The Comparative Physiology of the Neurohypophysis[1]

By H. HELLER, Bristol[2]

Recent studies concerned with the mechanism of secretion and elimination of the neurohypophysial antidiuretic principle (=ADH) have yielded sufficient information to allow reasonably safe conclusions as to the physiological role of this hormone in mammals. However, it is rarely realized that neurohypophysial principles–while similarly concerned with the metabolism of water–act by different mechanisms and on different anatomical sites in other vertebrate classes. It is proposed to discuss the evidence on these points and to attempt to give a more comprehensive picture of the function of the neurohypophysis in the vertebrate phyllum.

Phyletic distribution of neurohypophysial activities

Extracts having one or the other action of mammalian posterior pituitary preparations have been obtained from organs of certain invertebrates. For instance, BUTCHER, and BACQ and FLORKIN[3], obtained extracts from the sub-neural gland of ascidians which had a pressor effect in cats and stimulated the isolated guinea-pig uterus. GRAY and FORD[4], reported that extracts of crustacean eye-stalks had a similar effect on the water metabolism of frogs as mammalian posterior pituitary extracts, and ERSPAMER and PEROSA[5] found an antidiuretic principle in the salivary glands of certain octopods. It is to be doubted whether any of these factors are homologous to vertebrate neurohypophysial principles: the octopod factor differs chemically from mammalian ADH, we (HELLER and SMITH[6]), were unable to observe an antidiuretic, or oxytocic action of the crustacean principle, nor has the substance found in ascidians been shown to possess antidiuretic activity.

These findings cannot be said to rule out the possibility that posterior pituitary-like principles occur in species which lack a neurohypophysis. The occurrence of adrenaline in animals without an adrenal (HOGBEN[7]) may be remembered in this connection. Moreover, recent work suggests that posterior pituitary-like substances are formed in the supraoptic region of the hypothalamus, i.e. outside neurohypophysial tissue as characterized by the presence of pituicytes. BARGMANN[1], who examined the supraoptic nucleus in dogs and cats, describes its cells as containing secretory products which he was able to trace along or in the hypothalamico-hypophysial tract to the neurohypophysis. His findings are possibly the morphological counterpart of those of MELVILLE and HARE[2] who, in confirmation of ABEL[3], found that the supraoptic neurons of normal dogs contain 15 to 25% as much antidiuretic material as the neurohypophysis. Be this as it may, extracts having *all* the typical actions of mammalian posterior pituitary preparations have so far only been obtained from vertebrates with a clearly defined neurohypophysis. It will be seen from Table I that representatives of all vertebrate classes except cyclostome fishes have been investigated and that the main activities of neurohypophysial extracts occur in all these groups. This finding agrees satisfactorily with the comparative histology of the neurohypophysis: GRIFFITHS[4] reported that pituicytes occur in the *partes nervosæ* of all classes of vertebrates; he found them, except in the elasmobranchs, morphologically very similar throughout the series.

The amphibian water balance activity mentioned in Table I refers to that neurohypophysial factor which when injected into frogs kept in water, produces a temporary increase in the body water in these animals. This active principle has been shown by HELLER[5] and subsequently by others to be contained in the oxytocic fraction of mammalian posterior pituitary extracts, but it is probably not identical with the oxytocic principle (HELLER[6]). It seemed therefore advisable to list it separately.

The hormone content of the neurohypophysis of different classes and species of vertebrates

Table II shows that the amounts of antidiuretic hormone per mg neurohypophysial tissue are of a similar order of magnitude in different mammalian species. The proportion of oxytocic to antidiuretic activity is usually found to be the same as in the international standard powder which is prepared from pituitaries of cattle or pigs. Exceptions have been reported by GEILING

[1] Based on lectures delivered at New York University and Columbia University, New York, during the tenure of a Visiting Professorship at New York University Medical College in 1949.

[2] Professor of Pharmacology, University of Bristol, England.

[3] E.O.BUTCHER, J. exp. Zool. *57*, 1 (1930). – Z.M.BACQ and M.FLORKIN, Arch. int. Physiol. *40*, 422 (1935).

[4] S.W.GRAY and W.FORD, Endocrinology *26*, 160 (1940).

[5] V.ERSPAMER and L.PEROSA, Exper. *4*, 486 (1948)

[6] H.HELLER and B.SMITH, J. exp. Biol., *25*, 388 (1948).

[7] L.T.HOGBEN, *The comparative physiology of internal secretion*, p. 36 (Cambridge, University Press, 1927).

[1] W.BARGMANN, Z. Zellforsch. *34*, 610 (1949).

[2] E.V.MELVILLE and K.HARE, Endocrinology *36*, 332 (1945).

[3] J.J.ABEL, Bull. Johns Hopkin's Hosp. *35*, 305 (1924).

[4] M.GRIFFITHS, Proc. Linnean Soc. U. S. Wales *63*, 81 (1938).

[5] H.HELLER, Arch. exp. Path. Pharmak. *157*, 425 (1930).

[6] H.HELLER, J. Physiol. *100*, 125 (1941); Biol. Rev. *20*, 147 (1945).

Table I

Phyletic distribution of neurohypophysial activities (The quotations refer to the authors who demonstrated the activity first)

	Mammalian vasopressor	Mammalian antidiuretic	Oxytocic	Amphibian water balance
Elasmobranchs	?	HELLER[3]	HERRING[1]	HELLER[8]
Teleosts	HERRING[1]	HELLER[3]	HERRING[1]	BOYD and DINGWALL[9]
Amphibians	HERRING[1]	HELLER[3]	HOGBEN and DE BEER[6]	HELLER[10]
Reptiles	HERRING[1]	HELLER[4]	HOGBEN and DE BEER[6]	HELLER[4]
Birds	HERRING[1]	HELLER[3]	HOGBEN and DE BEER[6]	HELLER[3]
Mammals	OLIVER and SCHAEFER[2]	v. D. VELDEN[5]	DALE[7]	BRUNN[11]

[1] P.T. HERRING, Quart. J. exp. Physiol. *8*, 245 (1915).
[2] G. OLIVER and E.A. SCHAEFER, Proc. physiol. Soc., March 10 (1894).
[3] H. HELLER, J. Physiol. *99*, 246 (1941).
[4] H. HELLER, J. Physiol. *101*, 317 (1942).
[5] R. VAN DER VELDEN, Klin. Wschr. *50*, 2083 (1913).
[6] L.T. HOGBEN and G.R. DE BEER, Quart. J. exp. Physiol. *15*, 163 (1925).
[7] H.H. DALE, J. Physiol. *34*, 163 (1906).
[8] H. HELLER, Biol. Rev. *20*, 147 (1945).
[9] E.M. BOYD and M. DINGWALL, J. Physiol. *95*, 501 (1939).
[10] H. HELLER, J. Physiol. *100*, 125 (1941).
[11] F. BRUNN, Z. ges. exp. Med. *25*, 170 (1921).

and his coworkers[1], who assayed the pituitaries of various whales and of the armadillo. They found a low oxytocic activity in gland extracts from these species (10–40% oxytocic activity as compared with 100% of antidiuretic and pressor potency) when assayed against the international standard powder.

