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DISTRIBUTION OF NEUROHYPOPHYSIAL HORMONES IN MAMMALS

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Since the discovery that the vasopressin in the domestic pig differs from that in the ox posterior pituitary lobe by having lysine instead of arginine in position eight (Popenoe, Lawler & du Vigneaud, 1952), it has seemed desirable to undertake a systematic investigation of the distribution of mammalian neurohypophysial hormones.

8-arginine vasopressin (AVP) has since been shown to be present not only in the ox (du Vigneaud, Ressler & Trippet, 1953; du Vigneaud, Lawler & Popenoe, 1953), but also in man (Light & du Vigneaud, 1958), the horse (Acher, Chauvet & Lenci, 1959*a*), the sheep (Acher, Chauvet & Lenci, 1959*b*) and the fin-back whale, *Balaenoptera physalus* (Chauvet, Chauvet & Acher, 1963; Acher, Chauvet & Chauvet, 1964). In all these studies large amounts of the hormones were purified and their amino acid composition determined. This procedure cannot be used for the identification of neurohypophysial hormones in single pituitary glands but the hormones can be characterized by their chromatographic and pharmacological properties.

Van Dyke, Adamsons & Engel (1958) investigated the vasopressin in the dog, camel, cat, rabbit, sheep, man and macaque monkey by comparing its rat pressor and dog antidiuretic potencies; van Dyke, Engel & Adamsons (1963) characterized the vasopressin of the kangaroo, rat, gerbil and guinea-pig by the same method. The vasopressin of all these animals had the pharmacological properties of the 8-arginine analogue. This hormone has also been pharmacologically identified in the neurohypophysis of the American opossum (*Didelphis virginiana*) and the spiny anteater (*Tachyglossus aculeatus*) (Sawyer, Munsick & van Dyke, 1960). Bio-assays of a neurohypophysial extract from the North American collared peccary (*Tayassu angulatus*) indicated that 8-arginine vasopressin was present (Sawyer, 1961). This finding was surprising in view of the fact that the peccaries are more closely related to the domestic pig than the hippopotamus, which, by chromatographic and pharmacological means, was shown (Heller & Lederis, 1960) to elaborate a hormone with the properties of 8-lysine vasopressin (LVP).

The aim of this investigation was to study the occurrence of LVP, first, by extending the search to as many species of the even-toed ungulates (the *Artiodactyla*) as possible, secondly, by analysing the glands of some forms which are taxonomically related to this order of mammals and thirdly by including representatives of other groups of the Eutheria.

Some of these results have already been briefly communicated (Ferguson, Heller, Lederis & Pickford, 1962).

METHODS

Tables 1 and 2 show the taxonomic position of the mammalian species from which pituitaries were obtained and their provenance.

All glands (with few exceptions which are listed in the Results) were removed as soon as possible after death. They were immersed in anhydrous acetone, which was changed after 24 hr, and stored in acetone in a refrigerator until required. Whenever possible the posterior lobes only were extracted by homogenizing the tissue in 0·25 % acetic acid. The protein was precipitated either by immersion in boiling water for 3 min or by adding 50 % trichloroacetic acid, to give a concentration of T.C.A. of 2·5 %, a method which was found to be more efficient. After centrifugation the clear supernatant was pipetted off, and the trichloroacetic acid was removed by shaking three times with an excess of ether.

TABLE 1. Eutherian mammals, other than Artiodactyla, investigated

Super-order or Order	Species investigated	Common name	Provenance
Insectivora	*Erinaceus europaeus*	Hedgehog	United Kingdom
Chiroptera	*Eidolon helvum*	African fruit-bat	Uganda
Edentata	*Priodontes gigas*	Giant armadillo	Unknown
	Dasypus novemcinctus	Nine-banded armadillo	Unknown
Ferae			
Carnivora	*Meles meles*	Badger	United Kingdom
Paenungulata			
Hyracoidea	*Procavia capensis*	African hyrax	Kenya
Proboscidea	*Elephas maximus*	Indian elephant	Unknown
	Loxodontia africana	African elephant	Uganda
Mesaxonia			
Perissodactyla	*Equus burchelli*	Common zebra	Uganda
	Tapirus terrestris	Tapir	Peru
	Diceros bicornis	Black rhinoceros	Kenya

The paper chromatographic procedure introduced by Heller & Lederis (1958) was used initially, but was later replaced by column chromatography on carboxymethylcellulose (which was used to separate oxytocin from vasopressin) and agar-gel electrophoresis or thin-layer chromatography. The latter steps served to separate mixtures of 8-lysine and 8-arginine vasopressin. A 5 × 0·5 cm carboxymethylcellulose column was used, and the pituitary extract was added to the column in 0·2 M pyridine-acetic acid buffer at pH 7·0. Oxytocin passed freely through the column; it was collected, dried in a rotary evaporator, taken up in 0·25 % acetic acid and assayed on an isolated rat uterus. Vasopressin was eluted by changing the buffer to 2 M pyridine-acetic acid at pH 7·3. The eluate was dried *in vacuo*, and the residue taken up in 0·25 % acetic acid. Elution of vasopressin by pyridine-acetic acid, instead of ammonium acetate, as used by Ward & Guillemin (1957), made it possible to remove the buffer by rotary evaporation instead of repeated freeze-drying.

