J. Physiol. (1941) 99, 246–256 577.174.92:596

# THE DISTRIBUTION OF THE PITUITARY ANTIDIURETIC HORMONE THROUGHOUT THE VERTEBRATE SERIES

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(*Received* 19 *August* 1940)

BURGESS, HARVEY AND MARSHALL stated in 1933 that the antidiuretic hormone induces accelerated reabsorption in the avian and mammalian kidney only. Since the thin segment of the loop of Henle is present in the kidney of these classes of vertebrates only, they assumed that the thin segment is the site of the hormone's action. Gersh's [1934] histochemical observations on the site of water reabsorption in rats and rabbits supported this hypothesis.

Is there then a correlation between the phylogenetic development of the antidiuretic hormone of the posterior pituitary gland and the development of Henle's loop in the vertebrate kidney? Does the pituitary gland of lower vertebrates (on which extract of mammalian pituitary glands are reported to have no antidiuretic action) produce a principle identical with the antidiuretic hormone of the mammal? The posterior lobe, or, to use the more appropriate term, the pars nervosa of the pituitary gland of some lower vertebrates (teleost fishes, amphibians, reptiles), has been shown to contain a pressor principle [Herring, 1913], but no demonstration of the antidiuretic hormone, whether qualitative or quantitative, seems to have been attempted. Even data on the antidiuretic hormone content in higher vertebrates, i.e. birds or mammals, are very meagre. Quantitative determinations of the antidiuretic hormone of the pars nervosa of the pituitary gland seem to be recorded for human beings [Lampe, 1926] and cats only [Fisher, Ingram & Ranson, 1938]. The presence of a pituitary antidiuretic principle in birds has been noted by De Lawder, Tarr & Geiling [1934].

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The present series of experiments consists of quantitative determinations of the antidiuretic activity of pituitary glands of representatives of the following classes of vertebrates: mammals, birds, amphibians and fishes. In addition, an attempt has been made to compare the antidiuretic and pressor activity of such extracts. This seemed of particular interest as it has recently been shown [Heller, 1939, 1940] that a non-pressor but antidiuretic extract can be prepared from the posterior lobe of mammalian glands. The finding of a significant disproportion between the amounts of the two principles would be further evidence that these two substances are not identical.

## Methods

The choice of animals belonging to the various vertebrate classes was limited by the circumstances. Reptiles were unobtainable. All animals with the exception of the fishes were killed by decapitation. Since fish could not be obtained living, the pituitary glands of dead fishes which had been kept in a refrigerator for various lengths of time were used. The whole pituitary glands and the parts of the brain proximal to the gland were used for the preparation of extracts. The loss of active material in structures outside the hypophysis proper is thus excluded.

Extracts were prepared by mashing the material with 1·0 c.c. of 0·25 % acetic acid in 0·9 or 0·6 % saline and boiling for 6 min. The extract was then filtered and the residue washed twice with 1·0 c.c. of saline. The filtrate consisted of a clear colourless fluid. The extracts were assayed on the day of preparation. Extracts of approximately equal amounts of indifferent brain tissue prepared in an identical manner were used for control observations.

*Methods of assay.* Spinal cats were used for the estimation of pressor potency. Two methods were used for the antidiuretic assay. (1) Subcutaneous injection into rats [Burn, 1931]; this method, though quite satisfactory for assays of pure extracts, is open to the objection that impurities have been shown to retard, inhibit or in certain cases, augment the action of the posterior pituitary antidiuretic principle [Heller & Urban, 1935; Noble, Rinderknecht & Williams, 1939] presumably by changing the rate of absorption from the subcutaneous tissue. Estimations were therefore made by (2) intravenous injections into rabbits. Rabbits weighing 1·6–2·5 kg. were kept on a standard diet of bran, oats and cabbage. At the beginning of the experiment they were given 5 % of their body weight of tepid water by stomach tube. A second and if

necessary a third dose of water was given later. The antidiuretic hormone content of samples of unknown strengths was estimated in terms of doses of pure pitressin (Parke, Davis and Co.). The rabbits were first tested by injections of 1 milliunit (mU.)[1] of pitressin per rabbit. A series of dilutions of the unknown samples was prepared and matched with the response to the injection of pure pitressin. The method has the advantage of great sensitivity, the rabbit having been shown to react to as little as 0·5 mU. of the antidiuretic principle per animal [Heller, 1937; Walker, 1939]. A method of such sensitivity proved to be essential for the estimation of the antidiuretic hormone content of the glands of certain small animals. The method has the disadvantage that the water diuresis of some rabbits is sometimes inhibited spontaneously. However, these very occasional interruptions have never lasted longer than 30 min. and were of a different magnitude from those caused by the injection of 1·0 mU. of pure pitressin. This disadvantage was further reduced by the use in the same experiment of several (usually three) rabbits injected with doses of the same extract. The method, while not as accurate as that of Burn, when used for a pure preparation, yielded as satisfactory results as those obtained by Walker [1939]. It should be added that an antidiuretic action of the control extracts prepared from brain samples of each species of animal was in no case observed.