Table II

Antidiuretic hormone content of the pituitary glands of various mammalian species and of man*

Species	Hormone content in units			Reference
	per gland	per mg fresh gland	per 100 g body weight	
Man (adult)	14·6	0·76	0·022	HELLER and ZAIMIS[1]
(newborn)	0·38	0·17	0·019	HELLER and ZAIMIS
Dog	15·0	0·75	–	MELVILLE and HARE[2]
Cat	3·5–5·0	0·35–0·5	–	PHILLIPS and HARE[3]
Rabbit	1·8	0·25	0·080	SIMON and KARDOS[4]
Guinea-pig	0·9	0·36	0·200	SIMON and KARDOS[4]
Rat (adult)	0·75–1·1	0·60–0·90	0·350	HELLER[5]
(newborn)	0·002	0·03	0·019	HELLER[5]
Mouse	0·04	–	0·175	HELLER[6]

* All figures are approximative.

[1] H. HELLER and E.J. ZAIMIS, J. Physiol. *109*, 162 (1949).
[2] E.V. MELVILLE and K. HARE, Endocrinology *36*, 332 (1945).
[3] D.M. PHILLIPS and K. HARE, Endocrinology *37*, 29 (1945).
[4] A. SIMON and Z. KARDOS, Arch. exp. Path. Pharmak. *176*, 238 (1934).
[5] H. HELLER, J. Physiol. *106*, 28 (1947).
[6] H. HELLER, J. Physiol. *99*, 246 (1941).

Table III compares the antidiuretic and the amphibian water balance activities of the glands of several vertebrate classes. The unit of water balance activity has been defined (HELLER[1]) as the amount of (frog) water-balance activity contained in 0·5 mg of the international standard powder. The data suggest that the antidiuretic activity proponderates in mammalian glands while the water balance factor supervenes in the amphibian pituitary; results from more amphibian species are needed to make this fully acceptable. Proof for a class-specific difference in the quantitive distribution of neurohypophysial factors would be of considerable interest when related to the different effector mechanisms for the regulation of the water metabolism in the various vertebrate classes. However, it has first to be shown that class-specific effector mechanisms exist and to what extent they can be linked to the various activities of neurohypophysial extracts.

Sites of action of neurohypophysial principles in various vertebrate classes

Mammals and birds. STARLING and VERNEY[2] showed in experiments on the isolated dog kidney that the antidiuretic effect of mammalian posterior pituitary extracts was due to a renal mechanism and the results of MOLITOR and PICK[3], who found that ADH failed to have an inhibitory effect in animals with experimental toxic nephrosis suggested a tubular site of action. Later work (BURGESS, HARVEY and MARSHALL[4]; SHANNON[5]) confirmed this assumption and the view that neurohypophysial extracts inhibit water diuresis in mammals by modifying the rate of tubular water reabsorption has since been generally accepted. A tubular mechanism is also suggested by the characteristic ability of the mammalian kidney to concentrate the tubular fluid beyond the plasma osmotic

[1] E.M.K. GEILING, Paul Reed Rockwood Lecture, State Univ. Iowa, Iowa City (1940).

[1] H. HELLER, J. Physiol. *100*, 125 (1941).
[2] E.H. STARLING and E.B. VERNEY, Proc. R. Soc. B. *97*, 321 (1925).
[3] H. MOLITOR and E.P. PICK, Arch. exp. Path. Pharmak. *101*, 169 (1924).
[4] W.W. BURGESS, A.M. HARVEY, and E.K. MARSHALL, J. Pharmacol. *49*, 237 (1933).
[5] J.A. SHANNON, J. exp. Med. *76*, 371 (1942).

Table III

Water-balance activity and antidiuretic activity of pituitary glands of representatives of various classes of vertebrates (HELLER[1])

Vertebrate class	Animal	Average units of water-balance principle in one pituitary*	Average units of antidiuretic principle in one pituitary*	Ratio of water-balance to antidiuretic activities per gland
Elasmobranchs	Dogfish	0·2 (–)	0·0035 (–)	–
Teleosts	Cod	8·0 (–)	0·166 (–)	48·0/1·0
Amphibians	Frog	0·8 (4·1)	0·0035 (0·012)	228·0/1·0
Reptiles	Grass-snake	3·3 (3·6)	0·100 (0·095)	33·0/1·0
Birds	Pigeon	1·5 (0·43)	0·031 (0·007)	48·0/1·0
Mammals	Rat	0·4 (0·24)	1·075 (0·360)	0·4/1·0

* Hormone per 100 g animal in brackets.

[1] H. HELLER, J. Physiol. *99*, 246 (1941); J. Physiol. *100*, 125 (1941); J. Physiol. *101*, 317 (1942); Biol. Rev. *20*, 147 (1945).

pressure under the influence of ADH. In fact, it seems likely that the elaboration of a hypertonic urine by the mammalian kidney is in essence dependent on this principle. Admittedly, slightly hypertonic urines have been observed in SHANNON'S[1] experiments with dehydrated dogs suffering from experimental diabetes insipidus and DE BODO and PRESCOTT[2] appear to have obtained similar results, but these findings may have been due to a small residuum of hormone-producing tissue.

