

DIFFERENTIATION OF AN (AMPHIBIAN) WATER BALANCE PRINCIPLE FROM THE ANTIDIURETIC PRINCIPLE OF THE POSTERIOR PITUITARY GLAND

By H. HELLER (*Beit Memorial Research Fellow*)

From the Department of Pharmacology, Oxford

(Received 7 November 1940)

AN injection of mammalian posterior pituitary extract into an amphibian kept in water causes a gain of weight due to a temporary increase of the animal's body water. The smallest dose which produces a significant increase of weight in frogs (*Rana esculenta*) is approximately 0.1 unit or 100 milliunits (mU.) of a posterior pituitary preparation [Heller, 1930*a*]. (Boyd & Brown [1938], working with American frogs (*Rana pipiens*), obtained a comparable figure.) It has recently been shown [Heller, 1941*a*] that the pituitary gland of the frog contains an antidiuretic activity equivalent to only about 3.5 mU. pitressin. In view of the discrepancy between the small amount of antidiuretic principle found in the frog pituitary gland and the minimum amount of a (mammalian) posterior pituitary extract needed to produce an increase of body water it looks as though the antidiuretic hormone had little significance for the regulation of the water balance of amphibians.

Extracts of frog pituitary glands were therefore prepared and their action on the water metabolism of frogs tested.

METHODS

Frog assay of pituitary extracts. English frogs (*R. temporaria*) of an average weight of 20 g. were used. All experiments were done between March and October. The evening before the experiment started each frog was placed in a numbered beaker and immersed in tap water. The beakers were covered with glass plates to prevent undue losses of weight due to air currents. Changes of weight of frogs kept under these conditions at room temperature were small and inconsistent. For example, the mean changes of weight in 48 hr. of a series of fifty frogs were found to be

$-2.2 \pm 2.84\%$. Cross tests were used to compare the effect of any preparations on the water uptake of a series of frogs. As a rule five of a series of ten frogs were injected with one preparation, the other five with the other extract. After an interval of 48 hr. the groups were changed round and the animals injected with the preparation which had not been administered in the first experiment. Injections were made into a ventral lymph sac. The volume of each injection was uniformly 0.5 c.c. Weighings were made 1 hr. before the injection and subsequently at hourly or half-hourly intervals. Before each weighing the frogs were carefully dried and the bladder thoroughly emptied.

Oxytocic and pressor assay. Guinea pigs' uteri and spinal cats were used.

Preparation of pituitary extracts. With the exception of the fish pituitary glands which were obtained from dead fishes kept in a refrigerator, glands of freshly killed animals were used. If not otherwise stated (see p. 135) extracts were prepared from the whole pituitary gland including the pars tuberalis and the tuber cinereum. For details of extract preparation see a previous paper [Heller, 1941a]. Control extract of pieces of indifferent brain tissue of the various species of vertebrates used were prepared in an identical manner, but had no significant effect on the water uptake of frogs.

The commercial (mammalian) post-pituitary extracts employed were Messrs Parke, Davis & Co.'s pituitrin, pitocin and 'specially prepared' pitressin and B.D.H. posterior pituitary extract.

RESULTS

Is the 'water balance' principle identical with any of the known hormones of the posterior pituitary gland?

It will be noted (Figs. 1-3) that the weight of a series of frogs each injected with the extract of one frog pituitary gland increases for over 5 hr., reaching a maximum of over 10% above the initial weight. It was mentioned above that the antidiuretic activity of one frog pituitary gland equals about 3.5 mU. pitressin [Heller, 1941a]. Fig. 1 shows that the injection of 10 mU. of pitressin per frog has practically no effect on the water balance. An increase of the duration and intensity caused by the extract of a single frog pituitary gland can, therefore, hardly be explained by its content of antidiuretic activity. The dose of a commercial (mammalian) post-pituitary extract which has to be injected to obtain an effect comparable to that of the extract of a single frog pituitary gland, amounts to about 800 mU. (Fig. 2).

The objection could be raised that the action of commercial post-pituitary extracts is modified by the more complicated methods used for its preparation. Rat pituitary extracts, prepared in the same manner as