Agar-gel electrophoresis was performed in sodium carbonate-sodium bicarbonate buffer (0·04 M) at pH 10·5, using a current of 13 mA for 8 hr. Surface evaporation from the gel was minimized by covering it with a thin polyethylene sheet and by performing the experiment at +2° C. This method was replaced by thin-layer chromatography with cellulose as an adsorbent and butanol-acetic acid-water (6:2:2) as a solvent system (Ferguson, 1965). When small quantities of hormones (less than 5 i.u.) had to be separated, the crude extract was subjected to thin-layer chromatography since this procedure separates AVP, LVP and oxytocin.

TABLE 2. Artiodactyla investigated

Suborder and Species	Common name	Provenance
SUIFORMES		
Sus scrofa	European wild boar	Bavaria and Poland
Phacochoerus aethiopicus	Warthog	Uganda
Potamochoerus porcus	Bushpig	Kenya
Hylochoerus meinertzhageni	Giant forest hog	Kenya
Tayassu angulatus	Collared peccary	Arizona, U.S.A.
Tayassu pecari	White-lipped peccary	Peru
Hippopotamus amphibius	Hippopotamus	Uganda
TYLOPODA		
Lama glama	Llama	Peru
RUMINANTIA		
Syncerus caffer	African buffalo	Uganda
Alcephalus cokei	Kongoni	Uganda
Damaliscus korrigum	Topi	Uganda
Gorgon taurinus	Blue wildebeeste	Kenya
Kobus kob	Kob	Uganda
Tragelaphus scriptus	Bushbuck	Uganda

Interpretation of chromatographic results based on results obtained with authentic peptides. Hormone eluted from paper chromatograms at R_F 0·10–0·26 was regarded to have run to the LVP position, R_F 0·23–0·40 was regarded as the position of AVP and R_F 0·45–0·70 as that of oxytocin. The R_F values on thin-layer chromatograms were: LVP, 0·10–0·25; AVP, 0·30–0·50 and oxytocin, 0·55–0·90. In agar-gel electrophoretograms authentic LVP moved 2–5 cm from the origin and AVP 5–8 cm from the origin, towards the cathode. It was felt, however, that whenever possible chromatographic and electrophoretic results should be supported by bio-assays of the separated hormones.

Bio-assay methods. The rat pressor assay of Dekansky (1952) was used. Oxytocin was assayed on the isolated rat uterus by the method of Holton (1948) with the modification that virgin rats were injected with stilboestrol for 3 days before use (Follett & Bentley, 1964), and that Munsick's (1960) solution without magnesium was used as the suspension fluid. Oxytocic and pressor assays were of the standard (2+2) design; four blocks of four doses were used for each assay. AVP and LVP were distinguished by the quantitative differences in their effects on water or sodium transport across the isolated urinary bladder of *Bufo bufo* or *B. marinus*. The procedures used were those of Leaf, Anderson & Page (1958) and Bentley (1958, 1960). The responses to the unidentified hormone were compared with those of AVP. Equipressor doses of AVP and LVP were used of a size which ensured a marked response to AVP, while LVP gave little or none. An example of such an assay is shown in Fig. 1. Student's t test for small samples was used to estimate the significance of difference between the responses. All P values given in the text and the tables refer to the probability of there being no difference between the unidentified hormone and AVP. Since the difference in potency between the two active peptides in this assay is of the order of ten, errors in standardization by pressor assay did not affect the validity of the results.

Antidiuretic assays on dogs were done by Dr Mary Pickford, of the Department of Physiology, University of Edinburgh. Van Dyke, Engel & Adamsons (1956) have shown

that one pressor unit of LVP has about one-sixth of the antidiuretic activity of one pressor unit of AVP when given intravenously to dogs.

Standard preparations. Syntocinon (Sandoz, Ltd.) and Pitressin (Parke, Davis and Co.) standardized against the Third International Standard preparation were used as reference substances in pressor and oxytocic assays. AVP, purified in this Department from ox pituitaries, and synthetic LVP, provided by Sandoz Ltd., were used as reference substances in chromatograms and in the water transfer and natriferic (Bentley, 1960) assays on toad bladders.

