

THE POSTERIOR PITUITARY PRINCIPLES OF A SPECIES
OF REPTILE (*TROPIDONOTUS NATRIX*) WITH SOME
REMARKS ON THE COMPARATIVE PHYSIOLOGY
OF THE POSTERIOR PITUITARY
GLAND GENERALLY

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There is now general agreement that the posterior pituitary secretion of mammals aids in the conservation of body water by producing an increase of the tubular reabsorption of water, and thus leads to the production of a hypertonic urine. The physiology of posterior pituitary action in other classes of vertebrates has been neglected mainly because lower vertebrates, with the exception of birds, are unable to prepare a hypertonic urine. The mammalian mechanism of posterior pituitary action can, therefore, not apply.

However, there is one group of lower vertebrates for which a physiological importance of the posterior pituitary secretion for the regulation of their water balance has been suggested. Small doses of posterior pituitary extracts injected into frogs or other amphibians produce a temporary increase of body water. Steggerda [1937], working with several species of amphibians, showed a definite correlation between the magnitude of this effect and the natural habitat of the species. The more terrestrial the species the bigger the effect of a given dose of posterior pituitary extract on its water balance. There is ample evidence to prove that this effect is produced, partly at least, by an extrarenal mechanism, viz. by a change in the permeability of the amphibian skin [Brunn, 1921; Steggerda, 1931; Novelli, 1936].

Bearing these mechanisms in mind, how is one to comprehend a physiological action of the posterior pituitary gland in reptiles, i.e. in a class of vertebrates which are unable to excrete a hypertonic urine [Burian, 1910; Smith, 1932] and whose habitat and skin structure excludes an extrarenal mechanism similar to that of amphibians? There is a possibility that the posterior pituitary gland does not take part in the regulation of the water metabolism of reptiles. It was, therefore, of interest to determine whether the reptile pituitary gland contained any considerable amounts of those posterior lobe activities which are supposed to be concerned with the water metabolism of other vertebrate

classes. Herring [1913] reported the presence of a pressor principle in extracts of tortoise pituitary glands, but no demonstration of the antidiuretic and the 'water-balance' activity of reptile pituitary extracts has yet been made.

METHODS

Assay of antidiuretic activity. Intravenous injections into unanaesthetized rabbits were employed. The intravenous injection of saline or, in other words, an intravenous injection as such does not interfere with the normal water