It is clear that the conservation of body water under stress is much aided by the ability to prepare hypertonic urines, in so far as the ratio urinary solids per ml water excreted increases considerably. This is best seen in mammals suffering from lack of antidiuretic hormone as in experimental diabetes insipidus when tolerance to water deprivation is much decreased (DE BODO and PRESCOTT[2]), or again in the newborn rat whose kidney is less responsive to the hormone than that of adult animals (HELLER[3]). The internal environment of newborn rats, after withdrawal of fluid for 24 hrs. (as gauged by changes in plasma water concentration, hæmatocrit value and red cell count) is markedly different from that of normal animals, while that of adult rats under the same conditions of stress hardly differs from the controls (HELLER[4]). That is to say, the defence of the newborns against dehydration is less effective than that of the adult animals.

The ability to concentrate their urine seems to be differently developed in different mammalian species. Dehydrated albino rats, for instance, yield urines of lower freezing-point depressions than those of thirsting human beings (HELLER[4]), and the data of HOWELL and GERSH[5] and of SCHMIDT-NIELSEN, SCHMIDT-NIELSEN, BROKOW and SCHNEIDERHAN[6] show that some species of desert rodents excrete urine of much higher tonicity again than those elaborated by the albino rat, an indication that the maximum concentrating ability may be linked with environmental conditions. However, conservation of water by preparing a hypertonic urine is a mechanism limited to mammals and perhaps to birds. The thin segment of the loop of Henle is typically developed in mammals and birds only and these classes of vertebrates are said to be the only ones which respond to neurohypophysial extracts by increasing tubular water reabsorption against the osmotic gradient. It has therefore been suggested by E. K. MARSHALL that the thin segment is the tubular site of action of ADH. This attractive hypothesis has been queried by SPERBER[1] who pointed out that most nephrons in the avian kidney have no thin segment and that many of the thin segments which do occur, are very short (LINDGREN, HUBER, FELDOTTO[2]) but that nevertheless birds produce a urine from the ureter which has been described as a "thick, cream-coloured mucoid fluid" (HESTER, ESSEX and MANN[3]). It may be objected that the consistency of such urines does not allow any conclusion as to their osmotic pressure. Their concentrated appearance derives mainly from their content of uric acid, a large fraction of which has been shown to occur in a colloidal form (YOUNG and MUSGRAVE, YOUNG and DRYER[4]), i.e. in a state which contributes little to the total urinary osmotic pressure. Indeed D'ERRICO[5] reported freezing point determinations which indicate that the osmotic pressure of chicken urine is only little higher than that of plasma. The relatively poor development of the avian thin loop would be compatible with these findings.

[1] J. A. SHANNON, *loc. cit.*

[2] R. C. DE BODO and K. F. PRESCOTT, Fed. Proc. *5*, 1 (1946).

[3] H. HELLER (unpublished experiments).

[4] H. HELLER, J. Physiol. *108*, 303 (1949).

[5] A. B. HOWELL and I. GERSH, J. Mammal. *16*, 1 (1935).

[6] N. SCHMIDT-NIELSEN, K. SCHMIDT-NIELSEN, A. BROKOW, and H. SCHNEIDERHAN, J. cell. comp. Physiol. *32*, 331 (1948).

[1] I. SPERBER, Zool. Bidrag. Uppsala *22*, 249 (1944).

[2] H. LINDGREN, Z. Rat. Med. *33*, 15 (1868), quoted from I. SPERBER (1944). – G. C. HUBER, Anat. Rec. *13*, 305 (1917). – A. FELDOTTO, Z. mikr. anat. Forsch. *17*, 353 (1929).

[3] H. R. HESTER, H. E. ESSEX, and F. C. MANN, Amer. J. Physiol. *128*, 592 (1940).

[4] E. G. YOUNG and F. F. MUSGRAVE, Biochem. J. *26*, 941 (1932). – E. G. YOUNG and N. B. DRYER, J. Pharmacol. *49*, 162 (1933).

[5] G. D'ERRICO, Beitr. chem. Physiol. Pathol. *9*, 453 (1907), quoted from M. M. CRANE, Amer. J. Physiol. *81*, 232 (1927).

It is known, on the other hand, that carnivores which, on the whole, elaborate a more concentrated urine than most herbivores, have a relatively longer thin segment. Furthermore, SPERBER and KOEFOED[1] have remarked on the fact that the thin segment is better developed in desert than in other small rodents which would be in harmony with the finding of SCHMIDT-NIELSEN *et al.* already mentioned. Another objection against regarding the thin portion of Henle's loop as the site of action of the antidiuretic hormone has been made by H. W. SMITH[2] who, in a recent lecture (1950) considers that the flat epithelium of the thin segment is cytologically ill constituted for a function requiring as much metabolic energy as is presumably needed in water absorption against an osmotic gradient. This question touches the unknown cellular mode of action of the antidiuretic hormone which is most easily imagined as involving the active transport of water by an effect on an enzyme system in a segment of tubular epithelial cells. The results of NICHOLSON[3], who found that the dog kidney after poisoning with cyanide, loses its concentrating power while normal glucose reabsorption is retained, suggest that an oxidative system may be responsible for the performance of much of the osmotic work. It seems thus possible that the antidiuretic hormone exerts its typical action on the mammalian tubule by modifying the metabolism of the epithelial cells in some segment or segments, and that this effect in turn alters the rate of transfer of water across the cell membrane. The flatness of the epithelium of the thin segment is not necessarily incompatible with such a mechanism: admittedly epithelium on the only other site of the mammalian body where forces other than those of osmosis determine the rate of water transport viz. that of the small intestine (VISCHER and ROEPKE[4]) consists of high columnar cells but the investigations of SMITH[5] and BEVELANDER[6] on the branchial epithelium of fish grills demonstrate that considerable osmotic work can be performed by flat squamous cells[7].

Other modes of action of ADH in mammals have been considered. For instance, SCHAUMANN[8] has suggested that renal water reabsorption is increased because the neurohypophysial vasopressor principle constricts Henle's tube and thus prolongs the retention of the glomerular filtrate in the tubules. This hypothesis seems difficult to apply as it fails to account for the water movement against the osmotic gradient. All the same, a constrictor action of vasopressin on the mammalian nephron should not be too rigidly excluded; but it is more likely to be exerted on its vascular component. Opinion on the stabilithy of the glomerular filtration rate and renal plasma flow in mammals have changed during recent years. Several factors (body water load, protein and salt content of the diet) have been shown to influence the level of glomerular filtration in different mammalian species. Moreover, while the renal vessels of some species seem to be highly resistant to the action of posterior pituitary extracts, filtration rate (and renal plasma flow) in others changes in response to very small doses of neurohypophysial principles. For example SHANNON[1] showed that slow infusion of 1 to 350 mU. pituitrin per hour did not influence the renal blood flow in dogs. On the other hand marked changes of glomerular filtration rate were observed in rats after the administration of as little as 0·3 mU./kg vasopressin (DICKER and HELLER[2]). A glomerular antidiuretic effect can thus not be ruled out as participating in the physiological action of the neurohypophysis in some mammalian species or orders.