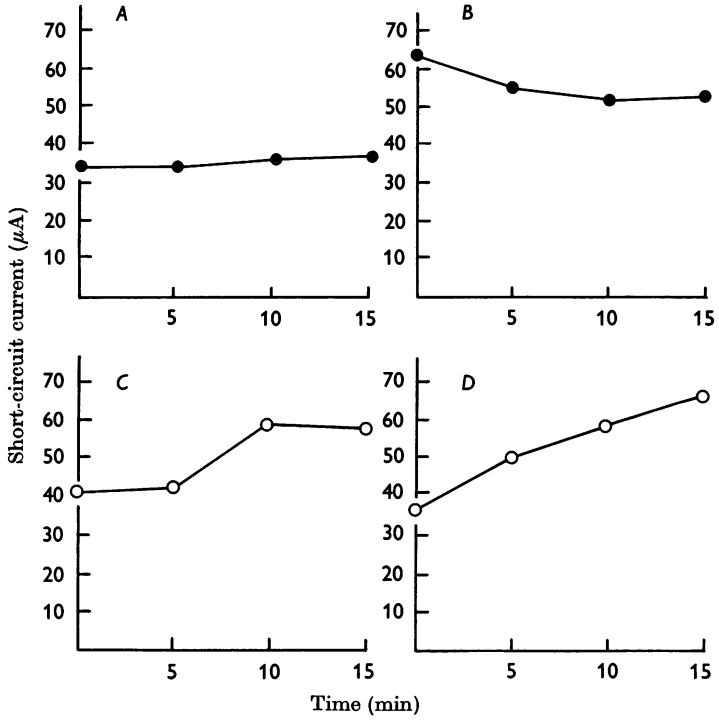

Fig. 1. Assay of slow-moving and fast-moving pressor component in eluates from chromatograms of a giant forest hog posterior pituitary extract. The effect of these substances on the short-circuit current across the isolated urinary bladder of *Bufo marinus* was measured and compared with equi-pressor doses of authentic arginine vasopressin (AVP) and lysine vasopressin (LVP). Concentration of hormone on serosal side 1 m.u. (rat pressor)/ml. in all instances. *A*. Slow-moving pressor principle of giant forest hog extract. *B*. Lysine vasopressin. *C*. Fast-moving principle of giant forest hog extract. *D*. Arginine vasopressin. There was no response with *A* and *B*, the responses with *C* and *D* were 21 and 23 μA, respectively.

Validity of methods. While most of the pituitary glands were removed as soon as possible after death, a few pituitaries were obtained from animals which had been deep-frozen after death. Estimations of the hormone content and the mole ratios in the latter animals are likely to be less accurate than in the former, since autolysis after death and thawing of the carcass may have led to losses of hormones.

Comparisons of the natriferic, water transfer (hydrosmotic) and antidiuretic activity of neurohypophysial hormones rest on accurate standardization by pressor assay. The Index

of Precision (λ) as calculated for ten randomly selected pressor assays was $0{\cdot}027 \pm 0{\cdot}003$ (S.E.) (Gaddum, 1953).

The identification of neurohypophysial hormones in mammals by pharmacological means is simplified by the fact that, so far, only AVP, LVP and oxytocin have been found in this group of vertebrates. The chromatographic and pharmacological methods used in this work characterize these three hormones with a high degree of certainty.

Calculation of mole ratios. Although oxytocin has a small pressor potency and the vasopressins in very large doses stimulate the uterus (Berde, 1963), these activities are so small that they fall within the limits of error of the respective assays. They have therefore been disregarded in the calculation of mole ratios. All mole ratios given are expressed as the ratio vasopressin to oxytocin.

RESULTS

Marsupialia

Posterior pituitary lobes from representatives of two Families of the Marsupialia were investigated.

Glands from the Australian opossum (*Trichosurus vulpecula*; Family: Phalangeridae), the wallaby (*Setonyx brachyurus*) and the red kangaroo (*Macropus rufus*), both of the Family Macropodidae, were studied. All specimens were obtained from Western Australia. Table 3 shows that the neurohypophysial extracts of all these animals contained hormones with the chromatographic and pharmacological properties of AVP and oxytocin. The mole ratio (mean for all the marsupials investigated) was $4{\cdot}8 \pm 0{\cdot}06$.

Eutheria

Tables 1 and 2 list the eutherian species investigated.

Insectivora, Chiroptera and Edentata

A single species of the Insectivora, the hedgehog, was studied. Two neural lobes were extracted together; the extract contained 9·6 i.u. (22·2 mμmole) pressor activity, and 4·6 i.u. (10·2 mμmole) oxytocic activity giving a mole ratio of 2·2. Oxytocin was eluted from its usual position on a thin-layer chromatogram, pressor activity was eluted in the AVP position. The presence of AVP was confirmed in a natriferic assay, i.e. the response was not significantly different from that of AVP ($P > 0{\cdot}4$).

Chiroptera. Five whole pituitaries of the African fruit bat were extracted together. The mean value per gland for pressor activity was 2·8 i.u (6·5 mμmole) and that for oxytocic activity was 1·4 i.u. (3·2 mμmole), with a mole ratio of 2·0. Thin-layer chromatography showed spots in the AVP and oxytocin position. A natriferic assay confirmed the presence of AVP ($P > 0{\cdot}8$).