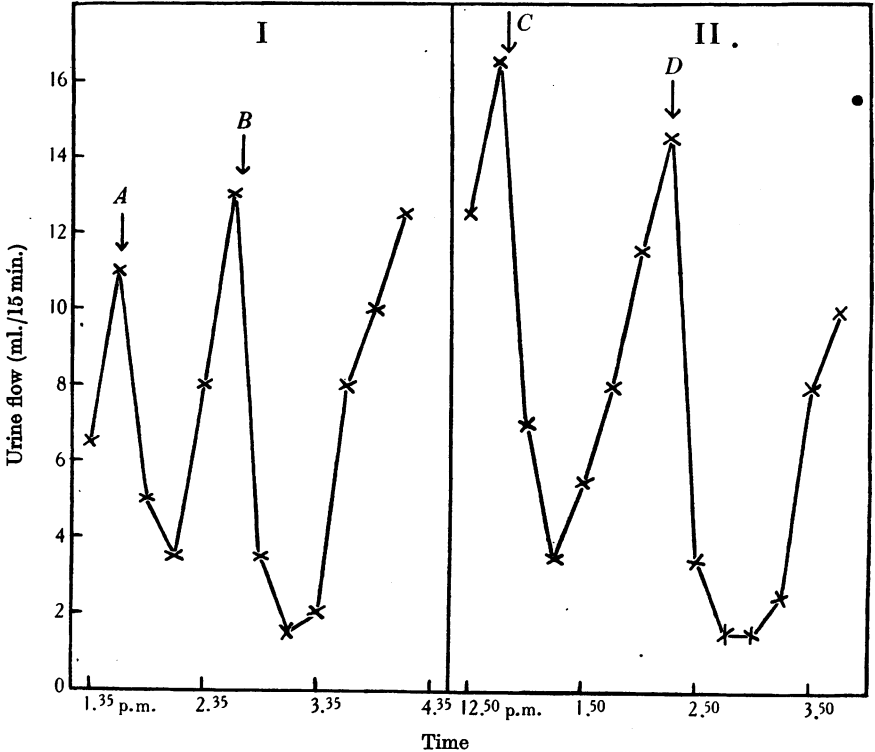


Fig. 1. Estimation of antidiuretic activity of grass-snake pituitary gland. Grass-snake no. 4, 50 g. ♂. I=rabbit no. 71. At 10.00 a.m. and at 12.10 p.m. 5% of body weight of water by stomach tube. A=1.0 mU. pitressin i.v. B=2.0 vol. % of grass-snake pituitary gland extract i.v. II=rabbit no. 89. At 10.04 a.m. and at 12.09 p.m. 5% of body weight of water by stomach tube. C=0.75 vol. % of grass-snake pituitary gland extract i.v. D=1.0 mU. pitressin i.v. The antidiuretic activity of the grass-snake pituitary extract was, therefore, equivalent to more than 50 mU. and to less than 130 mU. pitressin.

diuresis of a rabbit provided that care is taken not to excite the animal. To avoid excitement and struggling, it was found best to inject the rabbits without the help of a box or the hands of an assistant. Varying amounts of the extract of one reptile pituitary gland were matched with the response to a

standard dose of pitressin. Several rabbits were used to estimate the potency of any one grass-snake pituitary extract. Further details of the assay are given in the 'legend' to Fig. 1.

Assay of (amphibian) water-balance activity. English frogs (*Rana temporaria*) of an average weight of 20 g. were used. All experiments with frogs were done between April and September. The evening before the experiment started, each frog was placed in a covered beaker and immersed in tap water. Changes of weight of frogs kept under these conditions were small and inconsistent. Cross tests were used to compare the effect of any preparation on the water uptake of a series of frogs. Injections were made into a ventral lymph sac. The volume of each injection was made up to 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. Care was taken to perform the cross tests at approximately the same temperature. However, the method of assay cannot be considered very accurate, and the results obtained should be regarded as indicating an order of magnitude rather than as precise estimations of the water-balance activity. The unit of water-balance activity used in this paper was defined as the amount of (frog) water-balance activity contained in 0.5 mg. of the international (mammalian) standard powder. It should be noted that Boyd and his co-workers [Boyd & Mack, 1940] use a different unit of water-balance or water-retention activity.

Assay of pressor activity. Spinal cats were used.

Preparation of grass-snake pituitary extracts. A series of fifteen English grass-snakes (*Tropidonotus natrix*), comprising both sexes, were used for the experiments presented. The animals were killed by decapitation, the whole pituitary gland and the parts of the brain proximal to the gland were removed and acid extracts were prepared without delay. The details of extraction are given in a previous paper [Heller, 1941*a*]. Control extracts of pieces of indifferent brain tissue were prepared in an identical manner.

The commercial (mammalian) posterior pituitary extracts employed were pitressin (Messrs Parke, Davis & Co.) and posterior pituitary extract (B.D.H.).

RESULTS

The antidiuretic activity of grass-snake pituitary extracts. Preliminary 'range-finding' experiments are not quoted. The experiment shown in Fig. 1 is included in Table 1. It will be noted that the figures given for the antidiuretic activity of the snake posterior glands lie in a comparatively narrow range. (Six out of eight glands showed an average antidiuretic hormone content equivalent to between 75 and 100 mU. pitressin.) Figures of hormone content calculated per 100 g. of body weight are more dissimilar. However, they indicate an average antidiuretic hormone content which is higher than that of birds and amphibians but lower than that of mammals [Heller, 1941*a*].

TABLE 1. The antidiuretic activity of grass-snake pituitary glands. For description of method of estimation see 'Methods' and legend of Fig. 1

No.	Sex	Weight of animal in g.	Antidiuretic activity of pituitary gland in terms of mU. pitressin	Antidiuretic activity per 100 g. animal
1	♂	99	> 20 ~ 50	> 20 ~ 50
2	♀	82	~ 100 < 200	~ 120 < 240
3	♀	70	> 50 ~ 100	> 70 < 140
4	♀	50	> 50 < 130	> 100 < 260
5	♀	50	> 50 < 100	> 100 < 200
6	♀	182	> 60 < 100	> 35 < 50
7	♀	163	< 200	< 120
8	♀	121	> 50 < 100	> 40 < 80
9	♀	175	~ 150 < 200	~ 85 < 115

The (amphibian) water-balance activity of grass-snake pituitary glands. Seven grass-snake pituitary glands were used for the estimations of water-balance activity. The extracts of several glands were pooled for any one experiment. Fig. 2 records one of these experiments. It will be seen that the water-balance activity of one snake pituitary gland equalled approximately that of 3300 mU. of a mammalian posterior pituitary extract. Other experiments gave essentially similar results. The average antidiuretic activity of grass-snake posterior pituitary glands, as established in the previous section, can be taken as roughly 100 mU. per gland. The ratio of water balance to antidiuretic activity is, therefore, approximately 33 to 1. This ratio is similar to that found for birds and fishes [Heller, 1941 b], but of a different order of magnitude than that of mammals and amphibians (Table 2).

