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NEUROHYPOPHYSIAL HORMONES OF NON-MAMMALIAN VERTEBRATES

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Extracts of non-mammalian neurohypophyses, when tested in mammals or on mammalian tissues, produce the same pharmacological effects as mammalian posterior pituitary preparations. Table 1 shows the phyletic distribution of these activities. Relative to their oxytocic, pressor and antidiuretic potencies in mammals, however, non-mammalian extracts have been shown (Heller, 1941*b*; Lazo-Wasem & Weisel, 1952; Sawyer, 1957) to exert a much greater effect on the water balance of frogs. It was therefore suggested (Heller, 1941*b*) that non-mammalian vertebrates secrete a hormone which is different from, but chemically closely related to, oxytocin and vasopressin. Heller & Lederis showed in 1958 that small amounts of neurohypophysial peptides in crude extracts could be separated by paper chromatography and it was therefore decided to re-examine the earlier work on lower vertebrates.

The experiments to be described deal mainly with the chromatographic and pharmacological characterization of the active peptides in pituitary extracts from teleost fishes, amphibians, reptiles and birds. A peptide not demonstrable in mammalian neural lobes was found, and the second part of the paper describes attempts to purify it and to identify its chemical constitution. Preliminary accounts of some of these results have already been given (Pickering & Heller, 1959; Heller & Pickering, 1960).

Nomenclature. The term oxytocin is used in reference to the octapeptide characterized by du Vigneaud, Ressler & Trippett (1953) and Tuppy (1953). Konzett & Berde (1959) suggested that other active neurohypophysial peptides and their synthetic analogues should be expressed as derivatives of oxytocin. Thus arginine vasopressin, for example, becomes phe^3- arg^8-oxytocin. This nomenclature has been adopted in the present paper.

METHODS

Preparation of extracts

Pituitary glands were obtained from representatives of the following vertebrate classes: *Marine teleost fishes.* Pollock (*Pollachius virens*). A powder obtained by lyophilizing whole pituitaries was used. The fishes were collected and dissected by Dr Grace Pickford and her

TABLE 1. Phyletic distribution of pharmacological activities in vertebrate neurohypophyses

Class	Species	Oxytocic	Galactobolic*	Mammalian pressor	Mammalian antidiuretic	Frog water-balance
Elasmobranch fishes	Skate	+ (Herring, 1915; Hogben & de Beer, 1925)	+ (Herring, 1913)	− (Herring, 1913; Hogben & de Beer, 1925	+	+
	Dogfish				(Heller, 1941*a*)	(Heller, 1941*b*)
Teleost fishes	Cod	+ (Hogben & de Beer, 1925)	+ (Herring, 1913)	+ (Herring, 1913)	+ (Heller, 1941*a*)	+ (Boyd & Dingwall, 1939)
Amphibians	Frog	+ (Hogben & de Beer, 1925)	Not investigated	+ (Herring, 1913)	+ (Heller, 1941*a*)	+ (Heller, 1941*b*)
	Toad			(Herring, 1913)	(Jørgensen, 1950)	(Jørgensen, 1950)
Reptiles	Tortoise	+ (Hogben & de Beer, 1925)	Not investigated	+ (Herring, 1913)	+	+
	Grass snake			(Heller, 1942)	(Heller, 1942)	(Heller, 1942)
Birds	Fowl Duck	+ (Hogben & de Beer, 1925)	+ (Mackenzie, 1911)	+ (Herring, 1915)	+	+
	Pigeon				(Heller, 1941*a*)	(Heller, 1941*b*)
Mammals	All species investigated	+ (Dale, 1909)	+ (Ott & Scott, 1910)	+ (Oliver & Schafer, 1895)	+ (von den Velden, 1913)	+ (Brunn, 1921)

+ = present; − = not demonstrated; * = milk ejection.

assistants working under a grant from the U.S. National Science Foundation (NSF G-7262). The pituitaries were deep-frozen and sent to Professor A. E. Wilhelmi who treated them as follows: The glands were thawed in the cold room, transferred to the lyophilizer and cooled to $-20°$ C. After evacuation of the chamber, the material was warmed to a final operating temperature of 23° C. The dry powder was crushed and dispatched by air. 750 g fresh weight of pollock pituitaries gave 122 g dry powder.

Elasmobranch fishes. Two species of dogfish (*Squalus acanthias* and *Scyliorhinus caniculus*).

Fresh-water teleost fishes. Trout (*Salmo iridens*).

Amphibians. Frog (*Rana temporaria*) and toad (*Bufo bufo*).

Reptiles. Tortoise (*Testudo graeca*) and grass snake (*Tropidonotus natrix*).

Birds. Fowl (*Gallus domesticus*) and pigeon (*Columba palumbus*).

Fresh glands were obtained from all the above vertebrates. The neuro-intermediate lobes were dissected from all but the trout, from which whole pituitary glands were used.

Acetic acid extracts. The fresh glands or the dry powder obtained from pollock pituitaries were extracted with 0·25 % (v/v) acetic acid as described by Heller & Lederis (1959).