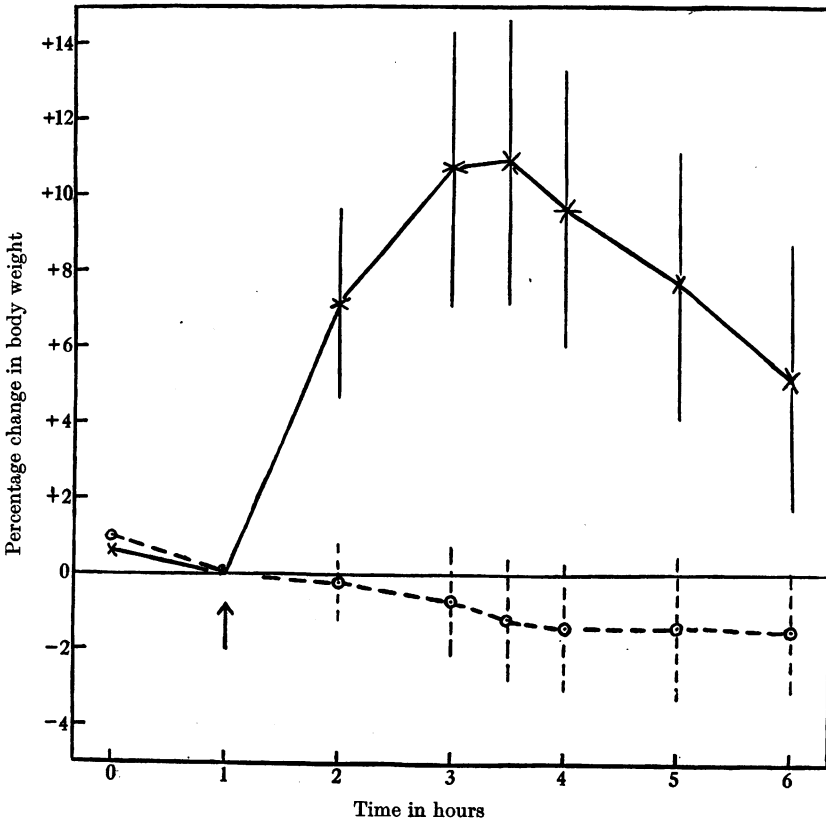


Fig. 1. The difference between the effect on the water balance of frogs of the extract of a single frog pituitary gland and of 10 mU. pitressin. Note that the maximum anti-diuretic activity of a frog pituitary gland was found to equal 5 mU. pitressin. X—X mean percentage changes in weight of twenty frogs injected with the extract of one frog pituitary gland each. O - - O same frogs injected with 10 mU. pitressin each. Injections at the time marked by arrow. The vertical lines indicate the standard error. In this and the following experiments the significance of the differences observed was investigated by Fisher's 't' test. The following figures were obtained for the present experiment: t (for maximum increases of weight) = 2.76, $P < 0.02$.

the frog pituitary extracts, were therefore assayed for their effect on the frog's water balance. Table 1 shows that the extract of one rat pituitary gland, which contains about 1000 mU. of the antidiuretic hormone [Heller, 1941 a], causes a smaller increase of the body weight of frogs than

the extract of one frog pituitary gland which contains the equivalent of 3.5. It seems therefore justifiable to assume that the principle causing the changes of the body water of frogs (water balance principle) and the

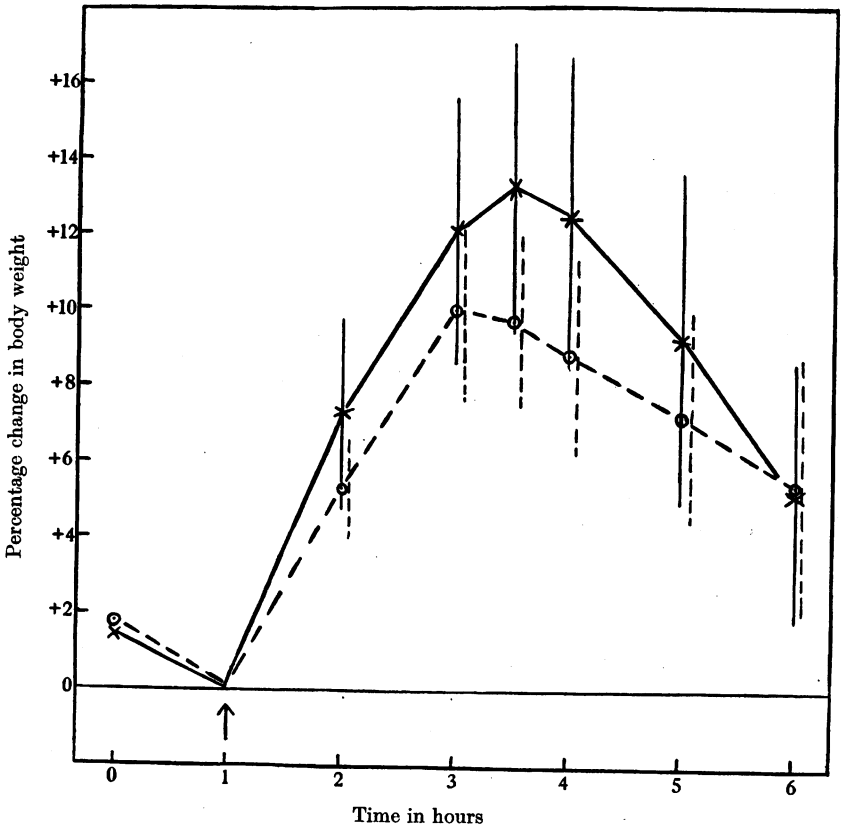


Fig. 2. The dose of a commercial (mammalian) posterior pituitary extract required to equal the water-balance activity of an extract of a single frog pituitary gland. X—X mean percentage changes in weight of twenty frogs injected with the extract of one frog pituitary gland each. O - - O same frogs injected with 800 mU. of B.D.H. posterior pituitary extract each. Injections at the time marked by arrow. The vertical lines indicate the standard error. The values for standard errors indicated by broken lines and belonging to the graph indicated by broken lines were obtained at the same relative times as those shown in full lines but are placed alongside for technical reasons. t (for maximum increases of weight) = 0.73, $P < 0.5$.

post-pituitary antidiuretic hormone are not identical. This conclusion agrees with earlier results [Heller, 1930*b*; Steggerda & Essex, 1934, and others], which proved that the vasopressor-antidiuretic fraction of post-

TABLE I. Comparison between the effect of frog and of rat pituitary extracts on the water balance of frogs. The extract of one rat pituitary gland which contains about 1075 mU. of the antidiuretic activity causes a smaller average increase of the body weight than the extract of one frog pituitary gland which contains about 3.5 mU. Series A = ten frogs injected with extract of one rat pituitary gland each. Series B = ten frogs injected with extract of one frog pituitary gland each. Weight immediately after injection = 100%, *t* (for maximum weight increases) = 3.34, *P* < 0.01

Time after injection (hr.)	Series A Percentage changes of body weight	Series B Percentage changes of body weight
1.0	+3.3 ± 1.33	+7.5 ± 2.51
2.0	+4.4 ± 1.08	+11.6 ± 3.47
2.5	+4.2 ± 0.73	+12.6 ± 2.19
3.0	+3.6 ± 0.94	+12.6 ± 2.03
4.0	+2.9 ± 1.13	+10.9 ± 2.55
5.0	+2.1 ± 0.86	+8.3 ± 2.39
24.0	-0.7 ± 2.24	-0.5 ± 1.80

pituitary extracts had a considerably weaker action on the water uptake of frogs than the oxytocic fraction.