Pressor assays of grass-snake pituitary gland extracts. The number of experiments performed was not sufficient to permit a satisfactory assessment of the average pressor activity of grass-snake pituitary glands. However, the five experiments done did show the presence of considerable amounts of pressor activity (Fig. 3). Moreover, it was noticed that the apparent pressor activity of any one grass-snake pituitary gland was much in excess of the antidiuretic activity of the same extract (cp. e.g. Fig. 3 and Table 1, no. 8). A similar, though not as marked, discrepancy had previously been observed between the pressor and the antidiuretic potency of pigeon posterior pituitary lobe extracts [Heller, 1941 a]. It is impossible to decide at present whether this discrepancy is due to a real difference between the concentration of the two activities in the extracts or whether it results from the combined errors of the methods of assay.

It will be observed that a not inconsiderable fall of blood pressure precedes the pressor effect of injections of snake pituitary extract (Fig. 3). This phenomenon occurred with all the extracts tested. It may be that this depressor effect is due to the presence of traces of a histamine-like substance. It is interesting to note that the depressor effect was only observed with grass-snake, and to a minor degree with frog pituitary extracts. It was not observed with

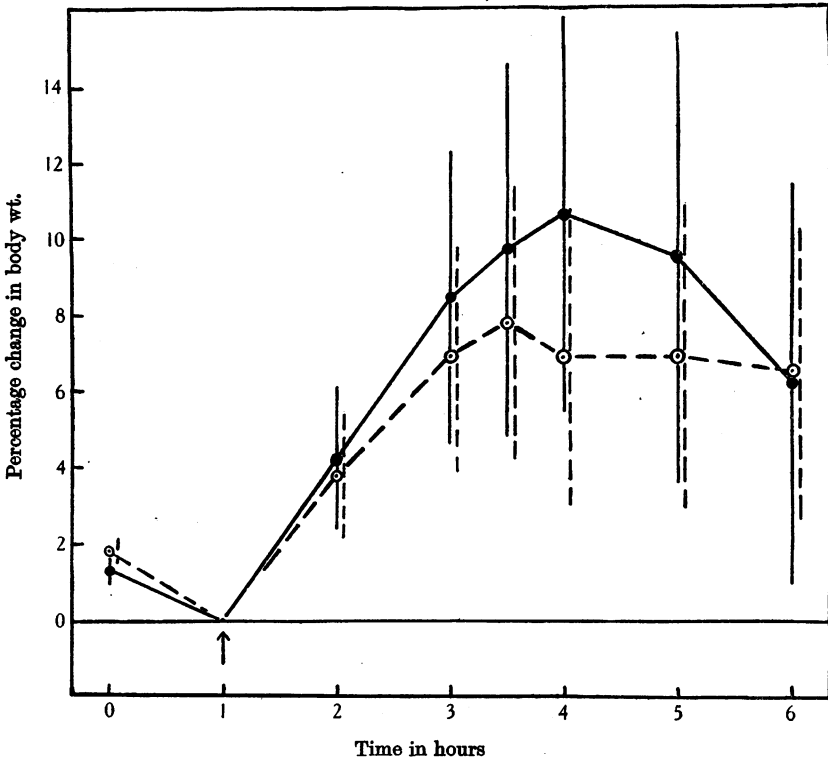


Fig. 2. Estimation of water-balance activity of grass-snake pituitary gland. ●—● mean percentage change in body weight of twenty frogs injected with 15 vol. % of an extract of one grass-snake pituitary gland each. ○----○ same frogs injected with 500 mU. of B.D.H. posterior pituitary extract each. The 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. Injections at the time marked by arrow. t (for maximum increases of weight) = 0.44, $P < 0.7$. It follows that the grass-snake pituitary glands contained about 3300 mU. of the water-balance principle.