Assay methods

Oxytocic assay. Heller & Lederis's (1959) modification of the pharmacopoeial procedure (British Pharmacopoeia, 1958) was used.

Vasopressor assay. Dekanski's (1952) modification of Landgrebe, Macaulay & Waring's (1946) method was used, but dibenyline was injected instead of dibenamine.

Antidiuretic assay. The method of Jeffers, Livezey & Austin (1942), employing intravenous injections into ethanol-anaesthetized rats, was used.

Galactobolic (milk-ejection) assay. The method of Cross & Harris (1952) and the recording system described by Fitzpatrick (1960) were used.

Water-balance assay. The method was essentially that introduced by Heller (1941*b*). Frogs were kept during the night in separate jars each containing water to a depth of about 1 in. (2·5 cm). Next morning the water was changed, the frogs' urinary bladders were emptied, and the animals were weighed to the nearest 0·05 g. Thereafter the bladders were emptied and the frogs weighed at hourly intervals. Injections were given into a ventral lymph sac 3 hr after the first weighing. The experiment was continued until the animals no longer increased in weight. Since Boyd & Brown (1938) had found that the rate of water uptake is influenced by changes in temperature, the experiments were carried out at 18° C in a thermoregulated room.

Responses to hormones were expressed as percentage increase in body weight. The unit of water-balance activity was originally defined (Heller, 1941*b*) as the activity of 0·5 mg International Standard Powder. However, since the vasopressin in the standard powder also exerts some water-balance activity, synthetic oxytocin (Syntocinon; Sandoz) was used as standard. In other words, the unit is now defined as the (frog) water-balance activity exerted by one (rat uterus) unit of Syntocinon.

Hen uterus (shell gland) assay. A laying hen was killed by decapitation and the contractions of a strip of the shell gland were recorded. The following suspension fluid was found most suitable (Heller & Lederis, 1960) (g/l.): NaCl, 6·6; KCl, 0·42; $CaCl_2$, 0·01; NaH_2PO_4, 0·1; Na_2HPO_4, 0·1; $NaHCO_3$, 2·5. The bath solution was maintained at 40° C.

Standard preparations. Pitressin (Parke, Davis and Co.); synthetic oxytocin (Syntocinon, Sandoz); phe^3-arg^8-oxytocin (arginine vasopressin, prepared from ox neurohypophyses by paper chromatography). All these preparations were standardized against the third International Standard Powder.

Index of discrimination. The calculation employed was a modification of that suggested by Gaddum (1955). The potencies of two substances were determined in terms of a standard

preparation and the index of discrimination was taken to be the ratio of these two potencies. If the two substances in question are identical, the index of discrimination will be unity whatever assay method is used.

Chemical methods

Paper chromatography. Both ascending and descending techniques were used. Solvents employed for the development of paper chromatograms were: (*a*) butanol–acetic acid–water (4:1:5 by vol.); (*b*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4 by vol.); (*c*) cellosolve–ammonium sulphate–water (24:20:56 by wt.); (*d*) α-picoline–water (6:4 by vol.). Two staining methods were used. The chromatograms were sprayed with 0·2 g ninhydrin/100 ml. butanol and then dried and heated to reveal the spots, and/or stained according to the method of Reindel & Hoppe (1954) as described by Heller & Lederis (1958). The peptides were eluted with NaCl solution 0·9 g/100 ml. The dried paper was cut into horizontal strips and each of these was again cut into small pieces which were placed in numbered tubes. An appropriate volume of NaCl solution was added to each tube and the contents thoroughly mixed. After filtering, half of the volume initially used was again added; the pieces of paper were finally squeezed over the filter.

Preliminary purification of pollock pituitary extracts. The procedure used was essentially that introduced by Acher, Light & du Vigneaud (1958) for the separation of mammalian neurohypophysial peptides. Sodium chloride was added to acetic acid extracts (pH 4·0) of pollock pituitary powder to a final concentration of 25 g/100 ml. The active protein complex precipitated was suspended in a minimal volume of water (about 10 ml.) and transferred to a dialysis bag. The salts, amino acids and free peptides were removed from the precipitate by dialysis against several 200 ml. portions of water for a total period of 3 hr. The precipitate was washed from the bag with, and dissolved in, acetic acid 0·25 % (v/v), and the active peptides were dissociated from the protein complex by the addition of trichloroacetic acid (TCA) to a final concentration of 10 g/100 ml. The precipitate obtained by this treatment was extracted with 0·25 % (v/v) acetic acid and re-precipitated with TCA. This procedure was repeated twice. The combined supernatants were added to Amberlite IR-45 resin (Standard grade) in the acetate form (3 g resin/5 ml. supernatant), and stirred until the pH rose to about 3·9; the resin was then removed by filtration. The filtrate was a solution of the active peptides free of TCA.