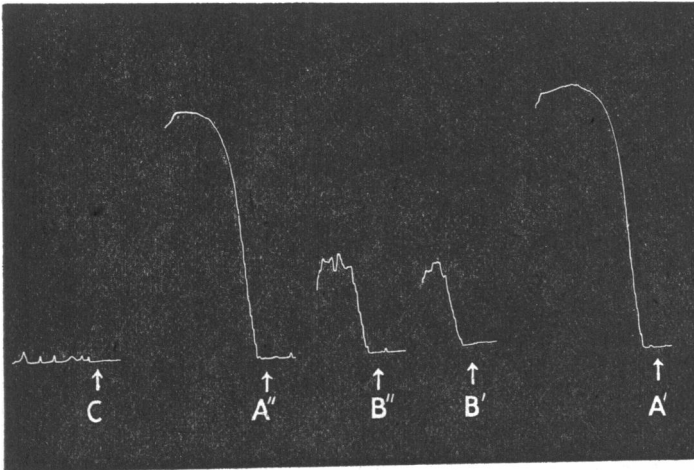


Fig. 3. The oxytocic activity of an extract of one frog pituitary gland. Isolated uterus of guinea-pig. Read from right to left. A', A'' = 8 mU. posterior pituitary extract B.D.H. B', B'' = 20 volume % of extract of one frog pituitary gland. C = control extract of indifferent frog brain. The extract of one frog pituitary gland contains less than 40 mU. of the post-pituitary oxytocic principle.

This suggests the possibility of an identity of the oxytocic hormone with the amphibian water-balance principle. The oxytocic potency of frog pituitary extracts was, therefore, determined. Fig. 3 establishes it at considerably less than 40 mU. (oxytocic) per one frog pituitary gland. These results suggest strongly that the effect on the water balance of

frogs exerted by the extract of frog pituitary glands is not due to their oxytocic activity. The difference between the action of an injection of 40 mU. of pitocin per frog and the effect of an injection of the extract of one frog pituitary gland per frog is clearly shown in Fig. 4.

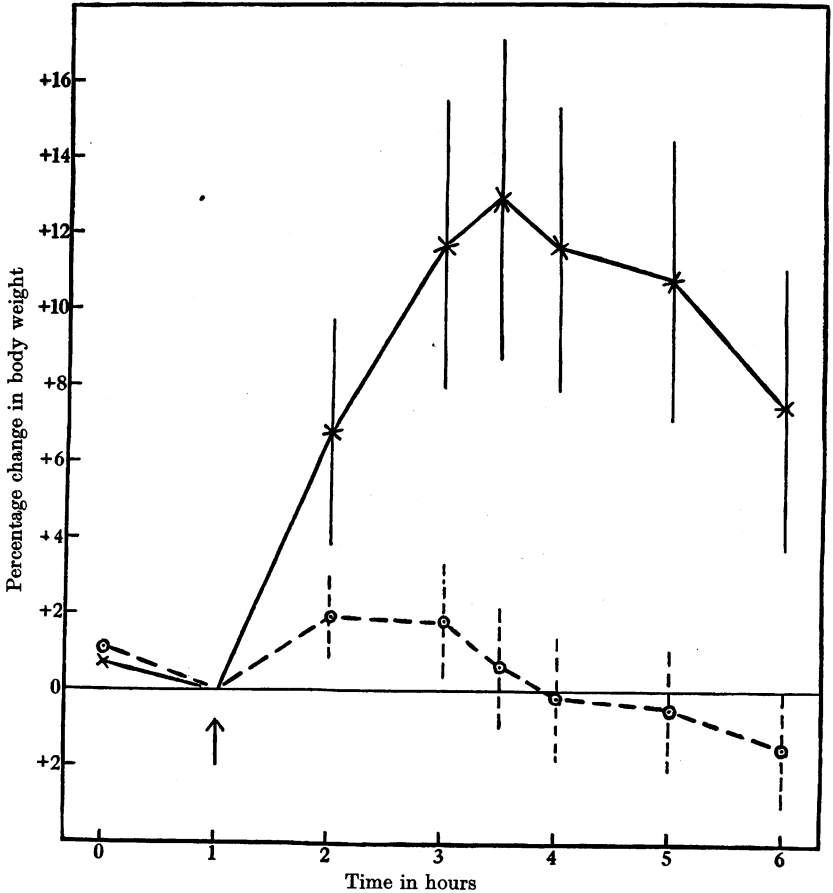


Fig. 4. The difference between the effect on the water balance of frogs of an extract of a single frog pituitary gland and of 40 mU. pitocin. Note that the oxytocic activity of a frog pituitary gland amounts to considerably less than the equivalent of 40 mU. pitocin (see Fig. 3). X—X mean percentage changes in weight of twenty frogs injected with extract of one frog pituitary gland each. O - - O same frogs injected with 40 mU. pitocin each. Injections at the time marked by arrow. t (for maximum increases of weight) = 2.58, $P < 0.02$.

The discrepancy between the average amounts of the antidiuretic principle (about 3.5 mU.) and of the oxytocic principle (less than 40 mU.) contained in the pituitary gland of the frog is more apparent than real.