## Results

### *The antidiuretic hormone content of the pituitary glands of various classes of vertebrates*

Preliminary "range-finding" experiments are not quoted. Experiments shown in figures are not included in the tables.

A. *Mammals* (Fig. 1, Tables I and II).

Fisher *et al.* found approximately 10 units of antidiuretic activity in the posterior pituitary lobe of the cat. This gives an antidiuretic hormone content of 400 mU. per 100 g. body weight if 2·5 kg. is assumed as the average weight of their cats. This figure is of the same order of magnitude as those shown for the rats and mice in the present series (Table VIII).

B. *Birds* (Fig. 2, Tables III and IV).

C. *Amphibians* (Fig. 3, Table V).

An attempt was made to estimate the antidiuretic potency of frog pituitary extracts by Burn's method of subcutaneous injections into rats. However, control experiments with extracts of indifferent brain tissue

[1] The term milliunit (mU.) is used to denote the activity of 0·0001 c.c. of pituitary (posterior lobe) B.P. extract.

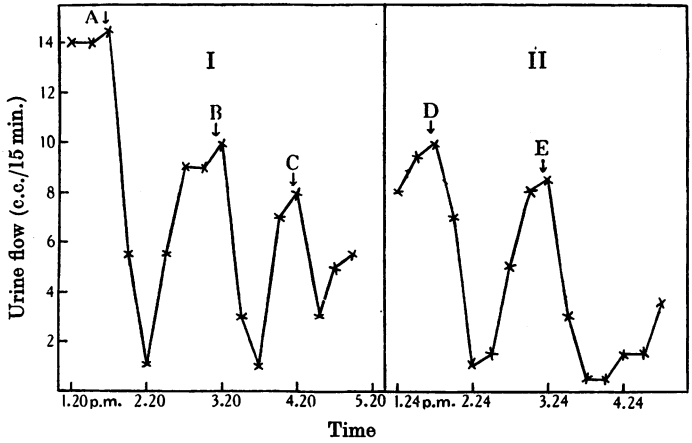


Fig. 1. Estimation of antidiuretic hormone content of a *rat* pituitary gland. Rat no. 6, 315 g. ♂. Extract of pituitary gland diluted to 1000 c.c. I = rabbit no. 56. At 10.23 a.m. and at 12.30 p.m. 5 % of body weight of water by stomach tube. *A* = 1·0 c.c. of rat pituitary extract i.v., *B* = 1·0 mU. pitressin i.v., *C* = 0·8 c.c. of rat pituitary extract i.v. II = rabbit no. 19. At 10.27 a.m. and at 12.32 p.m. 5 % of body weight of water. *D* = 1·0 mU. pitressin i.v., *E* = 1·25 c.c. of rat pituitary extract i.v. It will be noted that 0·8 c.c. of the rat pituitary extract (*C*) had a smaller and 1·25 c.c. (*E*) a greater inhibitory effect on the water diuresis than 1·0 mU. pitressin (*B* and *D*). The antidiuretic activity of the rat pituitary extract was equivalent, therefore, to less than 1250 and more than 800 mU. pitressin. 1·0 c.c. of the extract (*A*) has much the same effect as 1·0 mU. pitressin (*B*) indicating that the rat pituitary extract contained approximately 1000 mU. of an antidiuretic principle. A third rabbit was used in the same experiment and gave essentially similar results.