Reptiles. There is some difficulty in visualizing the physiological action of the neurohypophysis on the water metabolism of reptiles. This vertebrate class lacks, so far as known, the effector mechanisms of the phyletically adjacent classes. That is to say, reptiles show neither the rapid water movement through the skin which in amphibians is influenced by the neurohypophysial water balance factor nor are their renal tubules specifically adapted for water reabsorption beyond the plasma osmotic level (BURIAN, SMITH[3]). The latter fact has quite recently been confirmed by SHANNON[4] who used inulin clearances to investigate the response of the alligator kidney to dehydration. He found that the inulin U/P ratio in the dehydrated animals remained at a hypotonic level; nor did it alter much after rehydration, i.e. changes in urine flow were due exclusively to alteration of the glomerular filtration rate.

Thus renal regulation of water excretion in the reptile does not seem to follow the mammalian pattern. There are, however, several considerations which suggest that reptiles have a neurohypophysial mechanism which regulates the conservation of body water: (a) the neurohypophysis is a well-developed

[1] I. SPERBER, Zool. Bidrag. Uppsala *22*, 249 (1944). – H. KOEFOED, Scand. J. Clin. Lab. Invest. *1*, 340 (1949).

[2] H. W. SMITH, Bull. N. Y. Acad. Med. (in the press 1950).

[3] T. F. NICHOLSON, Biochem. J. *45*, 112 (1949).

[4] M. B. VISCHER and R. R. ROEPKE, Amer. J. Physiol. *144*, 468 (1945).

[5] H. W. SMITH, Quart. Rev. Biol. *7*, 1 (1932).

[6] G. BEVELANDER, J. Morphol. *57*, 335 (1935); Biol. Bull. *91*, 230 (1946).

[7] Note added on 8th Sept. 1950. The results of direct cryoscopy in kidney slices from dehydrated rats, reported by WIRZ, KUHN and HARGITAY at the XVIII International Physiological Congress at Copenhagen, support the concept that the thin segment is an important site of concentration.

[8] O. SCHAUMANN in HEFFTER's *Handbuch der experimentellen Pharmakologie* (Julius Springer, Berlin 1937, Ergänzungswerk III, p. 112).

[1] J. A. SHANNON, J. exper. Med. *76*, 371 (1942).

[2] S. E. DICKER and H. HELLER, J. Physiol. *104*, 353 (1946).

[3] R. BURIAN, Pflüg. Arch. ges. Physiol. *136*, 741 (1910). – H. W. SMITH, Quart. Rev. Biol. *7*, 1 (1932).

[4] J. A. SHANNON, 1st Macy Conference on Renal Function, in the press (1950).

structure in this vertebrate class (DE BEER[1]), (b) one species of reptile (*Tropidonotus natrix*) at least has been shown (HELLER[2]) to contain considerable amounts of the antidiuretic principle in its pituitary gland, (c) reptiles count some of the most aridly living species among its numbers, species therefore, in which a highly developed mechanism for the conservation of water can be expected. Assuming then that conservation of water is secured by the secretion of the neurohypophysis in reptiles as in other classes of vertebrates, on what effector mechanism could it be based? Some findings of BURGESS *et al.*[3] may supply the answer. These workers found that comparatively small subcutaneous doses of pitressin (1 mU/kg) have a pronounced antidiuretic action in a species of reptile, namely the alligator. On measuring glomerular filtration rate by means of xylose clearances they found further that even very small doses of pitressin decreased glomerular filtration almost proportionately to the urine flow. These results would be compatible with the assumption that conservation of body water in reptiles may be effected by a decrease in the volume of the glomerular filtrate.

A serious objection may be raised against regarding such a mechanism as of physiological importance. Decreasing the glomerular filtrate may mean not only a decrease of water lost in the urine but also a decrease of the glomerular excretion of metabolic waste products, clearly an unsatisfactory state if constriction of the renal vessels lasts for any length of time. However, it should be remembered that in reptiles the metabolic waste products and especially the most important one, uric acid, are eliminated not only be glomerular filtration but most likely to a much larger extent by tubular secretion (MARSHALL[4]), that reptiles have a renal-portal system, and that tubular secretion has been shown to be highly independent of glomerular activity (e.g. by PITTS, and CHAMBERS and KEMPTON[5] for the morphologically related avian kidney). Thus tubular secretion may be of greater importance for the reptile in which a glomerular antidiuresis is a physiological phenomenon than for a mammal in which conservation of water can be achieved without interference with the glomerular blood flow.

Amphibians. The temporary increase in body water which occurs when amphibians are injected with neurohypophysial extracts has been shown as already mentioned, to be due to the oxytocic fraction which appears to produce this effect by a double mechanism. Firstly there is good evidence for an increase of water intake through the skin: when both kidneys were removed in a series of frogs and some of the animals injected with posterior pituitary extract, it was found (BRUNN[1]) that all the nephrectomized animals, when kept in water, increased in weight but that the water intake of the injected frogs was much above that of the controls. Essentially the same results were obtained by BIASOTTI[2] who tied the ureters, and by HOUSSAY and POTICK, STEGGERDA, and REY[3] who ligatured the cloaca. A direct effect of neurohypophysial extracts on water movement through amphibian skin was demonstrated by NOVELLI[4] who, in experiments on toads, measured the fluid uptake of skin pouches with intact circulation. He reported that the skin pouches of animals injected with posterior pituitary extracts contained approximately 90% more fluid than the pouches of the control animals. This extrarenal effect of the oxytocic fraction on amphibian water metabolism seemed *a priori* unlikely to be the only cause for the retention of water (HELLER[5]). As HOWES[6] pointed out, if the increase in body weight is caused solely by the increased water uptake through the skin and renal water excretion was unaffected, it would be necessary to assume that the amphibian kidney works normally so near to the upper limit of its excretory capacity that it is unable to cope with an extra water inflow. However, there is no question that the frog's kidney has a considerable excretory reserve (KRAUSE, REY, GRANAAT, and HILLESUM[7]). The rate of urine flow should therefore, increase, if we assume that the extrarenal effect is the only one operating. This has been reported by early investigators (OEHME, BRUNN[8]) but later workers (HOUSSAY and POTICK, REY, PASQUALINI, HOWES[9]) demonstrated an inhibition of urine flow which could only be ascribed to an action on the kidney. A renal action of neurohypophysial extract is also implied in the results of BOYD and WHYTE[10] who found that the loss of water injected into frogs *kept out of water* could be inhibited by the administration of mam-