The extracts used for the determination of the oxytocic potency were crude and contained small amounts of impurities which had an oxytocic effect. A slight fall of blood pressure was regularly observed when the extract of a frog pituitary gland was injected into the vein of a spinal cat (Fig. 5). This finding indicates the presence of histamine-like impurities which enhance the oxytocic potency of crude frog pituitary extracts. Fig. 5 also shows that the pressor effect of the extract of a single frog pituitary gland is negligible. It is certainly much less than that caused

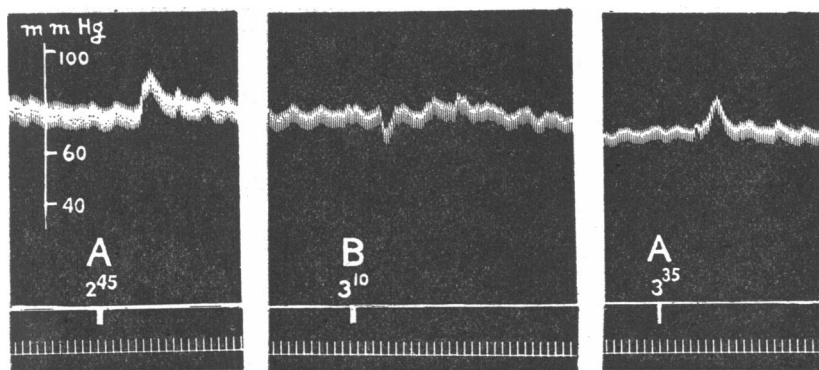


Fig. 5. Effect on blood pressure of spinal cat of extract of one frog pituitary gland. A = intravenous injection of 40 mU. pitressin. B = intravenous injection of extract of one frog pituitary gland. Time marker = 10 sec. The frog pituitary extract contains less than the equivalent of 40 mU. of vasopressor principle. Note slight fall of blood pressure after injection of frog pituitary extract indicating presence of histamine-like impurity.

by 40 mU. of post-pituitary mammalian extract, i.e. a twentieth of the amount which has been shown (Fig. 2) to be just sufficient to reproduce the effect on the water balance of an extract of a single frog pituitary gland.

The content of water balance principle of the pituitary glands of various vertebrate classes

The assumption is made here that the relation of the activity of the water-balance principle to that of the oxytocic principle is the same in the B.D.H. extract used as in the international standard. Both were prepared from ox glands by a similar process. The unit of the water-balance principle is, therefore, defined as the amount of (frog) water-balance activity contained in 0.5 mg. of the international standard powder. It follows from this definition and from Fig. 2 that the pituitary gland of the frog contains approximately 800 mU. of the water-balance

principle. The antidiuretic activity of frog pituitary glands has been shown [Heller, 1941*a*] to be equivalent to approximately 3.5 mU. of pitressin. The ratio of water-balance activity to antidiuretic activity in frog glands is, therefore, approximately 228 to 1.0. The content of water-balance activity of the pituitary glands of representatives of the following classes of vertebrates was also determined. A. *Mammals*. The pituitary gland of rats was found to contain approximately 400 mU. of the water-balance principle (Fig. 6). The antidiuretic activity of a rat pituitary

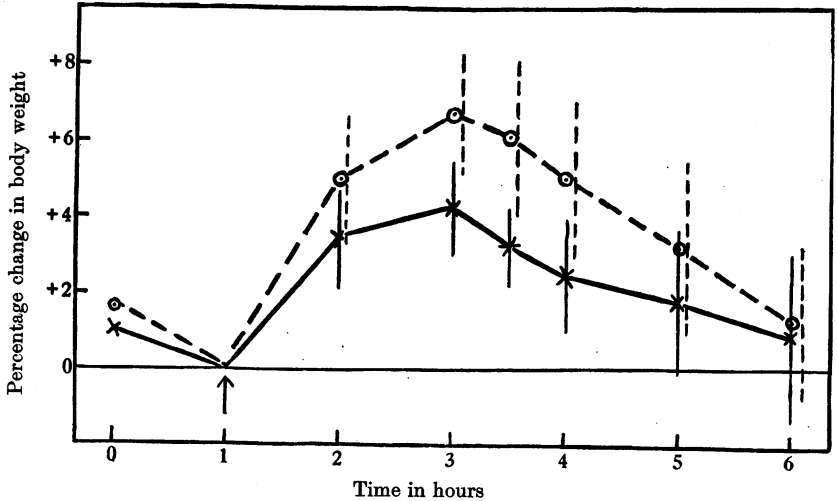


Fig. 6. Estimation of water balance activity of *rat* pituitary gland. X—X mean percentage changes in weight of ten frogs injected with extract of one *rat* pituitary gland each. O—O same frogs injected with 400 mU. of B.D.H. posterior pituitary extract each. Injections at the time marked by arrow. t (for maximum increases of weight) = 1.20, $P < 0.3$. It follows that *rat* pituitary glands contain an average of not more than 400 mU. of the water-balance principle.

gland has previously been shown to equal about 1075 mU. of pitressin [Heller, 1941*a*]. The ratio of water-balance activity to antidiuretic activity is thus 0.37 to 1.00. B. *Birds*. Pigeon pituitary glands contain about 1500 mU. of the water-balance principle (Fig. 7) and an average of 31.5 mU. of antidiuretic hormone per gland [Heller, 1941*a*]. The ratio of water-balance principle to antidiuretic principle is therefore 48 to 1.0. C. *Fishes*. It is known [Boyd & Dingwall, 1939] that extracts of fish pituitary glands are able to influence the water balance of frogs. However, quantitative determinations of the water-balance activity of fish pituitary glands are not extant. Fig. 8 gives an example of an experiment with cod pituitary extracts. It will be noted that the extract of one-tenth of a cod

pituitary gland had the same effect on the frogs' water uptake as the injection of 800 mU. of B.D.H. posterior pituitary extract. That is to say, the pituitary gland of the cod contained approximately 8000 mU. of the water-balance principle. The mean antidiuretic activity of a series of cod pituitary glands was shown to be equivalent to that of 166 mU. pitressin [Heller, 1941*a*]. The ratio of water-balance principle to antidiuretic principle is, therefore 48.0 to 1.0. This figure has to be accepted with the

