.); C, solution K_2 (0.5 ml., 225 units) and water (9 ml.); D, F, G, H, I (cathode), *N* ammonia (1 drop) and water (10 ml.). The dialysis was carried out for 20 hours at 5 milliamps. The p_H values of the compartments were finally: A and B, 2; C, 3.4; D, 4.2; F, 5.0; G, 5.8; H, 11; I, 13. A small amount of solid separated on the membrane of compartment C on the cathodic side; this was collected and dissolved in very dilute acetic acid (solution Y). Assays of the solutions gave the following results:

Solution	A	B	C	D	F	G	H	I	Y
Units	0	0.5	10	1	0	0	7	14	16

(iii) *In 4-compartment cell combined with norite adsorption*. The anode compartment (A) contained *N*/100 acetic acid (10 ml.). Compartment B contained solution K_4 (100 units per ml.; 189 units per mg.) which had been previously evaporated to dryness (8 ml.). Compartment C contained water (8 ml.), norite, and 1 drop of *N* NaOH. Compartment D contained *N*/100 NaOH (10 ml.). During the dialysis the temperature remained at 15° and the p_H of B fell by about 2 units to 3.6. Compartment C was maintained at p_H 11 by the addition of NaOH. The norite was collected, washed, eluted twice with 98% acetic acid, and the combined eluates were concentrated and diluted with water. The various solutions were assayed (Table VII).

Table VII.

Duration of dialysis hours	Current milliamps	Units at start	Amount of norite mg.	Units contained in				Norite
				A	B	C	D	
1.5 } 1 } 2.5 } 3.0 }	55 } 100 } 100 } 60 }	1000 275 1000	100 50 100	— Nil Nil	0.6 Trace 2	4.5 Nil 6	3.2 Nil 2	610* 125 517†

* 100 units per mg.

† 142 units per mg.; the cellophane membranes used in this experiment had been soaked in water for several days before use.

Electrodialysis at alkaline p_H values combined with norite adsorption.

Solution K_4 (1 ml., 1000 units, 5.30 mg., 189 units per mg.) was evaporated to dryness with water, diluted with water and submitted to electrodialysis in the centre chamber of a 3-compartment cell. The p_H of the centre compartment was maintained at 11 by means of NaOH, the temperature being 9°. Water was placed in the cathode chamber and also in the anode chamber, through which a continuous slow stream of water was passed to remove any material which entered the chamber. After the dialysis, the active material was adsorbed from the centre solution by norite, leaving no activity in the filtrate, and the norite was washed and eluted twice with 98% acetic acid. The eluates were evaporated to dryness under reduced pressure, and the residue was taken up in water for assay and for weight determination in an aliquot part, dried at 110°.

The amount of solid recovered was variable, and owing to the loss of activity the ratio activity/solid weight fell in each case (Table VIII). Recombination of the solutions from the three compartments did not restore the activity.

Table VIII.

No. of exp.	Duration of dialysis hours	Current milliamps	Weight of norite mg.	Units recovered	Solid recovered mg.	Units mg.
1	3.5	100	150	500	2.85	175
2	1.5	100	120	666	5.30	124
3	4.5	50	200	600	4.66	128
4	3.0	100	250	267	2.40	110

Before being used the cellophane membranes were soaked for several days in repeated changes of silica-distilled water, and in Exps. 2 and 4 the membranes were used in a blank experiment with acetic acid lasting $2\frac{1}{2}$ hours, in the hope of eliminating water-soluble material. Exp. 4 was carried out at 4° and p_H 9.5–10, a cellophane membrane being used on the cathode side of the centre compartment and a membrane of tough filter-paper on the anode side. It was hoped that these modifications would diminish the loss of activity and allow greater ease of passage of the proteose-like material to the anode. The anode and cathode compartments contained 12 and 13 units respectively.

Electrodialysis of solution containing oxytocic and pressor substances.

An extract of posterior lobe was concentrated and precipitated by ammonium sulphate and the precipitate extracted with acetic acid, as described by Kamm *et al.* [1928]. This extract (4.5 ml., containing 1080 oxytocic and the same number of pressor units) was evaporated to dryness, dissolved in water and electrodialysed in the centre-compartment of a 3-compartment cell for 3 hours at 50 milliamps and 10° . The centre compartment (B) was maintained at p_H 10–11, and the alkalinity of the cathode compartment (C) was reduced to the same value by means of a stream of carbon dioxide. The activities of these solutions and of the anode (A) liquid were assayed as follows. We are greatly indebted to Prof. J. H. Burn for making the pressor assays.

Compartment	...	A	B	C
Oxytocic units		3	610	47
Pressor units		2–2.5	1004	—

SUMMARY.

1. The ultraviolet absorption spectra of posterior lobe extracts and of purified hormone solutions, as at present available, are not characteristic of the hormone molecule but correspond to the substances which accompany it.

2. The nature of the spectra and their comparison with those of proteoses and peptones lead to the conclusion that the substances accompanying the hormone are peptide in character.

3. The effects of the following adsorbents on hormone solutions have been examined—silica gel, aluminium hydroxide $C\gamma$, fuller's earth, acid-treated talc, ignited talc, asbestos, purified norite.

4. The purer hormone solutions available were not purified further by these reagents.

5. An outline is given of a technique in which the concentration under diminished pressure and adsorption on a protein-ammonium sulphate precipitate, commonly used in purifying whole, aqueous extracts of posterior lobe, are replaced by adsorptions with fuller's earth and norite.

6. The hormone is inactivated by acetic anhydride.

7. At reactions more alkaline than p_H 8 the hormone remained in the centre compartment of an electrodialysis cell, whereas at reactions more acid than p_H 6 it migrated towards the cathode.

8. Electrodialysis under very varied conditions was not successful in raising the activity/weight ratio of purified hormone solutions, since removal of the proteose-like substances reduced the stability of the hormone towards alkalis.

9. Oxytocic and pressor hormones were not separated by electrodialysis at p_H 10–11.

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