Ion-exchange chromatography. The partially purified solution of active peptides from pollock pituitaries was added to a 10 × 1 cm column of Amberlite CG-50 (type II) in the hydrogen form. 0·1 M ammonium acetate (pH 5·0) was run through the column until the eluate had a pH of 5·0. The peptides were eluted from the column by a gradient of concentration and pH produced by the gradual introduction of 0·5 M ammonium acetate (pH 7·7) to a 50 ml. mixing chamber (Bock & Ling, 1954) containing the 0·1 M buffer; 2 ml. fractions of the eluate were collected with a Shandon automatic fraction collector.

Spectrophotometry. The optical densities of the eluates from the ion-exchange column were measured at 240 mμ in a Unicam S.P. 500 spectrophotometer using 5 mm cells.

Peptide hydrolysis. Approximately 1 mg of freeze-dried *highly purified peptide* (see p. 104) was placed in a thick-walled tube and 2 ml. of 6 N-HCl added. The air in the tube was displaced with nitrogen, the tube sealed and incubated for 48 hr at 110° C. The acid was removed from the hydrolysate by successive dilution and evaporation to dryness *in vacuo*.

Inactivation with sodium thioglycollate. Vogt's (1953) modification of the procedure of Ames & van Dyke (1951) was used.

RESULTS

Pharmacological activity of crude (acetic acid) extracts of non-mammalian neurohypophysial tissues

Quantitative estimations of the potency of extracts of non-mammalian neurohypophyses have not been carried out since Mackenzie (1911), Herring (1913), Hogben & de Beer (1925) and Heller (1941*a*, *b*, 1942) established that glandular extracts from all vertebrate classes produce the same qualitative effects as the mammalian posterior pituitary hormones. Oxytocic, pressor, antidiuretic and frog water-balance activities were therefore assayed in simple extracts of pituitary glands from teleost fishes, amphibians, reptiles and birds.

TABLE 2. Mammalian oxytocic and pressor potency of extracts of fresh pituitary glands from non-mammalian vertebrates

Species	No. of expts.	No. of glands	Mean activity (m-u./gland) Oxytocic (*O*) (isolated rat uterus)	Mean activity (m-u./gland) Pressor (*P*) (rat blood pressure)	*P/O*
Trout	2	36	81	75	0·93
Frog	6	383	32	13	0·25
Toad	1	24	45	42	0·94
Tortoise	1	6	58	37	0·64
Grass snake	2	22	365*	315*	0·86
Fowl	2	9	268	245	0·92
Pigeon	1	24	32	28	0·88

* Per 100 g body weight.

Extracts of pollock pituitary powder. Acetic acid extracts of this material were found to have the following mean potencies (m-u./mg dry powder) in five experiments: oxytocic activity, 604 ± 134; pressor activity 605 ± 74; antidiuretic activity, 577 ± 158; water-balance activity (one experiment only), 30,700. Referred to the oxytocic potency, the ratio of these activities was therefore 1·0 : 1·0 : 0·96 : about 50. The ratio antidiuretic activity : water-balance activity agrees well with that of 48 previously (Heller, 1941*b*) obtained in another teleost, the cod. All these activities were abolished by sodium thioglycollate.

Extracts of fresh glands from other vertebrates. Pollock pituitaries were the only ones available in quantity as a lyophilized powder, and extracts of freshly dissected glands from other vertebrates were therefore used. Table 2 shows the results of these assays.

Paper chromatography of non-mammalian pituitary extracts

Extracts of pollock pituitary powder. When pollock extracts were chromatographed on paper in the system butanol–acetic acid–water,

oxytocic activity could be eluted from two regions. The faster-moving substance was found (7 experiments) between R_F 0·5 and 0·6, i.e. in the same range as oxytocin. The slower-moving oxytocic substance was found at R_F 0·25–0·35 in all experiments, but some oxytocic activity was usually also found at an even lower R_F. The pressor and antidiuretic actions of the extracts were mainly associated with the slower-moving oxytocic substance.

In an attempt to discover whether the oxytocic and pressor effects from the lower region of unidimensional chromatograms were due to a single substance, acetic acid extracts of pollock pituitary powder were also subjected to two-dimensional paper chromatography. The paper was first

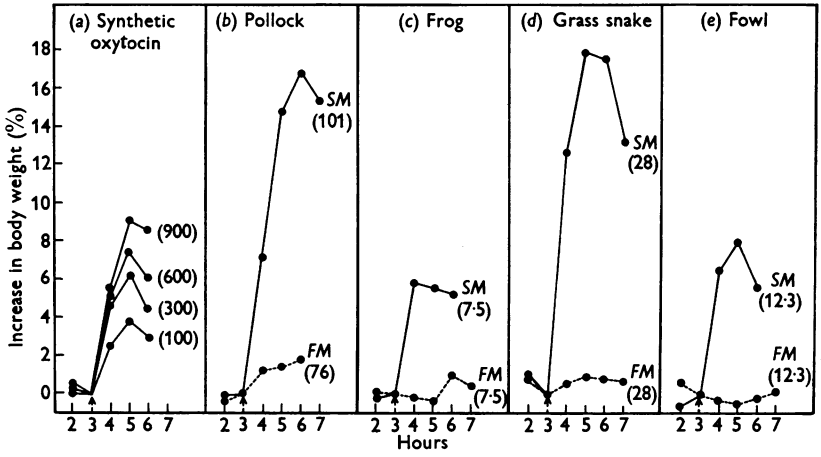

Fig. 1. Comparison of the water-balance potency of the fast-moving (*FM*) and slow-moving (*SM*) oxytocic substances (see text) from non-mammalian pituitary extracts with that of synthetic oxytocin. The results shown are the mean responses of five or six frogs. The figures in parentheses give the doses (m.-u./frog) in terms of (isolated) rat uterus activity. Injections at arrows. Note the disproportion between the oxytocic and the water-balance effect of *SM*.