TABLE 2. Water-balance activity and antidiuretic activity of pituitary glands of representatives of various classes of vertebrates

Class of vertebrate	Species of vertebrate used	Average amount of water-balance principle contained in one pituitary gland (in mU.). Figures in brackets = hormone content per 100 g. animal	Average amount of antidiuretic principle contained in one pituitary gland (in mU.). Figures in brackets = hormone content per 100 g. animal	Ratio of water-balance activity to antidiuretic activity per gland
Teleost fishes	Cod	8000 (—)	166 (—)	48.0/1.0
Amphibians	Frog	800 (4100)	3.5 (12)	228.0/1.0
Reptiles	Grass-snake	3300 (3600)	100 (95)	33.0/1.0
Birds	Pigeon	1500 (430)	31 (7)	48.0/1.0
Mammals	Rat	400 (240)	1075 (360)	0.4/1.0

extracts of mammalian and avian pituitary glands. All extracts were prepared immediately after the death of the animal. The occurrence of the histamine-like effect is, therefore, not due to post-mortem decomposition of the glandular material. It is quite conceivable, however, that the method of extraction removes a histamine-like substance in one instance, e.g. from snake pituitary glands and does not remove it in another, e.g. from mammalian glands. Grant & Jones's [1929] report on the presence of a vaso-dilator substance in the skin of frogs with similar but not identical properties to the histamine-like substance in mammalian skin, may be recalled in this connexion.

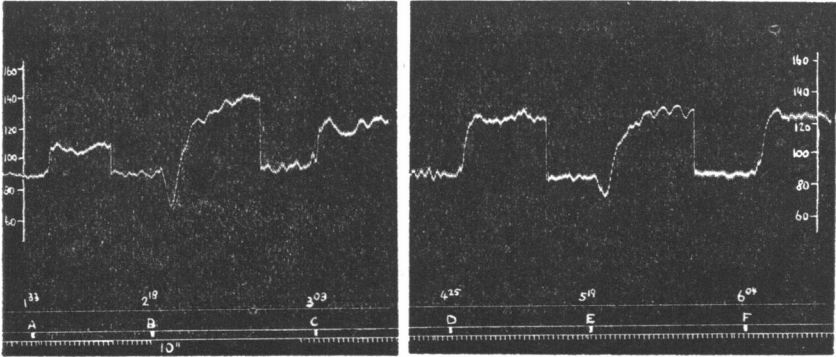


Fig. 3. Pressor activity of grass-snake pituitary gland extract. Spinal cat. Extract of grass-snake pituitary gland no. 8. *B* and *E* = intravenous injection of 25 vol. % of grass-snake pituitary gland extract. *A* = intravenous injection of 30 mU. pitressin. *C* = 50 mU. pitressin i.v. *D* = 70 mU. pitressin i.v. *F* = 90 mU. pitressin i.v. All injections made up to 1 c.c. The vasopressor activity of the whole gland equals approximately 320 mU. pitressin.

Mixtures of mammalian posterior pituitary extracts with amounts of histamine-acid phosphate having a proportionately equal depressor effect to the vaso-dilator impurities in snake pituitary extracts, had much the same pressor effect as the same amounts of pure posterior lobe extract without the histamine. It is, therefore, unlikely that the traces of depressor substance found in grass-snake pituitary extracts influenced the pressor assay to a significant degree.

DISCUSSION

It is clear from these experiments that the posterior pituitary glands of grass-snakes contain considerable amounts of active principles. This result agrees with the anatomy of the snake pituitary gland, the pars nervosa of which is a well-developed structure. Teleologically it would have been surprising if little or no activity had been found in the posterior pituitary gland of a snake, i.e. of a member of a vertebrate order which counts some of the most aridly living animals among its numbers, an order, therefore, in which a highly developed mechanism for the conservation of water would be expected.

The results of the quantitative determinations of the water-balance activity and of the antidiuretic activity of grass-snake pituitary glands will be found in Table 2, which includes the results obtained for pituitary gland extracts of representatives of the phylogenically adjacent classes of vertebrates [Heller, 1941*a*, 1941*b*]. However, no attempt will be made to draw conclusions from the quantitative data obtained for the different classes, as there are several considerations which render the significance of these values difficult to interpret. Some such factors as the errors of assay and the influence of captivity, involving possibly changes in the water uptake of the experimental animals [Geiling, 1940], may be of minor importance if the large differences found between the hormone content of glands of different classes are considered. However, two other considerations may be of more serious significance: (1) Only one or in some cases two species of any particular class of vertebrates have been investigated. It must be admitted that, so far, the differences between the hormone content of glands of different species have been found to be significantly smaller than the differences between the hormone content of glands of animals belonging to different classes of vertebrates. But data on more species, including if possible related species with a widely different habitat, are desirable. (2) It will be noted that the quantitative determinations of the posterior pituitary activities of the frog and of the rat suggest a relatively much higher concentration of the water-balance principle and the antidiuretic principle respectively for these than for the representatives of the other classes of vertebrates. On the other hand, it is known that amphibians and mammals, respectively, respond to these principles in a specific manner (Table 3).