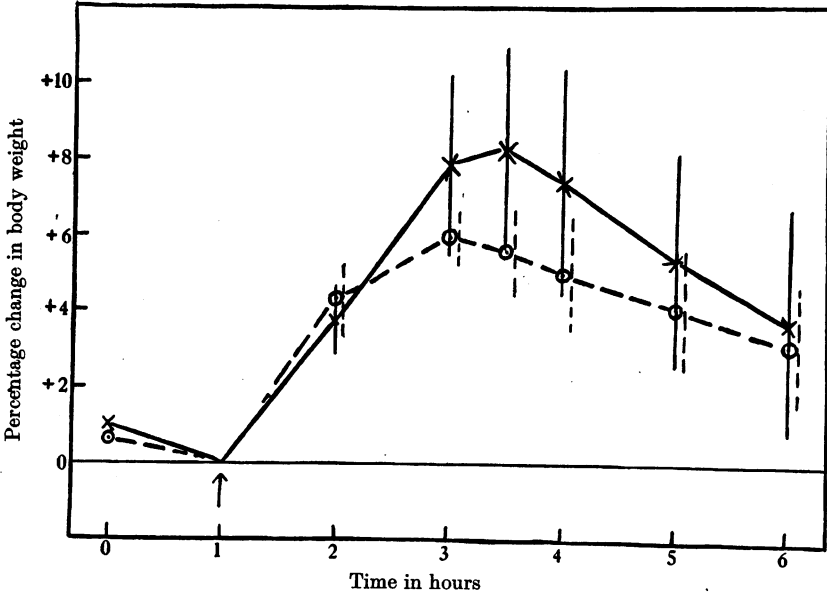


Fig. 7. Estimation of water-balance activity of *pigeon* pituitary glands. X—X mean percentage increases in weight of ten frogs injected with one-third of an extract of a *pigeon* pituitary gland each. O - - O same frogs injected with 500 mU. of B.D.H. posterior pituitary extract each. Injections at the time marked by arrow. *t* (for maximum increases of weight) = 0.79, *P* < 0.5. It follows that *pigeon* pituitary glands contain about 1500 mU. of the water-balance principle.

reservation that the cod glands used for the water-balance assay were those of fishes which had not been freshly killed. The amounts of water-balance activity found are, therefore, likely to be too small. However, the figures obtained are sufficient to show that the ratio of water-balance activity to antidiuretic activity in fish pituitary gland is of a different order of magnitude from that of mammalian pituitary glands.

When comparing the water-balance activity of mammalian and non-mammalian pituitary extracts the objection could be raised that the lower water-balance activity of the mammalian extracts is apparent

rather than real because their high content of vasopressor-antidiuretic principle interferes with the estimation of the water-balance activity. It is conceivable, for instance, that the absorption from the lymph sac is impeded by a high concentration of the vasopressor principle. If that

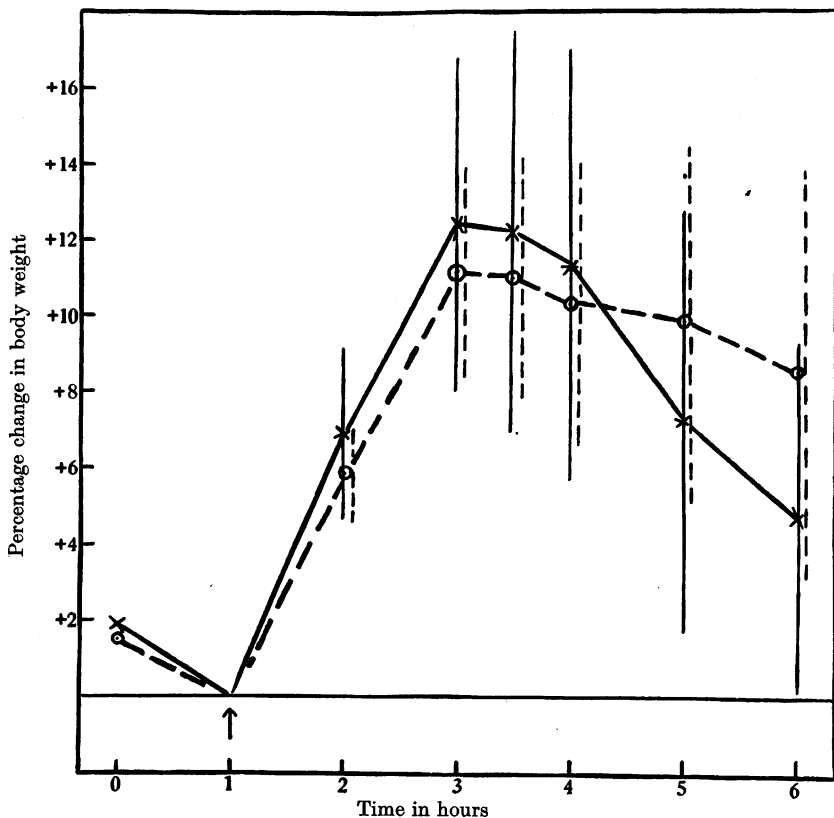


Fig. 8. Estimation of water-balance activity of *cod* pituitary glands. X—X mean percentage increases in weight of ten frogs injected with one-tenth of an extract of a *cod* pituitary gland each. O - - O same frogs injected with 800 mU. of B.D.H. posterior pituitary extract each. Injections at the time marked by arrow. t (for maximum increases of weight) = 0.24, $P < 0.9$. It follows that the *cod* pituitary gland contained about 8000 mU. of the water-balance principle.