developed with butanol–acetic acid–water (descending) and then with cellosolve–ammonium sulphate–water (ascending) at right angles to the first development. Oxytocic activity could still be eluted from two regions only. Pressor activity was again associated with the oxytocic substance which moved more slowly in both solvent systems. This suggested that the pollock pituitary extracts did not contain any arginine- or lysine-vasopressin, but indicated the presence of an unknown neurohypophysial peptide with a more pronounced oxytocic potency than the vasopressins. Figure 1*b* shows that the water-balance activity of the pollock gland extracts was mainly due to this unknown peptide.

Extracts of fresh glands from other vertebrates. Table 3 shows chromatographic results obtained with extracts of the pituitaries of: a fresh-water

teleost fish (trout), two suborders of anurans (frogs and toads), two orders of reptiles (tortoise and grass snake) and two species of birds (fowl and pigeon). In all these extracts two oxytocic substances were again found which could be eluted from the same regions as in the chromatograms of the pollock pituitary preparations. The pressor and most of the water-balance (Fig. 1) activities were again eluted from the same region as the 'slow-moving' oxytocic substance.

Purification of pollock pituitary extracts

After preliminary purification (see Methods), the two 'oxytocic' substances in the pollock pituitary extracts were separated by ion-exchange chromatography. Figure 2 shows the elution curves. Eluates from the first

TABLE 3. Chromatographic characteristics in the system butanol–acetic acid–water (4:1:5 descending) of the active principles in extracts of non-mammalian neurohypophysial tissue

Class and species	No. of expts.	R_F of oxytocic substances		R_F of pressor substance
		'Fast-moving'	'Slow-moving'	
Teleost fishes				
(*a*) Trout	1	0·53–0·69	0·26–0·38	0·26–0·38
(*b*) Pollock	7	0·50–0·60	0·25–0·35	0·25–0·35
Amphibians				
(*a*) Frog	3	0·50–0·55	0·25–0·35	0·25–0·35
(*b*) Toad	1	0·55–0·65	0·30–0·35	0·30–0·35
Reptiles				
(*a*) Tortoise	2	0·50–0·65	0·25–0·35	0·25–0·35
(*b*) Grass snake	2	0·50–0·60	0·25–0·35	0·25–0·35
Birds				
(*a*) Fowl	2	0·50–0·60	0·25–0·35	0·25–0·35
(*b*) Pigeon	1	0·55–0·60	0·30–0·35	0·30–0·35

active peak (maximum at tube 30), which was in the position of mammalian oxytocin (Acher *et al.* 1958), showed only oxytocic activity. Eluates from the second peak (maximum at tube 80) gave both oxytocic and pressor effects. With the proviso that the contents of every tenth tube only were assayed, the ultra-violet absorption curve resembles the curve of pharmacological activities, in that a single peak was obtained in the same region (tube 80) as for the 'oxytocic-pressor' peptide. There were, however, two peaks in the region of oxytocin, suggesting greater impurity.

The eluates from the region of the second active peak (tubes 75–95) were freeze-dried, dissolved in 2 ml. 0·25 % (v/v) acetic acid and further purified by paper chromatography in butanol–acetic acid–water. The active peptide was eluted with 0·25 % (v/v) acetic acid and lyophilized. This preparation will henceforth be referred to as the '*highly purified pollock peptide*'.

In one experiment the elution gradient was increased by the introduction of 0·75 M ammonium acetate (pH 7·7) to the mixing chamber, after the second active peak had emerged from the column. This procedure (Acher *et al.* 1958) would elute phe^3-arg^8-oxytocin (arginine vasopressin), but no further activity could be eluted.