TABLE 3. Effect of posterior pituitary extract on the water metabolism of different classes of vertebrates

	Extrarenal (‘water-balance’) effect	‘Glomerular’ antidiuresis	‘Tubular’ antidiuresis
Amphibians	Pronounced	Doubtful	None
Reptiles	Doubtful	Pronounced	None
Birds	Not investigated	Feeble	Feeble
Mammals	None*	None	Pronounced

* Boyd & Garand [1942] showed recently that pitocin, i.e. the posterior pituitary fraction containing the bulk of the amphibian water balance activity, is not more effective than pitressin but rather less so in retaining body water in a mammal, the albino rat. These results differ from those obtained in corresponding experiments on frogs [Heller, 1930; Boyd & Brown, 1938] and indicate that posterior pituitary extract does not have the same effect upon mammalian water balance as upon amphibian water balance.

It would, therefore, be tempting to assume a correlation between a class specific receptor apparatus and the quantity of the corresponding hormone produced by the gland. However, the apparent preponderance of a pituitary principle in the glands of a class of animal on which that principle exerts a specific action is open to another interpretation. It has been shown in the case of anterior pituitary hormones and of the gonadotrophic hormone, in particular,

that the effectiveness of the hormone in a foreign species tends to vary directly with the phylogenetic proximity of the donor and the recipient species [Creaser & Gorbman, 1939]. In some instances the loss of effectiveness proved to be so great as to lead to an apparent refractoriness by the recipient species to rather large doses of gonadotrophic material. It becomes clear, therefore, that if there is an appreciable variation of responsiveness of a given test animal to hormones obtained from different species or classes, then 'units' as determined by biological tests do not represent equivalent absolute units of hormones, but only the amount of hormone required to produce a given effect on the particular animal species or class under consideration. Though the possibility of species or class specificity of posterior pituitary principles seems so far not to have been suggested, it will be realized that the data indicating a preponderance of the antidiuretic principle in mammalian glands and of the water-balance principle in amphibian glands have been obtained in experiments with material from mammals and frogs respectively. The apparent high concentration of the antidiuretic principle in mammals and of the water-balance principle in the frog may, therefore, be an indication of the class specificity of these principles.

The difficulty of visualizing the mechanism of a posterior pituitary effect on the water metabolism of reptiles has been mentioned, and it was pointed out that, as far as we know, reptiles lack the effector mechanisms of the phyletically adjacent classes. That is to say, reptiles show neither the rapid water movement through the skin as, e.g. frogs, nor are their renal tubules specially adapted for water reabsorption beyond the iso-osmotic level. Assuming that water conservation is secured by the secretion of the posterior pituitary lobe in reptiles as in other classes of vertebrates, on what mechanism could it be based?

It should be remembered that, taking the vertebrate phylum as a whole, we know of three actions of posterior pituitary extracts which may influence the body water of animals: (1) The increase of tubular water reabsorption as present in mammals and birds. (2) The increase of the permeability of the skin to water as in amphibians. (3) The constrictor action on blood vessels and on the glomerular capillaries in particular. The third mechanism has so far not been drawn into the circle of physiological possibilities, but it is clear that an antidiuresis caused by a decrease of the glomerular filtrate would tend to conserve water. Such a 'glomerular antidiuresis' induced by posterior pituitary extracts has been occasionally observed in amphibians and in mammals [Adolph, 1936; Iversen & Bjering, 1934]. However, very large doses had to be given to produce it. The question arises whether the posterior pituitary vasopressor principle acts on reptilian kidneys in a similar manner, and whether the reptile glomerulus is more sensitive to its action than the glomerulus of other vertebrate classes.

Burgess, Harvey & Marshall [1933], who investigated the action of mammalian posterior pituitary extracts on the urinary secretion of various classes of vertebrates, found that even very large doses of pitressin (1000 mU. per kg.) did not produce an inhibition of urine flow in the frog (*R. catesbiana*). However, the antidiuretic action of 100 mU. per kg. in a reptile (the alligator) was very pronounced. Marshall and his co-workers investigated this antidiuretic action in the alligator further by measuring the xylose clearance, and found that 'even very small doses of pitressin decreased the glomerular filtrate almost proportionately to the urine flow'. This finding is in contrast to the antidiuretic action of posterior pituitary extracts in mammals which has been repeatedly shown to occur without a significant decrease in glomerular filtration.