were so one would expect 'pituintrin' to have a smaller effect on the water balance of frogs than the equivalent dose (equivalent as to oxytocic activity) of 'pitocin'. However, according to Boyd & Brown [1938] this is not the case. It can also be shown that the water-balance effect of frog pituitary extract is not significantly altered if amounts of a mammalian vasopressor-antidiuretic preparation are added which raise the anti-

diuretic potency of frog pituitary extracts to the level of mammalian pituitary extracts. An experiment on ten frogs gave the following figures: (a) maximum increase in body weight of frogs each injected with 50 mU. of 'specially prepared' pitressin = $1.4 \pm 1.73\%$. Weight at time of injection = 100%; (b) maximum increase in body weight of the same frogs each injected with the extract of one-third of a frog pituitary gland = $7.7 \pm 3.58\%$; (c) maximum increase in body weight of the same frogs each injected with the extract of one-third of a frog pituitary gland to which 50 mU. of pitressin had been added = $8.0 \pm 3.65\%$. The difference between (b) and (c) is not significant as $t=0.06$ and $P>0.9$.

The site of formation or storage of the water-balance principle in the pituitary gland

The experiments so far reported were performed with extracts of the whole pituitary gland. It was therefore impossible to say whether the water-balance principle was a posterior or an anterior pituitary hormone. The action on the water balance of frogs of mammalian posterior pituitary extract suggests the pars nervosa as the site of formation or storage in that class of vertebrates. In addition Biasotti showed in 1923 that mammalian anterior pituitary extract failed to influence the water balance of frogs. I repeated Biasotti's experiments using freshly prepared extracts of cat anterior pituitary lobes and obtained equally negative results. However, these findings do not necessarily apply to the pituitary glands of lower vertebrates. Frozen pigeon pituitary glands were therefore carefully divided into anterior and posterior lobe (the pars tuberalis and the tuber cinereum were included in the posterior lobe fraction), extracted in the usual way and injected into frogs. Fig. 9 shows an experiment on a series of twelve frogs. It will be noted that the anterior lobe extract had no discernible influence on the water balance. Essentially the same results were obtained with extract of the pars anterior of frog pituitary glands (Fig. 10). The pars anterior of frog glands was separated from the neuro-intermediate lobe in the following manner. The frog was decapitated and the skull opened from the dorsal surface. Using a dissection microscope, the olfactory lobes were pushed back with a blunt needle. The second and third nerves and the optic chiasma were cut and the brain pushed backwards until the pituitary gland became plainly visible. The anterior lobe was removed with the help of one sharp and one blunt needle, quickly rinsed in a drop of saline and put into a test tube containing 0.25% acetic acid. The remaining pituitary tissue was removed with a pair of fine pincers and the cranial cavity cleaned with a

small swab of cotton wool. The swab and the brain tissue proximal to the pituitary gland were added to the posterior lobe fraction. A comparison of Fig. 1 and Fig. 10 shows that extracts of the frog pars nervosa prepared

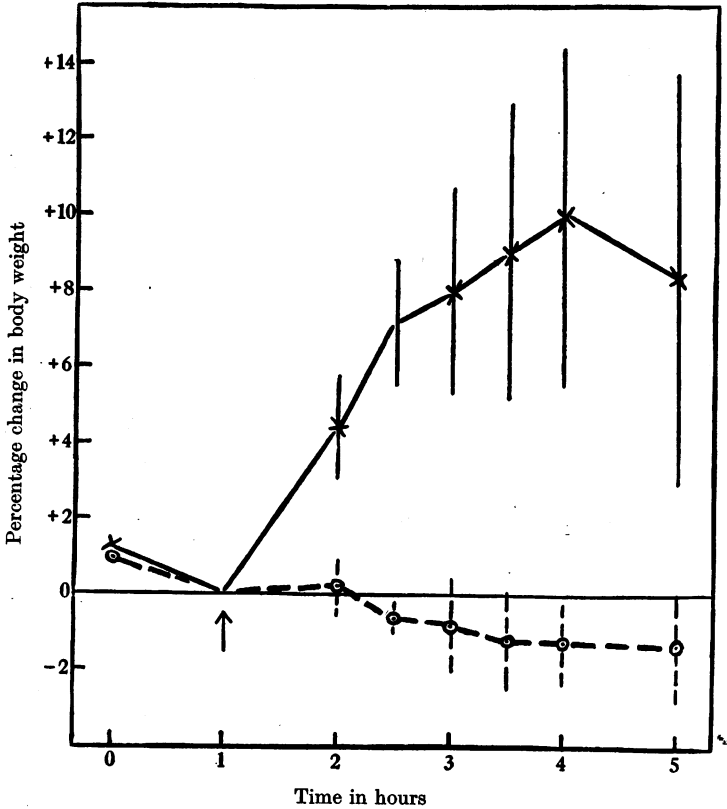


Fig. 9. The difference between the water-balance activity of *pigeon* anterior and posterior pituitary extracts. X—X mean percentage changes in weight of twelve frogs injected with extract of one-third of a *pigeon posterior* pituitary lobe each. O - - O mean percentage changes in weight of twelve frogs injected with extract of a *pigeon anterior* pituitary lobe each. Injections at the time marked by arrow. t (for maximum changes of weight) = 1.97, $P < 0.1$. Note that the anterior lobe extract fails to increase the water uptake of frogs.

in this manner have quantitatively much the same effect as those of the whole pituitary gland, indicating that little of the active material had been lost in the process of separation. Fig. 10 shows also that extracts of the anterior lobe had no noticeable effect on the water uptake of frogs.