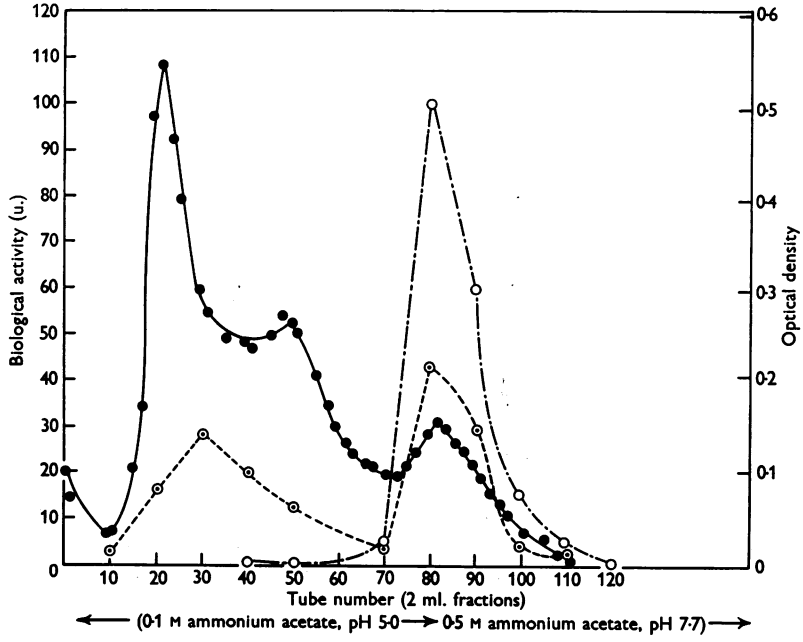

Fig. 2. Chromatography of pollock pituitary extracts on Amberlite CG-50. Elution curves: ●—● optical density at 240 mμ; ⊙···⊙ oxytocic activity; ○– – –○ pressor activity. For further details see text.

Identification of the slow-moving peptide in pollock pituitary extracts

The results so far reported suggested that the non-mammalian pituitary extracts contained a peptide which resembles oxytocin and at least one other peptide which, because of its pronounced oxytocic and frog water-balance activities, differed from phe^3-lys^8-oxytocin and phe^3-arg^8-oxytocin, i.e. from lysine- and arginine vasopressin.

While this work was in progress, Munsick, Sawyer & van Dyke (1959) reported that the pharmacological effects produced by extracts of fowl neurohypophyses were compatible with the assumption that such extracts contained oxytocin and arg^8-oxytocin (arginine vasotocin), an octapeptide synthesized by Katsoyannis & du Vigneaud (1958). The slow-moving pollock peptide was therefore compared with synthetic arg^8-oxytocin, which was kindly provided by Professors van Dyke and du Vigneaud. The two peptides were chromatographed, side by side, in three solvent systems and Fig. 4

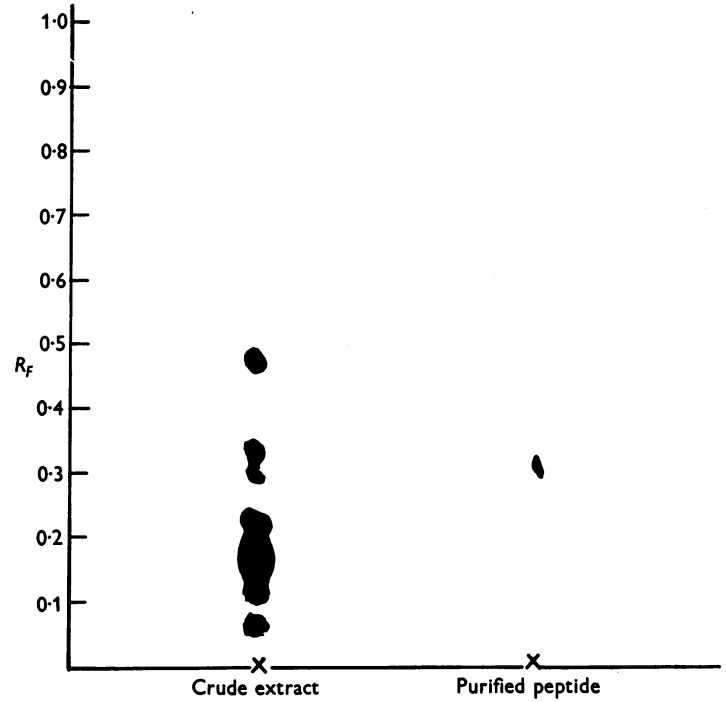

Fig. 3. Descending paper chromatograms, in butanol–acetic acid–water, of crude pollock extract and the *highly purified pollock peptide* (see text) dissolved in 0·25 % (v/v) acetic acid. Pressor activity of volumes of solutions applied: crude extract, 100 m-u.; solution of highly purified peptide, 700 m-u. Staining as described by Heller & Lederis (1958).

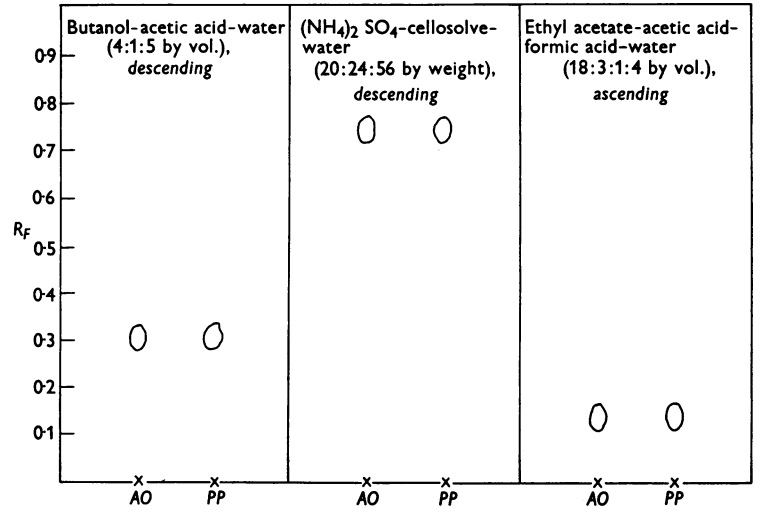

Fig. 4. Synthetic arginine^8-oxytocin (*AO*) and the *highly purified pollock peptide* (*PP*) were chromatographed, side by side, in three solvent systems. The figure shows drawings of the results.

shows that the R_F values for the two substances were the same in each solvent.