Edentata. Pituitary glands were obtained from two giant armadillos which had been deep-frozen after death; they were extracted together and

TABLE 3. Neurohypophysial hormones of Marsupialia

Species	Sex	Pressor activity		Oxytocic activity		Mole ratio	Method of separation	Positions on chromatograms	Results of natriferic assays
		i.u./ gland	mμmole/ gland	i.u./ gland	mμmole/ gland				
T. vulpecula	F	1·4	3·3	0·37	0·83	4·2	PC	AVP, oxytocin	AVP
	M	2·4	6·5	0·22	0·50	13·0	CMC, AG	AVP, oxytocin	AVP
	M	5·8	13·4	1·69	3·76	3·6	PC	AVP, oxytocin	AVP
	F	3·7	8·5	0·94	2·10	4·0	CMC, AG	AVP, oxytocin	AVP
S. brachyurus	M	3·7	8·5	0·71	1·60	5·3	PC	AVP, oxytocin	—
	—	1·65	4·5	0·90	2·0	2·3	TLC	AVP, oxytocin	AVP
M. rufus	—	9·5	21·0	2·50	5·60	3·9	PC	AVP, oxytocin	AVP
	—	14·4	34·6	2·40	5·3	6·3	PC	AVP, oxytocin	AVP
	—	15·0	34·6	3·6	8·0	4·3	TLC	AVP, oxytocin	AVP

PC = paper chromatography. TLC = thin-layer chromatography. AG = agar-gel electrophoresis. CMC = column chromatography on carboxymethylcellulose.

All responses of the unidentified vasopressin were not significantly different from those to authentic AVP.

contained 0·22 i.u (0·52 mμmole) pressor activity and 0·088 i.u. (0·20 mμmole) oxytocic activity. The mole ratio was 2·6. On a thin-layer chromatogram pressor activity was eluted from the AVP position and oxytocic activity from the oxytocin position. In a natriferic assay the vasopressin behaved like AVP, but there were insufficient results for statistical analysis. The extract of two whole pituitary glands from nine-banded armadillos (also deep-frozen after death) contained 0·18 i.u. (0·42 mμmole) pressor and 0·44 i.u. (0·10 mμmole) oxytocic activity; mole ratio 4·2. The pressor and oxytocic activities were eluted from a thin-layer chromatogram at the R_F of AVP and oxytocin respectively. There was not enough vasopressin for bio-assay.

Carnivora

A single badger pituitary was extracted. It contained 3·9 i.u. (9·0 mμmole) of pressor and 2·8 i.u. (6·2 mμmole) of oxytocic activity (mole ratio 1·45). Thin-layer chromatography yielded a pressor spot in the AVP position, and oxytocic activity in the oxytocin position. The pressor principle gave a natriferic response which was not significantly different from AVP ($P > 0·7$). These results are in keeping with those of van Dyke *et al.* (1958) in two other carnivores, the dog and the cat.

Paenungulata

Proboscidea. The posterior pituitary lobe of a female Indian elephant contained 19·6 i.u. (45·0 mμmole) pressor, and 35·0 i.u. (77·8 mμmole) to oxytocic activity, with a mole ratio of 0·58. Thin-layer chromatography gave active eluates at the R_F of AVP and oxytocin. Natriferic assays showed that the pressor principle behaved like AVP though there were insufficient results for statistical analysis.

The neurohypophysis of a young male African elephant contained 54·0 i.u. (124 mμmole) pressor activity and 51 i.u. (113 mμmole) oxytocic activity in a mole ratio of 1·1. On a thin-layer chromatogram the oxytocic component was found in the oxytocin and the pressor compound in the AVP position. The latter principle behaved also like AVP in a natriferic assay ($P > 0·3$).

Hyracoidea. A whole pituitary from a male African hyrax was extracted; it contained 0·066 i.u. (0·15 mμmole) pressor activity and, 0·39 i.u. (0·87 mμmole) oxytocic activity in a mole ratio of 0·17. Since this gland contained insufficient material to identify the hormones, five pituitaries from female animals which had been deep-frozen after death were pooled. The mean pressor activity per gland was 0·14 i.u. (0·33 mμmole) and the mean oxytocic activity 3·7 i.u. (8·2 mμmole), a mole ratio of 0·04. On a thin-layer chromatogram pressor activity was found in the AVP position,

oxytocin was eluted in the usual position. Natriferic assay of the vasopressin failed to show a significant difference from AVP ($P > 0{\cdot}4$).

Mesaxonia

The super-order Mesaxonia contains only one Order, the *Perissodactyla*, comprising the Equidae, the Tapiroidea and the Rhinocerotidae. Two zebra pituitaries were extracted, one contained 30 i.u. (69 mμmole) pressor activity and 72 i.u. (160 mμmole) oxytocic activity, the other 12 i.u. (27·7 mμmole) pressor and 29 i.u. (63·3 mμmole) of oxytocin. The mole ratios were 0·43 and 0·44, respectively. From a paper chromatogram pressor activity was eluted between R_F 0·16 and 0·30 and oxytocic activity between R_F 0·33 and 0·43. The vasopressin in the pituitary extract from the first animal behaved like AVP on natriferic assay. The crude extract of the other posterior lobe was subjected to thin-layer chromatography, and the results suggested the presence of AVP and oxytocin. This was confirmed by a natriferic assay ($P > 0{\cdot}3$).