[1] G. R. DE BEER, *The comparative anatomy, histology, and development of the pituitary body* (Edinburgh, Oliver Boyd, 1926).
[2] H. HELLER, J. Physiol. *101*, 317 (1942).
[3] W. W. BURGESS, A. M. HARVEY, and E. K. MARSHALL, J. Pharmacol. *49*, 237 (1933).
[4] E. K. MARSHALL, Physiol. Rev. *14*, 133 (1934).
[5] R. F. PITTS, J. cell. comp. Physiol. *11*, 99 (1938). – R. CHAMBERS and R. T. KEMPTON, J. cell. comp. Physiol. *3*, 131 (1933).

[1] F. BRUNN, Z. ges. exp. Med. *25*, 170 (1921).
[2] A. BIASOTTI, C. r. Soc. Biol. *88*, 361 (1923).
[3] B. A. HOUSSAY and D. POTICK, C. r. Soc. Biol. *101*, 940 (1929). – F. R. STEGGERDA, Amer. J. Physiol. *98*, 255 (1931). – P. REY, C. r. Soc. Biol. *118*, 949 (1935).
[4] A. NOVELLI, Riv. Soc. Arg. Biol. *12*, 163 (1936).
[5] H. HELLER, Biol. Rev. *20*, 147 (1945).
[6] N. H. HOWES, J. exp. Biol. *17*, 128 (1940).
[7] F. KRAUSE, Z. Biol. *87*, 167 (1928). – P. REY, C. r. Soc. Biol. Paris *118*, 1390 (1935). – D. GRANAAT and J. HILLESUM, Arch. neerl. Physiol. *22*, 268 (1937).
[8] C. OEHME, Z. ges. exp. Med. *9*, 112 (1919). – F. BRUNN, Z. ges. exp. Med. *25*, 170 (1921).
[9] B. A. HOUSSAY and D. POTICK, Riv. Soc. Arg. Biol. *5*, 66 (1929). – P. REY, C. r. Soc. Biol., Paris *118*, 1390 (1935). – R. Q. PASQUALINI, *Papel de la hipófisis en la regulación de la diuresis* (El Ateneo, Buenos Aires, 1938). – N. H. HOWES, J. exp. Biol. *17*, 128 (1940).
[10] E. M. BOYD and D. W. WHYTE, Amer. J. Physiol. *125*, 415 (1939).

malian posterior pituitary extracts. These authors stated further that the pituitary factor responsible for this inhibitory effect is chiefly contained in the oxytocic fraction. Proof of the direct renal vascular effect of this fraction has been recently obtained by W. H. SAWYER[1] who, when visualizing the glomerular blood flow in the exposed frog kidney, observed that pituitrin and pitocin were equally effective in causing constriction of the afferent arterioles and cessation of glomerular blood flow. The same dose of pitressin had no effect—which is in agreement with earlier results of BURGESS *et al.*[2].

It would thus appear that the renal effect of neurohypophysial extracts in amphibians resembles that in reptiles (in that a glomerular antidiuresis is produced) with the important difference that the inhibitory activity resides in the oxytocic and not in the vasopressor-antidiuretic fraction. Similarly, as in the reptilian kidney, glomerular antidiuresis as a means of conservation of body water would appear to be well compatible with the continuation of those renal functions of amphibians which are concerned with the elimination of metabolic waste products. The presence of a renal-portal system points again to a high degree of tubular "autonomy" and at the importance of secretory processes. It is therefore neither surprising that specific secretory cells have been described in the nephrons of various amphibian species (STEEN, BARGMANN[3]), nor that clearance experiments prove a high efficiency of the frog kidney for tubular secretion. It seems particularly significant that as in reptiles, the main nitrogenous metabolic waste product – though in this case urea – has been shown (MARSHALL and CRANE; CRANE, MARSHALL[4]), to be partly eliminated by tubular secretion.

Fishes: The neurohypophysis of fishes contains undoubtedly both the mammalian antidiuretic and the amphibian water balance factors. Comparatively large amounts of these principles have been found in marine teleosts (HELLER[5]), elasmobranch glands would appear to contain much less (HELLER[6]). However, attempts (admittedly not numerous) to demonstrate an effect of neurohypophysial extracts on the water metabolism of fishes have so far failed. BURGESS *et al.*[7] found that pitressin did not effect the volume of the urine in the catfish; BOYD and DINGWALL[1] did not observe a change in the body water content when five species of fresh water teleosts were injected with 5 to 20 units of pituitrin. Further experiments on fishes are very desirable and in particular an investigation of the action of neurohypophysial extracts on the teleost gill.

The neurohypophysis and mineral metabolism

Interest in the question whether doses of neurohypophysial hormones sufficiently small to be of a physiological order have a specific effect on renal electrolyte excretion has recently revived. But it cannot be said that we see clearly, in spite of the considerable volume of work published during the last two years. Earlier work was concerned almost exclusively with the effect of posterior pituitary extracts on chloride excretion and all investigators appear to have found an increase of chloride *concentration* in the urine. This would only show that the reabsorption of chloride is unaffected by the change in water reabsorption induced by ADH and suggests further that the two processes are highly independent. However, under certain—not very well defined—circumstances, posterior pituitary extracts increase the absolute amount of chloride excreted, that is to say they have a chloruretic effect. It seems not unlikely that one of the factors favouring this effect is a high chloride content of the diet (see e.g. UNNA and WALTERSKIRCHEN, HELLER and STEPHENSON[2]). The neurohypophysial fraction which exerts the chloruretic effect is, astonishingly enough, not the same in all mammalian species. There is good evidence that the principle responsible for the decreased tubular chloride absorption observed in dogs (ANSLOW, WESSON, BOLOMEY, and TAYLOR; SARTORIUS and ROBERTS[3]) resides in the vasopressor-antidiuretic fraction. However, it has been shown equally clearly (KUSCHINSKY and BUNDSCHUH; FRASER; DICKER and HELLER; SCHAUMANN and SCHMIDT; HELLER and STEPHENSON[4]), that the chloruretic effect of the posterior pituitary extracts on rats—when it occurs—is exerted by the oxytocic fraction. The slight diuretic action of oxytocin in this species is possibly secondary to this effect.