A sample of the *highly purified pollock peptide* was hydrolysed, and the hydrolysate was subjected to two-dimensional chromatography in (*a*) butanol–acetic acid–water (descending) and (*b*) α-picoline–water (ascending) at right angles. A mixture of the constituent amino acids of arg^8-oxytocin was chromatographed in the same manner. Both chromatograms were first stained with ninhydrin and then by the method of Reindel

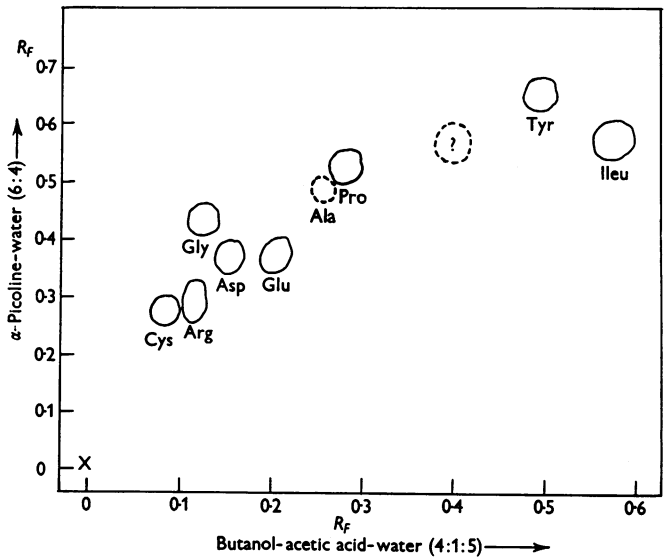

Fig. 5. Drawing of a two-dimensional paper chromatogram of a hydrolysate of a sample of the highly purified pollock peptide. The amino acids of arginine^8-oxytocin are as follows: $(Cys\text{-})_2$, Arg, Gly, Asp, Glu, Pro, Tyr, Ileu. All these amino acids were found in the hydrolysate, which showed in addition a faint spot corresponding to alanine and another, not stained by ninhydrin, probably due to a peptide fragment.

& Hoppe (1954). The hydrolysate contained all the amino acids of arg^8-oxytocin, but two additional 'spots' were found, a faint one in the position of alanine and another, not revealed by ninhydrin, which was probably due to a peptide fragment (Fig. 5). Three such experiments were done.

Pharmacological potency of the highly purified pollock peptide

Table 4 shows the potency of our purest preparation of the slow-moving peptide assayed against arginine vasopressin and oxytocin. The highly purified pollock peptide was also compared with synthetic arg^8-oxytocin (arginine vasotocin) and the results are shown in Table 5. The pollock peptide could not be differentiated from arg^8-oxytocin by any of the assay methods used: the index of discrimination was near unity in every instance.

The similarity of the potencies per microgram of the pollock peptide and synthetic arg^8-oxytocin suggests that the former was almost pure. Comparison of the frog water-balance potencies of the two peptide preparations was attempted in only one experiment, but Fig. 6 shows that the potencies were similar.

TABLE 4. Pharmacological potency of the '*highly purified pollock peptide*' (see text). Each value is the result of a (2+2) assay

	Potency (m-u./μg) of pollock peptide compared with	
Assay method	Arginine vasopressin	Oxytocin
Blood pressure (rat)	77; 67; 70	—
Antidiuresis (rat)	70; 72	—
Rat uterus	—	47; 33; 33
Milk ejection (rabbit)	—	112; 124; 120
Water-balance (frog)	—	3500; 2300; 2000
Hen uterus	1920; 2160	—

TABLE 5. Pharmacological comparison of the slow-moving peptide isolated from pollock pituitary extracts with synthetic arg^8-oxytocin. Each value is the result of a (2+2) assay

Assay method	Pollock peptide (m-u./μg)	Arg^8-oxytocin (m-u./μg)	Index of discrimination of means
Blood pressure (rat)	77; 67; 70	82; 67; 74	0·96
Antidiuresis (rat)	70; 72	76; 84	0·89
Rat uterus	47; 33; 33	48; 33	1·0
Hen uterus	1920; 2160	2000	1·03

Experiments with elasmobranch pituitary extracts

Perks, Dodd & Dodd (1960) found that neurohypophysial extracts from several species of elasmobranch fishes exerted greater milk-ejection effects than would be expected from their oxytocic activities. They suggested, therefore, that elasmobranchs elaborate an oxytocic principle which differs from oxytocin. They also confirmed the earlier finding (Heller, 1941*a*) that the antidiuretic activity of these extracts is very low.