The pituitary of a South American tapir contained 1·95 i.u. (4·5 mμmole) vasopressin and 1·85 i.u. (6·3 mμmole) oxytocin, in a mole ratio of 0·72. Thin-layer chromatography indicated the presence of oxytocin and AVP. The vasopressin behaved like AVP in a natriferic assay ($P > 0{\cdot}4$).

The neurohypophysis of a black rhinoceros contained 32 i.u. (74 mμmole) vasopressin and 44 i.u. (96 mμmole) oxytocin. The mole ratio was 0·77. In a thin-layer chromatogram vasopressin was eluted from the AVP position and the oxytocic activity at the R_F of oxytocin. A natriferic assay showed that there was no significant difference in activity between the vasopressin and AVP ($P > 0{\cdot}3$).

Artiodactyla

The artiodactyls or even-toed ungulates are usually subdivided into three suborders, the Suiformes, the Tylopoda and the Ruminantia.

Suiformes. Posterior pituitary glands from five European wild boars were dissected from animals which had been deep-frozen after death. These glands were analysed singly; the results are summarized in Table 4. The chromatographic and pharmacological behaviour of the hormones from four of these animals was compatible with the presence of LVP and oxytocin. A paper chromatogram of the pituitary extract from the fifth animal (Table 4, no. 2) separated three biologically active substances; one of these had a predominantly oxytocic, and the other two predominantly pressor actions. When tested for its antidiuretic activity in dogs the slower-moving principle behaved like LVP and the faster-moving one like AVP.

TABLE 4. Neurohypophysial hormones of the European wild boar (*Sus scrofa*)

Pressor activity		Oxytocic activity					
i.u./ gland	mμmole/ gland	i.u./ gland	mμmole/ gland	Mole ratio	Method of separation	Positions on chromatograms	Results of natriferic assays
13·2	46·2	12·0	26·7	1·7	PC	LVP, oxytocin	LVP ($P < 0·05$)
21·0	—	30·0	66·7	—	PC and AG	AVP, LVP, oxytocin	AVP and LVP*
31·5	110·0	27·0	60·0	1·8	AG	LVP, oxytocin	LVP ($P < 0·01$)
22·0	77·0	15·0	33·3	2·3	AG	LVP, oxytocin	LVP ($P < 0·05$)
15·7	55·0	22·0	49·0	1·1	AG	LVP, oxytocin	LVP ($P < 0·05$)

PC = paper chromatography. AG = agar-gel electrophoresis.

* Antidiuretic assays in dogs.

The extracts of five warthog pituitaries were investigated individually. The results are summarized in Table 5. Four of these glands contained hormones with the characteristics of oxytocin and LVP, but a paper chromatogram of extract No. 2 yielded two regions of pressor activity. The two pressor principles were tested for their action on the rate of water transfer on isolated *Bufo bufo* bladders; the response to the slow-moving compound was significantly different from that of AVP ($P < 0{\cdot}05$), the faster-moving pressor substance could not be distinguished from AVP ($P > 0{\cdot}5$). It was concluded that both AVP and LVP were present.

TABLE 5. Neurohypophysial hormones of the warthog (*Phacochoerus aethiopicus*)

Pressor activity		Oxytocic activity					
i.u./ gland	mμmole/ gland	i.u./ gland	mμmole/ gland	Mole ratio	Method of separation	Positions on chromato-grams	Results of natriferic assays
31·9	111·8	18·6	41·3	2·7	PC	LVP, oxytocin	—
21·6	—	9·3	20·7	—	PC	AVP, LVP, oxytocin	AVP, LVP ($P < 0{\cdot}05$)
15·9	55·7	12·4	27·4	2·0	CMC, AG	LVP, oxytocin	LVP ($P < 0{\cdot}01$)
31·5	110	20·0	50·0	2·2	CMC, AG	LVP, oxytocin	LVP ($P < 0{\cdot}05$)
33·0	116	25·6	56·9	2·0	CMC, AG	LVP, oxytocin	LVP ($P < 0{\cdot}10$)

PC = paper chromatography. AG = agar-gel electrophoresis. CMC = column chromatography on carboxymethylcellulose.

A single African bush pig neurohypophysis contained 0·65 i.u. (1·5 mμmole) vasopressin, and 1·6 i.u. (3·8 mμmole) oxytocin, a mole ratio of 0·4. On a thin-layer chromatogram vasopressin was eluted in the LVP position and oxytocic activity from the oxytocin position. The natriferic response of the vasopressin was significantly different from that of AVP ($P = 0{\cdot}005$).