It seems sufficiently clear from these results that in the species of vertebrates investigated the posterior lobe must be regarded as the site of formation or storage of the water-balance principle.

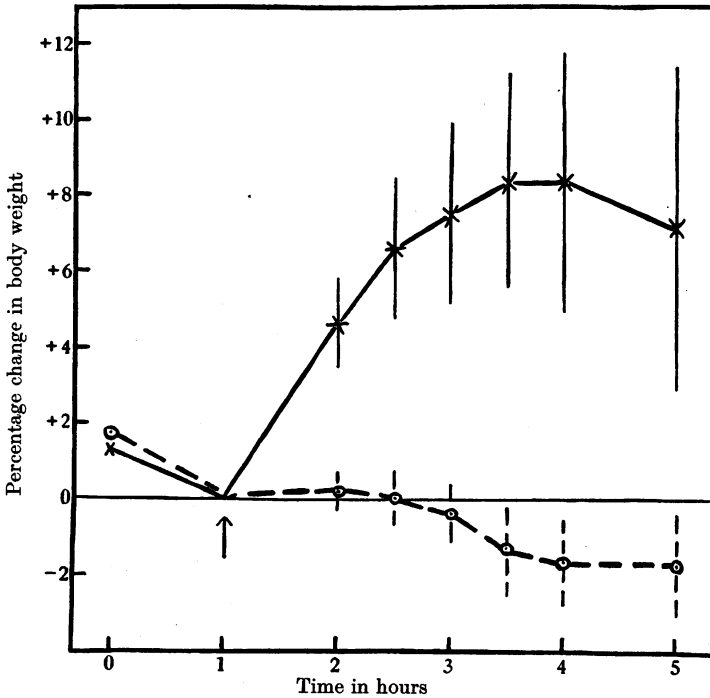


Fig. 10. Difference between the water-balance activity of frog anterior and posterior pituitary extracts. X—X mean percentage changes in weight of twenty frogs injected with extract of one frog posterior pituitary lobe each. O - - O same frogs injected with extract of one anterior pituitary lobe each. Injections at the time marked by arrow. t (for maximum increase of weight) = 2.76, $P < 0.02$. Note that anterior lobe extract fails to increase the water uptake of frogs.

DISCUSSION

The results of the present enquiry show that the effect of pituitary extracts of lower vertebrates on the water balance of the frog is not proportional to their antidiuretic, vasopressor or oxytocic activity. Considering the magnitude of these disproportions it is difficult to see how the pituitary principle which causes an increase of the body weight of amphibians (water-balance principle) can be identical with either of the known posterior pituitary hormones. A chemical difference between the antidiuretic and the water-balance principle is indicated by the finding [Heller, 1930*b* and others] that the oxytocic and not the antidiuretic

vasopressor fraction of posterior pituitary extracts contains the bulk of the water-balance activity.

The water-balance principle was found in the pituitary glands of all vertebrates (mammals, birds, amphibians and fishes) investigated. A comparison of the effect of anterior and posterior lobe extracts of mammals, birds and amphibians showed clearly that practically all of the water-balance principle is formed or stored in the posterior lobe or its anatomical equivalent. The fact that bird anterior lobe extracts fail to influence the water balance shows further that the water-balance principle cannot be identical with the melanophore hormone. Birds have no intermediate pituitary lobe and De Lawder, Tarr & Geiling [1934] have shown that in this vertebrate class the melanophore hormone can be extracted from the anterior lobe. The results of the experiments with bird anterior lobe extracts are in agreement with Oldham's [1936] observation that highly purified (mammalian) melanophore hormone preparations fail to influence the water balance of frogs.

The quantitative distribution of the antidiuretic and of the water-balance activities in the glands of the various classes of vertebrates differs. Table 2 (last column) shows the approximate ratio of antidiuretic

TABLE 2. Comparison of 'water-balance' activity and antidiuretic activity of pituitary glands of representatives of various classes of vertebrates. The unit of the water-balance principle is defined as the amount of water-balance activity contained in one international unit of a mammalian post-pituitary extract. Figures for antidiuretic activities taken from an earlier paper [Heller, 1941 a]

Species of vertebrate used	Milliunits of water-balance principle contained in one pituitary gland. (Figures in brackets = hormone content per 100 g. animal)	Milliunits of antidiuretic principle contained in one pituitary gland. (Figures in brackets = hormone content per 100 g. animal)	Ratio of water-balance principle to antidiuretic principle per gland
Rat	400 (238)	1075 (356.0)	0.37/1.00
Pigeon	1500 (429)	31 (7.2)	48.00/1.00
Frog	800 (4080)	3.5 (11.8)	228.00/1.00
Cod	8000 (—)	166 (—)	48.00/1.00

to water-balance principle in the pituitary glands of various classes of vertebrates. It will be seen that the pituitary glands of mammals contain very large amounts of an antidiuretic principle [Heller, 1941 a] but relatively little of the amphibian water-balance activity. On the other hand, extracts of pituitary glands of lower vertebrates contain large amounts of the water-balance principle but have little antidiuretic activity. The position of mammals with regard to the two post-pituitary activities is thus in sharp contrast to that of any other class of vertebrates. Table 2

shows also that, surprisingly enough, as far as the hormone content of their posterior pituitary lobe is concerned, birds must be classed with the lower vertebrates.