With all reservation as to future findings in other species of reptiles it can be pointed out, therefore, that the experimental evidence in the one reptile so far investigated does not contradict the hypothesis that water conservation in this vertebrate class 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 means 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 glomerular capillaries lasts for any length of time. However, it should be remembered that, in contrast to mammals, the metabolic waste products and especially uric acid of reptiles are eliminated not only by glomerular filtration but to a much larger extent by tubular secretion [Marshall, 1932], and that tubular secretion has been shown to be highly independent of glomerular activity (e.g. by Pitts [1938] for the avian kidney). Thus tubular secretion may be of greater importance for a 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.

Accepting the possibility of a glomerular action of the posterior pituitary secretion in the reptile, how would it fit into a scheme of the known effects of posterior pituitary extracts on the water metabolism of the various classes of vertebrates? It will be seen (Table 3) that the data on reptiles agree with the findings on the other classes in that the prevalence of a class specific effector mechanism is equally indicated. Birds form an apparent exception, as there appear to be two important mechanisms of posterior pituitary action. This exception can possibly be explained by the fact that the kidney of birds consists anatomically of a mixture of mammalian-like and of reptilian-like nephra.

The difficulty of drawing generalized conclusions from the scattered and

incomplete evidence will be appreciated. However, three points worth mentioning are beginning to emerge.

1. The findings mentioned in this and in the other papers of this series [Heller, 1941 *a*, 1941 *b*] suggest that all the three posterior pituitary activities possibly concerned with the regulation of the metabolism of water are present in all classes of vertebrates.

2. The effect of one posterior pituitary activity in preference to the others and dependent on an effector mechanism specific for the class appears to prevail in each class of vertebrates.

3. The comparative physiology of the posterior pituitary action will probably be best understood by the application of a teleological concept, namely that of conservation of water.

SUMMARY

1. The water balance, antidiuretic and pressor activity of pituitary extracts of a species of reptile (*Tropidonotus natrix*) has been determined.

2. The physiology of the reptile posterior pituitary gland in relation to the metabolism of water is discussed.

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REFERENCES

- Adolph, E. F. [1936]. *Amer. J. Physiol.* **117**, 366.
 Boyd, E. M. & Brown, G. M. [1938]. *Amer. J. Physiol.* **122**, 191.
 Boyd, E. M. & Garand, N. D. [1942]. *Endocrinology*, **30**, 433.
 Boyd, E. M. & Mack, E. C. [1940]. *Endocrinology*, **26**, 153.
 Brunn, F. [1921]. *Z. ges. exp. Med.* **25**, 170.
 Burgess, W. W., Harvey, A. M. & Marshall, E. K. [1933]. *J. Pharmacol.* **49**, 237.
 Burian, R. [1910]. *Pflüg. Arch. ges. Physiol.* **136**, 741.
 Creaser, C. W. & Gorbman, A. [1939]. *Quart. Rev. Biol.* **14**, 311.
 Geiling, E. M. K. [1940]. *Endocrinology*, **27**, 309.
 Grant, R. T. & Jones, T. D. [1929]. *Heart*, **14**, 339.
 Heller, H. [1930]. *Arch. exp. Path. Pharmacol.* **157**, 323.
 Heller, H. [1941 *a*]. *J. Physiol.* **99**, 246.
 Heller, H. [1941 *b*]. *J. Physiol.* **100**, 125.
 Herring, P. T. [1913]. *Quart. J. exp. Physiol.* **6**, 73.
 Iversen, P. & Bjerling, T. [1934]. *Arch. exp. Path. Pharmacol.* **175**, 681.
 Marshall, E. K. [1932]. *Proc. Soc. exp. Biol., N.Y.*, **29**, 971.
 Novelli, A. [1936]. *Rev. Soc. Argent. Biol.* **12**, 163.
 Pitts, R. F. [1938]. *J. cell. comp. Physiol.* **11**, 99.
 Smith, H. W. [1932]. *Quart. Rev. Biol.* **7**, 1.
 Steggerda, F. R. [1931]. *Amer. J. Physiol.* **98**, 255.
 Steggerda, F. R. [1937]. *Proc. Soc. exp. Biol., N.Y.*, **36**, 103.