TABLE I. The antidiuretic hormone content of *rat* pituitary glands. For description of method of estimation of antidiuretic potency see "Methods" and legend of Fig. 1

| No. | Sex | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin |
|---|---|---|---|
| 1 | ♂ | 370 | > 500 <1000 |
| 2 | ♂ | 320 | > 800 <1200 |
| 3 | ♂ | 271 | >1000 <1200 |
| 4 | ♂ | 277 | >1200 <1500 |
| 5 | ♂ | 260 | > 800 <1000 |

TABLE II. The antidiuretic hormone content of *mouse* pituitary glands

| No. | Sex | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin |
|---|---|---|---|
| 1 | ♂ | 17·2 | >20 <40 |
| 2 | ♀ | 20·5 | >30 <50 |
| 3 | ♂ | 19·7 | >30 <50 |
| 4 | ♀ | 20·5 | >30 <60 |
| 5 | ♂ | 18·4 | >30 |
| 6 | ♂ | 27·9 | >20 <40 |
| 7 | ♂ | 21·4 | >30 <50 |

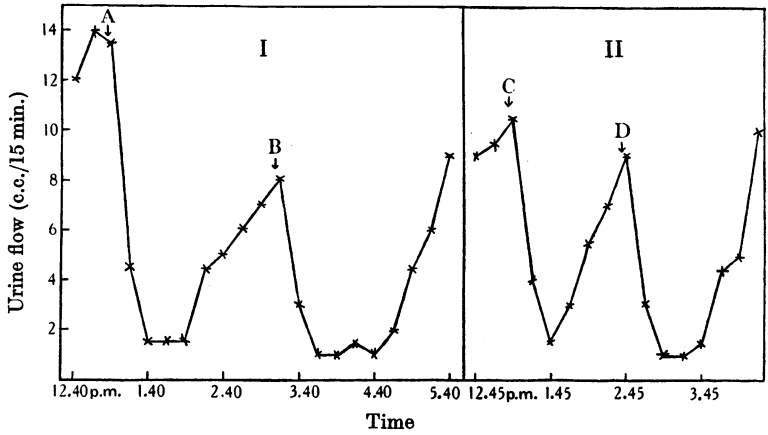


Fig. 2. Estimation of antidiuretic hormone content of a *pigeon* pituitary gland. Pigeon no. 13, 445 g. ♀. Extract of pituitary gland diluted to 20·0 c.c. I=rabbit no. 52. At 10.18 a.m. and 12.00 p.m. 5 % of body weight of water by stomach tube. $A$=1·0 mU. pitressin i.v., $B$=1·0 c.c. of pigeon pituitary extract i.v. II=rabbit no. 72. At 10.21 a.m. and 12.04 p.m. 5% of body weight of water. $C$=0·5 c.c. of pigeon pituitary extract i.v., $D$=1·0 mU. pitressin i.v. 1·0 c.c. of the pigeon pituitary extract had a greater and 0·5 c.c. a smaller inhibitory effect on the water diuresis than 1·0 mU. of pitressin. The antidiuretic activity of the extract was, therefore, equivalent to more than 20 and less than 40 mU. pitressin. A third rabbit, injected with doses of the same extract, gave essentially similar results.

TABLE III. The antidiuretic hormone content of *pigeon* pituitary glands

| No. | Sex | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin |
|---|---|---|---|
| 1 | ♂ | 415 | >25 <50 |
| 2 | ♂ | 487 | >20 <40 |
| 3 | ♂ | 436 | <50 |
| 4 | ♀ | 474 | >20 <40 |
| 5 | ♂ | 381 | >20 <40 |
| 6 | ♀ | 434 | >20 <40 |

TABLE IV. The antidiuretic hormone content of *drake* pituitary glands

| No. | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin |
|---|---|---|
| 1 | 1850 | >80 <160 |
| 2 | 1800 | >70 <100 |
| 3 | 1930 | >50 < 80 |

were not satisfactory, and the responses to injections of pituitary extracts when compared with those obtained by the rabbit method indicated a much higher antidiuretic potency (Table V, Exp. 1).