When elasmobranch pituitary extracts were chromatographed on paper in butanol–acetic acid–water (descending), oxytocic activity could be eluted from two regions. One of these was at the R_F of oxytocin (0·5–0·6) and the other at R_F 0·35–0·45. Four such experiments were done, two with *Squalus acanthias* and two with *Scyliorhinus caniculus* pituitary extracts.

The water-balance activity of dogfish pituitary extract was assayed in two experiments and found to be approximately 40 m-u./gland. The ratio water-balance activity:rat-uterus activity was about 4. These extracts therefore seemed to contain a principle which has more water-balance activity than oxytocin, but is much less potent than arg^8-oxytocin.

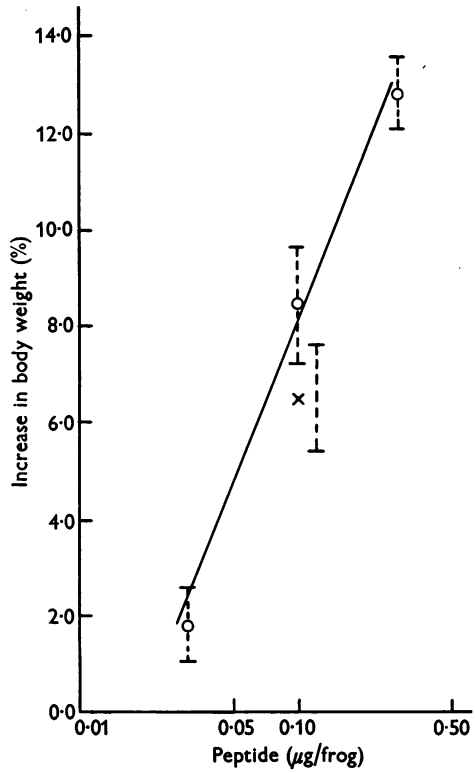

Fig. 6. Comparison of the water-balance potency of the highly purified pollock peptide (○) with that of synthetic arginine^8-oxytocin (×). Lack of the latter material prevented further experiments. Each point shows the mean and S.E. for 5 frogs. The vertical line indicating S.E. of the results with arg^8-oxytocin is displaced to the right for technical reasons.

DISCUSSION

The results presented suggest that a peptide resembling oxytocin is widely distributed throughout the vertebrate phylum. Chromatographic and pharmacological evidence for its presence was found in two species of elasmobranch fishes, a species of fresh-water and one of marine teleosts, frogs and toads, snakes and tortoises, fowls and pigeons, and in mammals. It has been shown to be oxytocin in the fowl (Acher, Chauvet & Lenci, 1960) but its identity in other vertebrate classes has yet to be established. Neurohypophysial extracts from teleosts, amphibians, reptiles and birds were also shown to contain another active peptide which in a teleost fish, the pollock, has been identified as arginine^8-oxytocin (arginine vasotocin). The independent pharmacological observations of Sawyer, Munsick & van Dyke (1959) are in full agreement with these results.

No evidence could be obtained for the presence of arg^8-oxytocin in extracts of elasmobranch pituitaries. These extracts contained two oxytocic principles, one with the chromatographic characteristics of oxytocin and the other differing, both chromatographically and pharmacologically, from all identified neurohypophysial peptides.

Arg^8-oxytocin accounted for the bulk of the pressor-antidiuretic and frog water-balance activity of pollock pituitary extracts. The pressor-antidiuretic and frog water-balance potency of the other non-mammalian

TABLE 6. Phyletic distribution of neurohypophysial hormones

Vertebrate class	No. of species investigated	Oxytocin (and peptides resembling oxytocin)	Phe^3-arg^8-oxytocin (arginine vasopressin)	Phe^3-lys^8-oxytocin (lysine vasopressin)	Arg^8-oxytocin (arginine vasotocin)	Unknown peptide
Mammals						
(*a*) Domestic pig and hippopotamus*		+	−	+	−	−
(*b*) All others investigated	13	+	+	−	−	−
Birds	2	+	−	−	+	−
Reptiles	3	+	−	−	+	−
Amphibians	4	+	−	−	+	−
Teleost fishes	2	+	−	−	+	−
Elasmobranch fishes	2	+	−	−	−	+
Cyclostomes†	1	−	−	−	+	−

Note: − = absent or not investigated; * see Heller & Lederis (1960); † see Sawyer *et al.* (1959).