The posterior lobe of an African giant forest hog contained 25·0 i.u. (88 mμmole) vasopressin and 12·3 i.u. (27·3 mμmole) oxytocin in a mole ration of 3·2. Pressor activity was eluted from a thin-layer chromatogram at R_F 0·2–0·3, and the oxytocic activity at R_F 0·6–0·8, in the position of oxytocin. The natriferic activity of the vasopressin was significantly different from that of AVP ($P < 0{\cdot}01$) and behaved like authentic LVP when tested on the same preparation. The pituitary of a second giant forest hog contained 19 i.u. of pressor activity, and 15·9 i.u. (35·3 mμmole) oxytocin. Two thin-layer chromatograms showed pressor regions in the positions characteristic of LVP and AVP. Oxytocin was present in its usual position. In natriferic assays the slower-moving pressor principle

had a significantly different potency from that of AVP ($P < 0{\cdot}05$), the fast-moving pressor principle behaved like AVP ($P > 0{\cdot}2$). It would seem that the posterior lobe of the first giant forest hog contained LVP and oxytocin, and that of the second AVP, LVP and oxytocin.

An extract of three pooled collared peccary glands were put at our disposal by Professor W. H. Sawyer. On paper chromatography, pressor spots were found in both the AVP and the LVP position, with oxytocin at the usual R_F. In assays measuring the effect on sodium transfer across the isolated bladder of *B. bufo*, the response to the slower-moving pressor principle was different from that to AVP ($P < 0{\cdot}01$). There was insufficient material to test the faster-moving component, but Professor Sawyer (Personal communication) had previously estimated its antidiuretic activity in dogs as 1·46 i.u./ml. with a rat pressor potency of 1·3 i.u./ml. which suggests that the extract contained not only LVP but also AVP.

The pituitary of a young white-lipped peccary was extracted, and contained 9·9 i.u. pressor activity and 15·6 i.u. (34·7 mμmole) oxytocic activity. Two pressor spots were found on a paper chromatogram, one at R_F 0·10–0·18, and the other at R_F 0·22–0·26, corresponding to the positions of AVP and LVP. The oxytocic activity was in the oxytocin position. The presence of the two vasopressins was confirmed by antidiuretic assay in dogs (see Heller, 1963). The pituitary of a pregnant female white-lipped peccary contained 41·0 i.u. (95 mμmole) pressor and 26·0 i.u. (56 mμmole) oxytocic activity, in a mole ratio of 1·7. Vasopressin was eluted at R_F 0·35–0·45 and oxytocin at R_F 0·70–0·90, from a thin-layer chromatogram. The remaining crude extract was subjected to column chromatography on carboxymethylcellulose, and the eluted vasopressin was run in a further thin-layer chromatogram, from which it was again eluted in the AVP position. A natriferic assay confirmed the presence of AVP.

The neural lobe of a lactating hippopotamus contained 34·7 i.u. (122 mμmole) pressor and 29·3 (65 mμmole) oxytocic activity; a mole ratio of 1·9. Two paper chromatograms were run, vasopressin was eluted at R_F 0·23–0·40 on one, and at 0·30–0·36 in the other; oxytocin was eluted at R_F 0·55–0·60 and 0·40–0·50, respectively. Although it appeared in the AVP position in both chromatograms, the eluted vasopressin gave responses in assays on the isolated urinary bladder of *B. bufo* (water transfer) which differed significantly from those of authentic AVP ($P < 0{\cdot}001$). It was concluded that the hippopotamus gland contained LVP and oxytocin.

Tylopoda. This suborder contains the camels which have been investigated by van Dyke *et al.* (1958) and the llamas. Results obtained from two posterior pituitaries of *Lama glama* (Table 6) indicate that this species carries AVP and oxytocin.

TABLE 6. Neurohypophysial hormones of a tylopod (the llama) and five ruminants

Species	Pressor activity		Oxytocic activity		Mole ratio	Method of separation	Positions on chromatograms	Results of natriferic assays
	i.u./ gland	mμmole/ gland	i.u./ gland	mμmole/ gland				
Lama glama	14·4	33	4	9	3·6	PC	AVP, oxytocin	AVP
	14·4	33	6	13	2·5	PC	*LVP, oxytocin	AVP
Syncerus caffer	6·4	15	6	12	1·2	PC	AVP, oxytocin	—
	33	76	53	117	0·65	TLC	AVP, oxytocin	AVP
Alcephalus cokei	24	65	21	46	1·4	PC	AVP, oxytocin	AVP
	45	104	27	60	1·7	TLC	AVP, oxytocin	AVP
Damaliscus korrigum	33	76	25	55	1·4	PC	AVP, oxytocin	AVP
Kobus kob	11	25	8	17	1·5	TLC	AVP, oxytocin	AVP
	14	33	12	28	1·2	TLC	2 pressor spots, oxytocin	AVP
Tragelaphus scriptus	15	35	11	24	1·4	TLC	AVP, oxytocin	AVP

PC = paper chromatography. TLC = thin-layer chromatography.