In normal men the intravenous injection of 5 to 100 mU. of vasopressin has been reported (CHALMERS,

[1] W.H. SAWYER (Dept. of Biology, Harvard University), personal communication.

[2] W.W. BURGESS, A.M. HARVEY, and E.K. MARSHALL, J. Pharmacol. *49*, 237 (1933).

[3] W.B. STEEN, Anat. Rec. *61*, 45 (1935). – W. BARGMANN, Z. Zellf. microsc. Anat. *25*, 764 (1937).

[4] E.K. MARSHALL and M.M. CRANE, Amer. J. Physiol. *70*, 465 (1924). – M.M. CRANE, Amer. J. Physiol. *81*, 232 (1927). – E.K. MARSHALL, J. cell. comp. Physiol. *2*, 349 (1932).

[5] H. HELLER, J. Physiol. *99*, 246 (1941a); *100*, 125 (1941).

[6] H. HELLER, J. Physiol. *99*, 246 (1941a); Biol. Rev. *20*, 147 (1945).

[7] W.W. BURGESS, A.M. HARVEY, and E.K. MARSHALL, J. Pharmacol. *49*, 237 (1933).

[1] E.M. BOYD and M. DINGWALL, J. Physiol. *95*, 501 (1939).

[2] K. UNNA and L. WALTERSKIRCHEN, Arch. exp. Path. Pharm. *181*, 681 (1936). – H. HELLER and R.P. STEPHENSON, Nature *165*, 189 (1950).

[3] W.P. ANSLOW, L.G. WESSON, A.A. BOLOMEY, and J.S. TAYLOR, Fed. Proc. *7*, 3 (1948). – O.W. SARTORIUS and K. ROBERTS, Endocrinology *45*, 273 (1949).

[4] G. KUSCHINSKY and H.E. BUNDSCHUH, Arch. exp. Path. Pharmakol. *192*, 683 (1939). – A.M. FRASER, J. Physiol. *101*, 236 (1942). – S.E. DICKER and H. HELLER, J. Physiol. *104*, 353 (1946). – O. SCHAUMANN and L. SCHMIDT, Arch. exp. Path. Pharm. *205*, 367 (1948). – H. HELLER and R.P. STEPHENSON, Nature *165*, 189 (1950).

LEWIS, and PAWAN[1]) to leave the absolute chloride output unaffected, or to decrease it slightly. Similar doses of oxytocin failed to have a chloruretic effect.

It is evident that chloride cannot be the only ion influenced, but older reports on the effect of posterior pituitary extract on the excretion of ions other than chloride are rather contradictory (see VAN DYKE[2]). The problem has recently been reinvestigated in man, the dog, and the rat with the emphasis on the effects on sodium and potassium excretion. It could thus be shown by ANSLOW and his coworkers[3] and by SARTORIUS and ROBERTS[3] that in dogs vasopressin causes an increase in the renal excretion of sodium. This natriuretic effect had been previously reported by SHANNON[4] who, however, obtained it after injection of small doses of undifferentiated posterior pituitary extract (pituitrin). It is not quite clear from SARTORIUS and ROBERTS' results whether the vasopressin natriuresis is linked with the changes of glomerular filtration rate which they reported, i.e. with renal vascular changes caused by the hormone. SARTORIUS and ROBERTS[3] found also that vasopressin increased potassium excretion in doses of 0·8 mU./kg/dog and the same action of vasopressin was observed in rats by HELLER and STEPHENSON[5], who used somewhat similar doses (0·3 mU./kg) in their experiments. We found, however, that in marked, contrast to the findings in dogs, vasopressin depressed sodium excretion in normal adults rats. SCHAUMANN[6] had independently observed the same effect with larger doses of the vasopressor-antidiuretic fraction. It is of interest that similar observations have recently been made in man: CHALMERS, LEWIS, and PAWAN[7] found that small intravenous doses of vassopressin *decreased*, if anything, renal sodium excretion in normal subjects. It would seem, therefore, that man resembles in this respect the rat rather than the dog. SCHAUMANN[8] found also that oxytocin exerted a marked natriuretic in rats; HELLER and STEPHENSON[9] using considerably smaller doses failed to observe a significant effect of oxytocin on sodium excretion in their series. Table IV summarizes the data just reported.

It had been previously shown by DICKER and HELLER[10] that doses of 0·3 mU. oxytocin/kg rat and amounts as low as 0·03 mU. vasopressin/kg rat produce well-marked changes in glomerular filtration rate and renal plasma flow, but there is insufficient evidence to decide at present whether the changes in tubular electrolyte absorption are correlated with these renal vascular actions of the two neurohypophysial fractions. The question is clearly of considerable interest; we shall not be able to speak of a specific or primary action of the neurohypophysial principles on tubular electrolyte absorption in various mammalian species until we know that the effects observed have not been elicited by renal vascular changes, be they either variations in the tone of the glomerular vessels or alterations in intrarenal blood distribution. An additional consideration is the fact that almost all investigations concerned with the effect of posterior pituitary extracts on electrolyte excretion have been performed on animals after administration of considerable amounts of water. Absorption of water from the alimentary tract and its distribution through the tissues involves, as shown by my colleague S. E. DICKER[1] in rats, well-defined phasic changes of sodium, potassium and chloride in plasma and tissues. The possibility cannot be excluded that such changes when modified by the neurohypophysial principles have a bearing on the rate of renal excretion of these electrolytes through the occurrence of chloruresis in non-hydrated dogs injected with small doses of pituitrin (UNNA and WALTERSKIRCHEN[2]) speaks against this assumption.