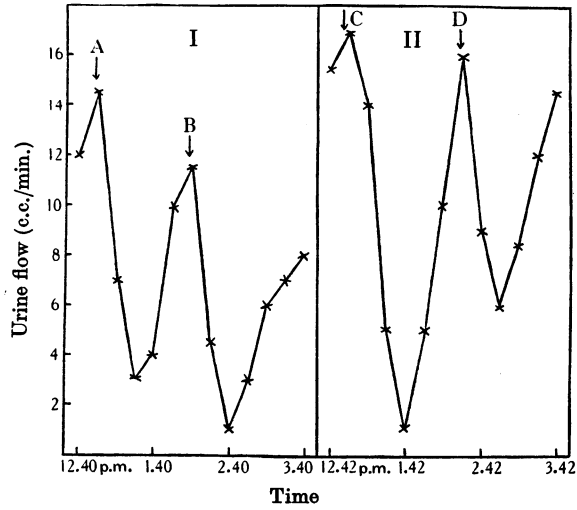


Fig. 3. Estimation of antidiuretic hormone content of a *frog* pituitary gland. Frog no. 12, 20·9 g. ♂. Extract of pituitary gland diluted to 2·0 c.c. I = rabbit no. 77. At 10.25 a.m. and at 12.07 p.m. 5 % of body weight of water by stomach tube. *A* = 0·5 c.c. of frog pituitary extract i.v., *B* = 1·0 mU. pitressin i.v. II = rabbit no. 93. At 10.28 a.m. and at 12.10 p.m. 5 % of body weight of water. *C* = 1·0 c.c. of frog pituitary extract i.v., *D* = 1·0 mU. pitressin i.v. The antidiuretic activity of the frog pituitary extract was equivalent to less than 4 and more than 2 mU. pitressin.

TABLE V. The antidiuretic hormone content of *frog* pituitary glands. Exp. 1, by rat method (see text). In Exps. 2 and 3 the extracts of two frog pituitary glands were pooled

| No. | Sex | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin |
|---|---|---|---|
| 1 | — | Four frogs | >10 |
| 2 | ♂, ♂ | 22·0, 28·0 | <4 |
| 3 | ♂, ♂ | 19·7, 22·0 | <6 |
| 4 | ♀ | 38·0 | > 2 <4 |
| 5 | ♂ | 23·0 | > 3 <6 |
| 6 | ♀ | 54·0 | > 3 <6 |
| 7 | ♀ | 20·8 | > 2 <4 |
| 8 | ♂ | 21·1 | > 2 <4 |

D. *Fishes.* (*a*) *teleosts* (Fig. 4, Table VI), (*b*) *elasmobranchs* (Table VII).

The figures given for the antidiuretic hormone content of cod, dogfish and skate pituitary glands are likely to be too low as the pituitary glands of those animals were not extracted in a fresh state.

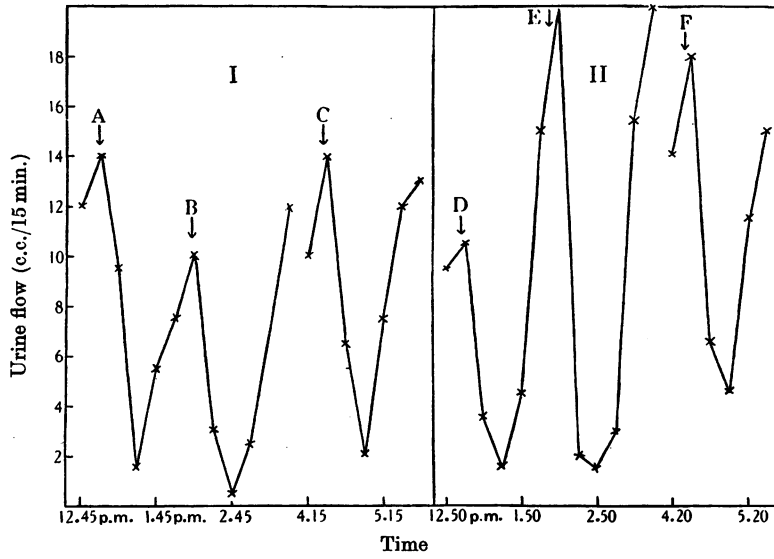


Fig. 4. Estimation of antidiuretic hormone content of a *cod* pituitary gland. Extract of pituitary gland diluted to 100 c.c. I = rabbit no. 52. At 10.15 a.m., 12.15 p.m. and 3.36 p.m. 5 % of body weight of water by stomach tube. *A* = 1·0 mU. pitressin i.v., *B* = 1·0 c.c. of cod pituitary extract i.v., *C* = 0·34 c.c. of cod pituitary extract i.v. II = rabbit no. 19. At 10.20 a.m., 12.20 p.m. and 3.38 p.m. 5 % of body weight of water. *D* = 1·0 mU. pitressin i.v., *E* = 0·5 c.c. of cod pituitary extract i.v., *F* = 0·34 c.c. of cod pituitary extract i.v. The antidiuretic activity of the cod pituitary extract amounted to clearly less than 300 mU. pitressin (*C*, *F*) but to more than 100 mU. pitressin (*B*). The effect of 0·5 c.c. of the diluted extract (*E*) was slightly greater than that of 1·0 mU. pitressin (*D*). Considering this and the pronounced inhibitory effect produced by as little as 0·34 c.c. (*C*, *F*) it seems justifiable to assume that the antidiuretic hormone content of this extract was equivalent to slightly over 200 mU. pitressin.