All responses of the unidentified vasopressin were not significantly different from those to authentic AVP.

* An aberrant chromatographic result.

Ruminantia. This suborder contains two infra-orders. One, the Tragulina, is represented by the rare chevrotain; the other infra-order is that of the Pecora, several representatives of which were studied in the present investigation (Table 6). All these glands contained AVP and oxytocin.

DISCUSSION

The results of this investigation suggest that the neurohypophysial hormones of the main groups of mammals, other than the Suiformes, are arginine, vasopressin and oxytocin. All the wild species of the Suiformes investigated carried either LVP or AVP, or a mixture of the two, together with oxytocin, with the exception of the bush pig and the hippopotamus in which LVP only was found. Since very few posterior lobes of the latter two species have been examined, it is impossible to be certain that AVP does not occur. However, AVP has never been found in the neural lobe of the domestic pig although thousands of glands have by now been investigated. It seems possible, therefore, that domestication has eradicated the AVP gene from this population.

AVP has been identified in mammals only and is likely to have arisen by mutation of the gene for arginine vasotocin leading to the replacement of isoleucine by phenylalanine in position 3. The mutation of the AVP-producing gene in its turn gave rise, again by a single step, to the production of LVP. This second mutation, in view of its occurrence in all the subdivisions of the Suiformes, seems to have arisen in the ancestors of the pig-like animals. If this is so, the mutation must have occurred before the end of the Eocene period, when it is thought that the Anthracotheres, the ancestors of the hippopotamus, became separated from a common pig-peccary stock (Simpson, 1945; Romer, 1955). Munsick and his colleagues (Munsick, Sawyer & van Dyke, 1958) have compared the antidiuretic activity in pigs of equipressor doses of AVP and LVP (the pressor assays were done in rats). They concluded that in terms of rat pressor units, the potencies of the two hormones were about the same. This means, however, that the 'molecular potency' of LVP is about three-quarters that of AVP as an antidiuretic agent in the pig, assuming rat pressor potencies of 270 i.u./mg for LVP and 400 i.u./mg for AVP (du Vigneaud, Bartlett & Jöhl, 1957; Boissonas, Guttmann, Berde & Konzett, 1961). It seems, therefore, that the renal receptors of the pig are not fully adapted to the mutant hormone.

The most likely explanation for the perpetuation of a gene which gives rise to a less active hormone is that in the pig-like animals the difference in potency between AVP and LVP as antidiuretic hormones is insufficient to be of evolutionary significance, especially since the amounts of hormone

released under physiological conditions are almost certainly very small relative to the total amounts stored in the neurohypophysis. Alternatively, the mutation to LVP may have persisted because it endows the animal with an unknown adaptive advantage. If the ancestors of the Suiformes lived under aquatic or semiaquatic conditions like the hippopotamus, a highly active antidiuretic hormone may not have been needed. Moreover, the finding that individuals in some wild species of pig-like animals carry both AVP and LVP in the gland suggests that they are the heterozygotes produced by the crossing of lines which produce one hormone only. Animals which have both hormones would obviously meet even less difficulty in the regulation of their water economy than the homozygote mutant carrying LVP. If the latter were at an adaptive disadvantage, the heterozygote would be in a better position to perpetuate the gene for LVP.

The constancy of the chemical structure of neurohypophysial hormones throughout the mammalian species which have been studied contrasts strongly with the relative mutability of other peptide hormones such as insulin (Sanger, 1960) or ACTH (Harris, 1960) in the same vertebrate class. This indicates that amino acid substitutions may occur in large peptide-hormone molecules without materially affecting their biological activity, whereas very few changes can occur in the molecule of oxytocin or vasopressin without impairment of biological activity: the smaller the peptide molecule, the greater the molecular specificity.

Follett (1963) has calculated the mole ratio of the two neurohypophysial hormones stored in the neurohypophyses of a variety of vertebrates and found that within certain groups this ratio is remarkably constant. The present findings agree with Follett's conclusions and support the concept that the mole ratio of the hormones in the neural lobe is under genetic control. The evolutionary pressure which tends to keep the mole ratios constant is not known, but two alternative hypotheses may be offered to explain the mechanism by which this ratio is maintained. First, vasopressin and oxytocin may be synthesized in separate neurones (Heller, 1961). If so, the proportion of vasopressin-producing to oxytocin-producing neurones would determine the mole ratio. The genetic control would be exercised over an embryological organizer which controls the differentiation of hypothalamo-neurohypophysial neurones. The alternative is that both vasopressin and oxytocin are synthesized in the same neurones, and that their ratio is determined by a control system of genes which regulate their production. Normally, synthesis and release of the two hormones must be closely correlated, thus maintaining the ratio. The mole ratio remains constant even when the absolute amounts of the neurohypophysial hormones stored varies. For example, 30 i.u. arginine vasopressin and 72 i.u. oxytocin (mole ratio 0·43) was found in one zebra and

12 i.u. arginine vasopressin and 29 i.u. oxytocin (mole ratio 0·44) in another. The absolute amounts stored in any single species appear to be related to body weight.