Table IV

Effects of the vasopressor-antidiuretic and of the oxytocic fraction on the renal excretion of chloride, sodium and potassium by different mammalian species

Species	Vasopressin			Oxytocin		
	Cl	Na	K	Cl	Na	K
Man	0 (–)	– (0)	?	0	0	?
Dog	+	+	+	0	?	?
Rat	0 (–)	– (0)	+	+	0	+*

0 unchanged + increased – decreased

* H. HELLER and R. P. STEPHENSON (1950).

There is thus no convincing evidence that the neurohypophysial principles play a role of importance in the physiological regulation of the metabolism of chloride, sodium, and potassium. This impression is supported by the fact that in diabetes insipidus, i.e. under conditions when the neurohypophysis is almost fully depleted of antidiuretic and oxytocic activity, (HEWER and HELLER; CAVALLERO and ZANCHI[3]), the plasma electrolyte levels and the sodium and potassium clearances are found to be normal (MCGAVACK, BOYD and GELVIN, HOLMES and GREGERSEN, WIL-

[1] T. M. CHALMERS, A. A. G. LEWIS, and G. L. PAWAN, J. Physiol., in the press (1950).

[2] H. B. VAN DYKE, *The Physiology and Pharmacology of the Pituitary Body* (University of Chicago Press, Chicago, 1936).

[3] W. P. ANSLOW, L. G. WESSON, A. A. BOLOMEY, and J. S. TAYLOR, Fed. Proc. *7*, 3 (1948). – O. W. SARTORIUS and K. ROBERTS, Endocrinology *45*, 273 (1949).

[4] J. A. SHANNON, J. exp. Med. *76*, 371 (1942).

[5] H. HELLER and R. P. STEPHENSON, Nature *165*, 189 (1950).

[6] O. SCHAUMANN, Experientia *5*, 360 (1949).

[7] T. M. CHALMERS, A. A. G. LEWIS, and G. L. PAWAN, J. Physiol., in the press (1950).

[8] O. SCHAUMANN, Exper. *5*, 360 (1949).

[9] H. HELLER and R. P. STEPHENSON, Nature *165*, 189 (1950).

[10] S. E. DICKER and H. HELLER, J. Physiol. *104*, 353 (1946).

[1] S. E. DICKER, Biochem. J. *43*, 453 (1948).

[2] K. UNNA and L. WALTERSKIRCHEN, Arch. exp. Path. Pharmacol. *181*, 681 (1936).

[3] T. F. HEWER and H. HELLER, J. Path. Bact. *61*, 499 (1949). – C. CAVALLERO and M. ZANCHI, private communication.

LIAMS, and HENRY[1]). If the posterior pituitary participates in the regulation of electrolyte metabolism why is severe and chronic deficiency of its active principles not reflected in changes of plasma levels and renal clearance?

While much work has been directed at the problem of clarifying the effects of the neurohypophysis on the electrolyte metabolism of mammals, evidence on the same subject is exceedingly scanty for other vertebrate classes: BOYD and WHYTE[2] concluded from experiments on frogs out of water and injected with 1% sodium chloride solution, in which they recorded the interval required to reach the initial weight after injections of pituitrin, pitressin or pitocin, that pitressin had the greater stimulating effect on salt excretion. JORGENSEN, LEVI, and USSING[3] investigated the action of posterior pituitary extract and its fractions on sodium and chloride uptake and loss in axolotls. They found that vasopressin induced an increased uptake of salt through the skin of these animals while oxytocin promoted a rapid *loss* of sodium and chloride. JOHNSEN and USSING[4] showed later (1949) that anterior pituitary extracts had much the same effect as vasopressin, and USSING[5] concluded that corticotrophic hormone present as an impurity may have been responsible for the effect observed in the experiments with the neurohypophysial fraction. He does not state, however, whether his corticotrophic extracts were tested for contamination with posterior pituitary principles, which occur frequently in adenohypophysial extracts and, particularly in the corticotrophic fraction. (SEE DICKER and HELLER[6].)

Concluding remarks

Based on the facts and hypotheses presented, it seems permissible to set out a tentative scheme of the distribution of the effect or mechanisms of the neurohypophysis as related to the water metabolism of different classes of vertebrates. Such a survey contains obviously important lacunæ but some generalizations do emerge: the neurohypophysial principles influence the water metabolism of all vertebrate classes investigated, though further work is needed to ascertain that they do so in physiological doses in lower vertebrates. The effects in amphibians, reptiles and birds can be interpreted as directed at the same regulatory aim as that already established in mammals, viz. at the conservation of body water. The effector systems seem to differ from vertebrate class to vertebrate class, phyletically adjacent classes sharing the same mechanisms to some degree. For instance the renal tubular effect is very prominent in mammals and is possibly also present in birds. The glomerular antidiuretic effect appears to occur in amphibians, reptiles, and probably birds, i.e. in the type of vertebrate kidney in which the tubules are well adapted to the secretion of metabolic waste products; it disappears or loses in importance in the mammal.

Table V

Effects of neurohypophysial extracts on the water metabolism of different classes of vertebrates (V = effect exerted mainly by vasopressor-antidiuretic fraction, O = effect exerted mainly by oxytocic fraction)

	Extrarenal effect	Renal effects	
	Increased water uptake through skin	Changes in glomerular filtration rate (glomerular antidiuresis)	Increase in tubular water reabsorption (tubular antidiuresis)
Fishes (Teleosts) . . .	none	not investigated	none
Amphibians . .	pronounced (O)	present (O)	none
Reptiles . . .	doubtful (O)	pronounced (V)	none
Birds	none	present (V)	feeble (V)
Mammals . . .	none	in some species? (V)	pronounced (V)

In conclusion, two further remarks: the concept that the same hormone fulfills the same purposive function by acting on morphologically non-homologous effectors in different groups of vertebrates is not quite foreign to endocrinology. As pointed out by OGDEN, (1950)[1] it obtains in the case of the adenohypophysial lactogenic hormone which acts on the crop glands of the pigeon and on the mammary glands of mammals. Conversely, it seems quite probable that the same endocrine principle, when secreted in two different vertebrate classes may be adapted to a different purpose. Thus the oxytocic fraction of neurohypophysial extract appears to be concerned with certain effects on water and mineral metabolism but that does not exclude the possibility that in mammals it is adapted to an entirely different action, namely a physiological effect on the pregnant uterus.