TABLE VI. The antidiuretic hormone content of *cod* pituitary glands. Cod heads only were obtained. In Exp. 3 the extracts of two pituitary glands were pooled

| No. | Antidiuretic hormone content of pituitary gland in milliunits pitressin | |
|---|---|---|
| 1 | >100 | <200 |
| 2 | >200 | <300 |
| 3 | > 50 | <150 |
| 4 | >100 | <200 |

TABLE VII. The antidiuretic hormone content of pituitary glands of *elasmobranch fishes*. In Exps. 1 and 2 the extracts of two pituitary glands were pooled

| No. | Weight of animal in g. | Antidiuretic hormone content of pituitary gland in milliunits pitressin | |
|---|---|---|---|
| | (*a*) Dogfish | | |
| 1 | 1222, 1035 | >2·5 | <5 |
| 2 | 1099, 824 | >2·0 | <5 |
| | (*b*) Skate | | |
| 3 | 511 | >1 | |
| 4 | 610 | >1 | |

*Pressor assays of pigeon and frog pituitary gland extracts*

Pressor assays were made with extracts of bird and frog pituitary glands. However, no significant difference between the antidiuretic and the pressor potencies of bird pituitary extracts was observed (Fig. 5). The average pressor activity of five pigeon glands equalled that of 69+19 mU. pitressin compared with an antidiuretic activity of about 30 mU. per gland (seven animals). The experiments with frog pituitary glands were inconclusive. The values for the antidiuretic hormone content

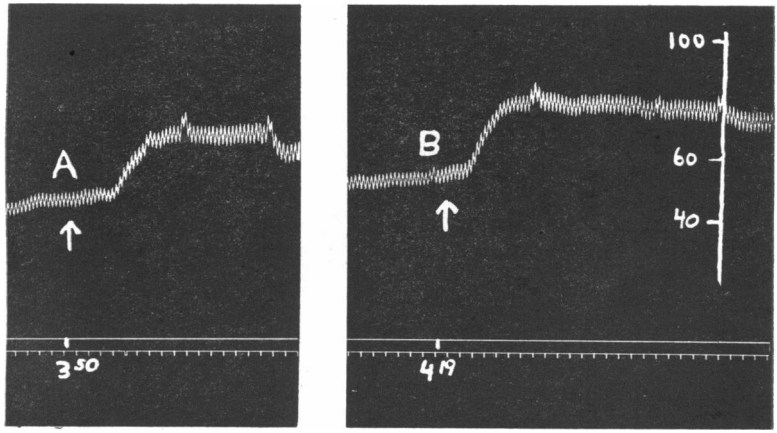


Fig. 5. Pressor activity of *pigeon* pituitary extract. Spinal cat. Extract of pigeon pituitary gland diluted to 3·0 c.c. *A* = intravenous injection of 1·0 c.c. of pigeon pituitary extract. *B* = intravenous injection of 20 mU. pitressin. The vasopressor activity of the whole gland equals approximately 60 mU. pitressin.

of cat and rat pituitary glands agree with Simon & Kardos's [1934] estimations of the average pressor activity of pituitary extracts of these animals. Simon & Kardos found an activity of 312 mU. for the cat and 445 mU. for the rat (per 100 g. body weight). The corresponding figures for the antidiuretic hormone content are 400 and 356 mU. respectively.

## Discussion

The pars nervosa of the pituitary gland is present throughout the vertebrate series [De Beer, 1926] and a pressor principle has been found in the pars nervosa of all classes of craniates with the exception of the most primitive groups, viz. cyclostomes and elasmobranch fishes. The present series of experiments demonstrates the presence of an antidiuretic principle in birds, amphibians and teleost and elasmobranch

fishes. Cyclostome fishes and reptiles were not investigated. The finding of an antidiuretic activity in elasmobranch glands is in apparent contrast with the absence of a pressor principle, as stated by Herring. However, this can be easily explained by the greater sensitivity of the antidiuretic assay.