Information is now available about the type and mole ratios of neurohypophysial hormones from representative species of all mammalian Orders except the Dermoptera (flying lemurs), Pholidota (pangolins), Tubulidentata (aardvarks) and Sirenia (sea cows).

TABLE 7. Distribution of vasopressins in the Artiodactyla

Suborder-	Family	Species	Type of vasopressin
Suiformes	Suidae	*Sus scrofa*	LVP or AVP and LVP
		Phacochoerus aethiopicus	LVP or AVP and LVP
		Potamochoerus porcus	LVP
		Hylochoerus meinertzhageni	LVP or LVP and AVP
	Tayassuidae	*Tayassu angulatus*	LVP, AVP
		Tayassu pecari	AVP or LVP and AVP
	Hippopotamidae	*Hippopotamus amphibius*	LVP
Tylopoda	Camelidae	*Lama glama*	AVP
		Camelus dromedarius	AVP†
Ruminantia	Cervidae	*Capreolus capreolus*	AVP*
	Giraffidae	*Giraffa camelopardalis*	AVP*
	Bovidae	*Syncerus caffer*	AVP
		Alcephalus cokei	AVP
		Damaliscus korrigum	AVP
		Gorgon taurinus	AVP
		Kobus kob	AVP
		Tragelaphus scriptus	AVP

* Chromatographic results only (K. Lederis, personal communication).

† Adamsons, Engel, van Dyke, Schmidt-Nielsen & Schmidt-Nielsen (1956).

Generally speaking, this investigation may be regarded as an attempt to apply biochemical criteria—in this instance the chemical structure and the mole ratios of a set of peptide hormones—to the taxonomic arrangement of groups of vertebrates. The results so obtained can be compared with the traditional classification based on morphological characteristics. Species variations in the structure of other peptide hormones are well known, but the neurohypophysial hormones are the only ones which have been studied sufficiently widely to permit such a comparison. Tables 7 and 8 show that grouping as defined by amino acid composition and mole ratios compare well with morphological classifications. For example, all Perissodactyla appear to have mole ratios below unity whereas the ratios in Marsupialia seem to be uniformly high. From the differences between ruminants and tylopodes (Table 8) it would seem that even mole ratios of suborders may be characteristic. The distribution of the vasopressin in taxonomic groups appears to be equally characteristic: so far lysine vasopressin has been found exclusively in the Suiformes and in every species of this suborder investigated. AVP appears to be the only vasopressin in the other Artiodactyla of which over ten species have been scrutinized.

TABLE 8. Examples of the distribution of mole ratios of neurohypophysial hormones of mammals. The ratio arginine vasopressin to oxytocin is shown.

Order: Marsupialia	*Didelphis virginiana*	2·9*
	Trichosurus vulpecula	6·2
	Setonyx brachyurus	3·8
	Macropus rufus	4·8
Order: Perissodactyla	*Equus equus*	0·93†
	Equus burchelli	0·44
	Tapirus terrestris	0·72
	Diceros bicornis	0·77
Order: Artiodactyla		
Suborder: Ruminantia	*Syncerus caffer*	0·91
	Alcephalus cokei	1·5
	Damaliscus korrigum	1·4
	Gorgon taurinus	1·3
	Kobus kob	1·4
	Tragelaphus scriptus	1·4
Suborder: Tylopoda	*Lama glama*	3·1
	Camelus dromedarius	3·6‡

* Calculated from the results of Sawyer (1961).

† Calculated from the results of Acher *et al.* (1959*a*).

‡ Calculated from the results of Adamsons *et al.* (1956).

SUMMARY

1. The distribution of lysine vasopressin and arginine vasopressin in the even-toed ungulates, the Artiodactyla, and in some Orders taxonomically related to them has been studied. The neurohypophysial hormones of some of the more 'primitive' mammals have also been investigated.

2. Lysine vasopressin, as characterized by chromatographic and pharmacological methods, was found in the posterior pituitary glands of all the species of pig-like animals examined, namely the European wild boar, the warthog, the bush pig, the giant forest hog, the collared and the white-lipped peccary and the hippopotamus. However, some glands of the wild boar, the warthog and the giant forest hog contained both vasopressins, while others contained lysine vasopressin only. Extracts of single posterior lobes of the white-lipped peccary contained either lysine and arginine vasopressin or arginine vasopressin only.

3. Arginine vasopressin only was found in neurohypophysial extracts of representatives of the other two suborders of the Artiodactyla, the Tylopoda and the Ruminantia.

4. Arginine vasopressin only was also found in the glands of species from eight other mammalian orders.

5. Chromatographic evidence suggested the occurrence of oxytocin in all the posterior pituitary extracts studied.

6. Mole ratios (vasopressin:oxytocin) calculated from these results led to a grouping of, and within, the various Orders which agrees with the accepted taxonomic classification.

7. The genetic and evolutionary implications of these results are discussed.

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