Zusammenfassung

Es wird zunächst gezeigt, daß sich die typischen Wirkungen von Säugetier-Hypophysenhinterlappenextrakten in der Neurohypophyse aller bisher untersuchten Wirbeltierklassen nachweisen lassen. Das Vor-

[1] T.H.MCGAVACK, L.J.BOYD, and P.GELVIN, J. clin. Endocrin. *2*, 551 (1942). – J.H.HOLMES and M.I.GREGERSEN, Amer. J. Med. *4*, 503 (1948). – R.H.WILLIAMS and C.HENRY, Ann. intern. Med. *27*, 84 (1947).

[2] E.M.BOYD and D.W.WHYTE, Amer. J. Physiol. *125*, 415 (1939).

[3] C.B.JORGENSEN, H.LEVI, and H.H.USSING, Acta physiol. Scand. *12*, 350 (1946).

[4] V.K.JOHNSEN and H.H.USSING, Acta physiol. Scand. *17*, 38 (1949).

[5] H.H.USSING, Physiol. Rev. *29*, 127 (1949).

[6] S.E.DICKER and H.HELLER, J. Pharmacol. *83*, 11 (1945).

[1] E.OGDEN, Texas Reports on Biol. and Med., in the press.

kommen homologer Wirkstoffe in Wirbellosen konnte bis heute nicht überzeugend bewiesen werden. Die Extrakte von allen Wirbeltierhypophysen enthalten demnach ein diuresehemmendes, gefäßverengerndes und uteruserregendes Hormon (oder mehrere derartige Hormone) und steigern die Wasseraufnahme von Amphibien. Diesem qualitativ gleichen Verhalten stehen aber anscheinend Unterschiede in der quantitativen Verteilung der Hinterlappenwirkstoffe gegenüber. So läßt sich zeigen, daß z. B. in Säugern das diuresehemmende Hormon überwiegt, während Froschneurohypophysen hauptsächlich den in der Oxytocinfraktion enthaltenen Wirkstoff enthalten, welcher den Wasseraustausch von Amphibien beeinflußt («water balance principle»).

Weiterhin wird gezeigt, daß Neurohypophysenextrakte in allen Wirbeltierklassen den Wasserhaushalt beeinflussen können. Der Sinn dieser Wirkungen ist stets derselbe, nämlich auf Einsparung von Wasser gerichtet. Der Wirkungsmechanismus und damit der hormonale Angriffspunkt wechselt aber von Wirbeltierklasse zu Wirbeltierklasse. So wird die Antidiurese in Säugetieren vorwiegend durch Erhöhung der tubulären Wasserresorption, auch gegen das osmotische Druckgefälle, erzeugt. In Vögeln ist dieser Mechanismus bedeutend schwächer entwickelt; Antidiurese infolge Nierengefäßverengung (und damit Erniedrigung der Filtratmenge) ist mitbeteiligt. Bei Reptilien scheint dieser Erfolgsweg von überwiegender Wichtigkeit zu sein; er ist anscheinend auch bei Amphibien vorhanden. Diese Art von Antidiurese läßt sich also bei jenen Wirbeltierklassen zeigen, bei denen sich erstens infolge der Verdopplung der zuführenden Nierengefäße eine gewisse Autonomie der Harnkanälchen annehmen läßt und bei denen zweitens die aktive tubuläre Sekretion von Stoffwechselendprodukten d.h. vor allem von Harnsäure und Harnstoff nachzuweisen ist. Bei Amphibien läßt sich überdies eine Wirkung des Hinterlappens auf die Wasseraufnahme durch die Haut, somit ein extrarenaler Angriffspunkt feststellen. Der Nachweis einer Beeinflussung des Wasserhaushaltes von Fischen durch Neurohypophysenextrakte ist bisher nicht gelungen.

Abschließend wird erörtert, inwieweit die vorliegenden Untersuchungen eine Beteiligung der Neurohypophysenhormone an der Regulierung des Mineralhaushaltes sicherstellen lassen. Ein Vergleich der Einwirkung kleiner Dosen auf die renale Ausscheidung von Chloriden, Natrium und Kalium in verschiedenen Säugetierarten spricht aus mehreren Gründen gegen eine physiologische Rolle der Neurohypophyse im Mineralstoffwechsel und führt zu der Annahme ihrer primären Bedeutung für die Regulierung des Wasserhaushaltes. Die Wirkung der neurohypophysären Wirkstoffe auf die Elektrolytausscheidung der niedrigeren Wirbeltierklassen muß allerdings noch weiter untersucht werden.

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. – Für die kurzen Mitteilungen ist ausschließlich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed by their correspondents.

The Chemistry of Biphenyl

The semiquantitative theoretical treatment of chemical reactivity, based upon the LCAO molecular orbital approximation, which has been carried through for several hydrocarbons[1], is equally applicable to the hydrocarbon biphenyl. Biphenyl is an alternant hydrocarbon[2] so the calculated π-electron densities all reduce to unity, just as in the case of biphenylene[3]. In such cases use must be made of the second order terms known as atomic self-polarizabilities[4]. The polarizabilities for the three positions capable of being substituted in biphenyl are listed in Table I. The results indicate chemical reactivities of these positions in the order 2, 4, 3.

[1] R.D. BROWN, Trans. Faraday Soc. *44*, 984 (1948); *ib. 45*, 296 (1949); *ib. 46*, 146 (1950).

[2] C.A. COULSON and G.S. RUSHBROOKE, Proc. Camb. Phil. Soc. *36*, 193 (1940).

[3] R.D. BROWN, *ib. 45*, 296 (1949).

[4] C.A. COULSON and H.C. LONGUET-HIGGINS, Proc. Roy. Soc., A, *191*, 39 (1947).

This result is in good agreement with the known chemistry of biphenyl, but REMICK[1] has suggested on qualitative grounds that the low yield of the ortho

Table I

Polarizabilities

Position	π $(-1/\beta)$
2	·423
3	·396
4	·411

isomer on nitration of biphenyl is due to the greater reactivity of the para-position. The present results

[1] A.E. REMICK, *Electronic Interpretations of Organic Chemistry*, p. 103 (1943) (John Wiley & Sons, New York).