Table VIII reveals a striking difference between the amounts of antidiuretic principle present in mammals and that of all other groups of vertebrates. The antidiuretic hormone content (calculated per 100 g. of animal) of any mammalian pituitary gland investigated was at least

TABLE VIII. Antidiuretic activity of vertebrate pituitary glands in terms of milliunits pitressin

| Vertebrate class or subclass | Species | No. of animals used | Average weight of animals in g. | Milliunits per gland | Milliunits per 100 g. animal |
|---|---|---|---|---|---|
| Mammals | Rat | 6 | 302·0 ± 41·0 | >920·0 <1230·0 | >305·0 <407·0 |
| | Mouse | 7 | 20·8 ± 2·2 | > 27·0 < 48·0 | >129·0 <230·0 |
| Birds | Pigeon | 7 | 439·0 ± 32·2 | > 21·0 < 41·7 | > 4·8 < 9·5 |
| | Drake | 3 | 1865·0 ± 81·3 | > 60·0 < 90·0 | > 3·2 < 4·8 |
| Amphibians | Frog (English) | 6 | 29·6 ± 13·6 | > 2·3 < 4·7 | > 7·8 < 15·8 |
| Teleost fishes | Cod | 6 | About 6000 | >116·0 < 216·0 | About 3·0 |
| Elasmobranch fishes | Dogfish | 4 | 1045·0 ± 94·0 | > 2·3 < 4·5 | > 0·2 < 0·4 |
| | Skate | 2 | 561·0 ± 62·0 | > 1·0 | > 0·2 |

eight times larger than that of any lower class of vertebrates. The question as to the possibility of a correlation between the phylogenetic development of Henle's loop and the post-pituitary hormonal mechanism can thus be answered in the affirmative.

The small amounts of the antidiuretic hormone found in bird hypophyses (Tables III, IV and VIII) suggest a relatively unimportant position of the antidiuretic hormone in the water metabolism of this class of vertebrates. It will be remembered in this connexion that the avian kidney consists of a mixture of the reptilian and mammalian type of nephron and that Henle's loop reaches full development only in the mammal.

Frog pituitary glands contain, per 100 g. body weight, about twice as much of the antidiuretic substance as bird pituitary glands (Table VIII). Is there a significance in the presence of these relatively large amounts in the glands of the more primitive group of vertebrates and in a species in which, according to most authors, an antidiuretic response to injections of post-pituitary extracts cannot be obtained? The possibility must be admitted that the pituitary gland of the frog forms a substance which, though having an antidiuretic effect in the mammal,

has no function in the frog. However, it will be recalled that the antidiuretic hormone has *two* actions in the mammal: (*a*) it regulates the excretion of water; (*b*) it increases the excretion of certain ions. It has recently been shown by Boyd & Whyte [1939] that posterior pituitary extracts exert a similar action on the mineral metabolism of the frog. Boyd & Whyte showed that the active principle thus concerned is contained in the pitressin (antidiuretic-pressor) fraction of commercial post-pituitary extract. It seems significant that it influences the chloride excretion of frogs in very small doses (5 mU. pitressin per 10 g. frog). Considering the amounts of antidiuretic hormone found in the pituitary glands of frogs (about 1·5 mU. per 10 g. frog) and the "unphysiological" method of application of the hormone (injection into a lymph sac), these doses would seem to be within the physiological limit. The question arises, therefore, whether the action of the post-pituitary antidiuretic hormone on the mineral metabolism is not developmentally the older one, the action on renal water reabsorption only appearing with the parallel development of Henle's loop in the mammalian nephron.

This hypothesis does not necessarily mean that the water metabolism of lower vertebrates is not under the hormonal influence of the posterior pituitary gland, but reasons will be given in a later paper to attribute that influence to an active principle not identical with the mammalian antidiuretic hormone.

## Summary

1. Quantitative estimations of the antidiuretic activity of pituitary extracts of representatives of the following classes of vertebrates have been made: mammals, birds, amphibians, teleost and elasmobranch fishes. An antidiuretic activity was found in the pituitary glands of all these groups.

2. The pituitary glands of different species of the same class of vertebrates were found to contain roughly the same amount of antidiuretic activity per 100 g. body weight.

3. There is a pronounced difference between the antidiuretic hormone content of mammalian glands and that of all other classes of vertebrates. Mammalian pituitary bodies contained at least eight times as much of the antidiuretic principle (calculated per 100 g. body weight) as the glands of any non-mammalian species (Table VIII).

4. A relation between the phylogenetic development of Henle's loop and the amounts of antidiuretic hormone produced by the posterior

pituitary is suggested, thus correlating the development of an anatomical structure with that of a hormonal mechanism.

The idea of this paper arose during a discussion with Dr C. S. Jang; it is therefore as much his as mine. I wish to thank Prof. J. H. Burn for providing facilities in his department.

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