TABLE 7. Comparison of the potency of neurohypophysial hormones (m-u./μg), assayed against the third International Standard Powder

Assay method	Arg^8-oxytocin (arginine vasotocin). Mol.wt. = 1036	Phe^3-arg^8-oxytocin (arginine vasopressin). Mol.wt. = 1056	Phe^3-lys^8-oxytocin (lysine vasopressin). Mol.wt. = 1028	Oxytocin. Mol.wt. = 993
Rat uterus	40	20–25[1]	15–20[1]	360[2], 415[3]
Blood pressure (rat)	71	400–450[1]	270–340[1]	< 10[1], 3·8[3]
Antidiuresis (rat)	71	400–450[1]	110–140[4]	Diuretic[5]
Milk ejection (rabbit)	119	70–80[1]	50–60[1]	371[3]
Hen uterus	1630	320[1]	29[1]	29[1]
Water-balance (frog)	2600	—	—	(360)
Urinary bladder (frog)	7800[2]	21[2]	< 5[2]	360[2]

[1] van Dyke (1959); [2] Sawyer (1960); [3] Berde, Cerletti & Konzett (1960); [4] Thorn (1959); [5] Berde & Cerletti (1956); Brunner, Kuschinsky & Peters (1956); Peters (1959).

pituitary extracts, with the exception of elasmobranchs, was also mainly due to a peptide with the chromatographic and pharmacological characteristics of arg^8-oxytocin.

Table 7 compares the potencies per microgram of arg^8-oxytocin with those of the mammalian hormones. Since the molecular weights of all

these peptides are much the same (Table 7), the ratio of the activities per microgram virtually equals the ratio per molecule. While many of the results shown are only approximate, the table indicates clearly that, per molecule, arg^8-oxytocin has much greater effects on the hen uterus and frog water-balance than the mammalian hormones. In other words, the non-mammalian tissues show a marked specificity for the endogenous peptide.

It is known that the water balance of frogs is more strongly affected by oxytocin than by vasopressin (Heller, 1930) whereas the reverse applies in the toad (Jørgensen, 1950; Ewer, 1951). This led to the suggestion that the neurohypophyses of these anuran suborders contained different hormones. The results now presented, however, support the assumption that both suborders elaborate the same peptide, namely arg^8-oxytocin, which may therefore be regarded as the amphibian *water-balance principle* postulated by Heller (1941*b*). Arg^8-oxytocin is intermediate structurally between oxytocin and arginine vasopressin (phe^3-arg^8-oxytocin), differing from each of these hormones by a single amino acid. It is therefore not surprising that amphibians respond to the mammalian hormones, but it may be conjectured that the 'fit' of the peptide molecules to the receptors varies in different anurans.

There is good evidence that the water-balance principle is of physiological importance. The pituitary gland of frogs is depleted of the water-balance principle when the animals are dehydrated (Levinsky & Sawyer, 1953; Jancsó, 1955) and it may also be assumed that the release of arg^8-oxytocin aids in maintaining tissue hydration in amphibians by inhibiting water loss through the skin (Boyd & Whyte, 1938), by decreasing urine flow and by increasing the absorption of depot-water from the urinary bladder (Ewer, 1952; Sawyer, 1960). Moreover, arg^8-oxytocin is probably also the main antidiuretic hormone of reptiles and birds. The significance of the effects of this peptide on other non-mammalian organs, e.g. on the reptilian and avian oviduct, has yet to be determined.

Our results suggest that, with regard to their neurohypophysial hormones, vertebrates fall into two groups: first, a large group comprising cyclostomes, fresh-water and marine teleosts, amphibians, reptiles and birds (Table 6) which elaborate arg^8-oxytocin and a second group, namely elasmobranchs and mammals, which secrete peptides which do not include arg^8-oxytocin. Elasmobranchs are unique in that they actively reabsorb urea from their renal tubules, being thus able to utilize a physiological uraemia for osmoregulation. (It will be interesting to see whether this renal tubular function is influenced by the unidentified hormones in their pituitary.) Mammals are characterized by a renal concentrating mechanism which, with the aid of vasopressin, enables them to produce a

hypertonic urine. It is therefore not unlikely that the neurohypophysial hormones produced by each vertebrate class are related to its specific pattern of osmoregulation.

It will be realized that such general conclusions can be only tentative at present. First, because, so far, only a few species within each vertebrate class have been investigated and, secondly, because further work may reveal the occurrence in vertebrate neurohypophyses of hormones additional to those already described.

SUMMARY

1. Paper chromatography of neurohypophysial extracts from teleost fishes, amphibians, reptiles and birds showed the presence of at least two oxytocic substances. These were designated 'slow-moving' and 'fast-moving' according to their behaviour on paper chromatograms developed in butanol–acetic acid–water.

2. The 'fast-moving' substance resembled mammalian oxytocin in some of its biological and chromatographic characteristics.

3. Pressor and antidiuretic activity was mainly associated with the 'slow-moving' oxytocic substance even after two-dimensional chromatography.

4. The marked frog water-balance effects exerted by non-mammalian pituitary extracts were shown to be due mainly to the 'slow-moving' oxytocic substance.

5. The 'slow-moving' oxytocic substance was isolated by ion-exchange chromatography from pituitary extracts of a teleost fish, the pollock. The purified compound was identified as arginine^8-oxytocin (vasotocin) by amino-acid analysis and by chromatographic and pharmacological comparison with the synthetic peptide.

6. Paper chromatography of elasmobranch pituitary extracts suggested that these glands contain a peptide which resembles oxytocin and another oxytocic principle which differs from all other identified neurohypophysial peptides. The water-balance potency of the unidentified peptides was much lower than that of arg^8-oxytocin.

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