The very different antidiuretic potency of mammalian and non-mammalian pituitary extracts does not appear to influence the assay for water-balance activity. Frog pituitary extracts to which amounts of mammalian antidiuretic hormone (pitressin) had been added which raised their antidiuretic potency to the level of a mammalian extract had much the same water-balance effect as normal frog pituitary extracts. On quoting figures for the ratio of antidiuretic to water-balance activity of pituitary extracts of different vertebrate classes the reservation must be made that antidiuretic activity was estimated by intravenous injection into rabbits whereas water-balance activity was determined by injection into a lymph sac of frogs. However, in view of the recent report of Ogden & Sapirstein [1940] that unspecific augmentor effects can be obtained equally with intravenous as with subcutaneous injections of posterior pituitary preparations, no attempt was made to estimate water-balance activity by another than the usual method.

It is impossible to decide at present whether the different proportions of antidiuretic and of water-balance activity found in the pituitary glands of the various classes of vertebrates indicate the distribution of a purely antidiuretic principle and of a factor which influences the 'water balance' only. The existence of an antidiuretic principle with a weak action on the water balance and of a water-balance principle with a weak antidiuretic action is a possible alternative. In other words there is no experimental evidence to prove that any of the posterior pituitary factors are secreted as separate chemical entities (Heller, 1941 *b*) though it may be possible to prepare them as such. The possibility must therefore be envisaged that the posterior pituitary glands of different classes (and even different species, see Geiling & Oldham, 1937) of vertebrates elaborate a secretion which exhibits the same activities but—by some unknown modification of the molecule—contains them in different proportions.

Our knowledge of the importance of the antidiuretic and of the water-balance activities for the water metabolism of the different classes of vertebrates is very fragmentary. The action of the antidiuretic hormone on the kidney of the mammal is clearly established, so is the action of the water-balance principle on the water metabolism of amphibians, but the effect on the water metabolism of either of the two principles on any of the other classes of vertebrates is either unknown or highly controversial. It will be noted (Table 2) that the amount of water-balance principle in

mammalian posterior pituitary lobes was smaller than that found in the glands of any lower vertebrate. However, this small amount may not be negligible and future research may reveal its physiological significance. On the other hand, it cannot be excluded that the presence of the water-balance factor in the mammalian gland is in the nature of a 'vestigial hormone' and that the post-pituitary regulation of the water metabolism in the mammal has been taken over by the antidiuretic hormone.

SUMMARY

1. Considerations are advanced for believing that the pituitary principle causing an increase of body water in the frog (water-balance principle) is not identical with any of the known posterior pituitary hormones. This assumption is based on the following findings:

A series of frogs injected with the extracts of one frog pituitary gland each showed a maximum gain of body weight of $12.4 \pm 3.94\%$ (s.e. of mean of seventy observations). The pituitary gland (including the pars tuberalis and the tuber cinereum) of the frog contains the following average amounts of the post-pituitary principles: an average of 3.5 mU. of antidiuretic activity, less than 40 mU. of vasopressor activity and less than 40 mU. of oxytocic activity. However, approximately 800 mU. of a mammalian posterior pituitary extract (containing the antidiuretic and vasopressor and oxytocic activities in equal proportions), had to be injected to produce an effect comparable to that caused by the injection of the extract of a single frog pituitary gland (Fig. 2).

2. The fact that the extract of a single frog pituitary gland contains sufficient of the water-balance principle to cause a marked change of the water balance of a frog suggests a physiological importance of this pituitary principle for the water metabolism of amphibians.

3. It would appear that the water-balance principle is a regular constituent of the pituitary gland of all classes of vertebrates.

4. A comparison of the effects of cat, pigeon and frog anterior and posterior lobe extracts shows that the water-balance principle is formed (or stored) in the posterior lobe of the pituitary gland.

5. The ratio of antidiuretic to water-balance activity contained in the pituitary gland differs in different groups of vertebrates. The results of the present inquiry suggest a unique position of the mammal, the pituitary gland of which contains large quantities of the antidiuretic factor but relatively little of the water-balance principle. The reverse is the case for the pituitary gland of lower vertebrates (birds, amphibians and fishes;

Table 2). A difference between the hormonal control of the water metabolism of mammals and of that of the other vertebrate classes is suggested.

I wish to thank Prof. J. H. Burn for providing facilities in his department, and Sir H. Dale and Prof. J. H. Gaddum for a valuable suggestion. I am much indebted to Dr Stanley White of the Parke, Davis Company for the supply of hormone preparations used in these experiments.

REFERENCES

- Biasotti, A. [1923]. *C.R. Soc. Biol., Paris*, **88**, 36.
 Boyd, E. M. & Brown, G. M. (1938). *Amer. J. Physiol.* **122**, 191.
 Boyd, E. M. & Dingwall, M. (1939). *J. Physiol.* **95**, 501.
 De Lawder, A. M., Tarr, L. & Geiling, E. M. K. [1934]. *J. Pharmacol.* **51**, 142.
 Geiling, E. M. K. & Oldham, F. K. [1937]. *Trans. Ass. Amer. Physicians*, **52**, 132.
 Heller, H. [1930a]. *Arch. exp. Path. Pharmac.* **157**, 298.
 Heller, H. [1930b]. *Arch. exp. Path. Pharmac.* **157**, 323.
 Heller, H. [1941a]. *J. Physiol.* **99**, 246.
 Heller, H. [1941b]. *Nature, Lond.*, **147**, 178.
 Ogden, E. & Sapirstein, L. O. [1940]. *Proc. Soc. exp. Biol., N.Y.*, **45**, 573.
 Oldham, F. K. [1936]. *Amer. J. Physiol.* **115**, 275.
 Steggerda, F. R. & Essex, H. E. [1934]. *Proc. Soc. exp. Biol., N.Y.*, **32**, 425.