J. Physiol. (1939) 95, 501–507 612.492.8:612.014.461.3

THE EFFECT OF PITUITARY (POSTERIOR LOBE) EXTRACT ON THE BODY WATER OF FISH AND REPTILES

By ELDON M. BOYD and MALCOLM DINGWALL, Jr.

From the Department of Pharmacology, Queen's University, Kingston, Canada

(*Received* 29 *December* 1938)

In recent publications from this laboratory [Boyd & Brown, 1938; Boyd & Whyte, 1938, 1939; Boyd & Smith, 1938] it has been found that pituitrin causes a retention of water in the body of frogs. Steggerda [1937] demonstrated that other amphibia gave a similar reaction. The experiments described below were designed to investigate the possibilities of a corresponding reaction in the subphyla adjacent to amphibia, namely fishes and reptiles.

Experiments on fish

The species of fish studied included shiners (*Notropis cornutus* (Mitchill)), catfish (*Ictaluras lacustris* (Walbaum)), perch (*Perca flavescens* (Mitchill)), sunfish (*Eupomatis gibbosus* (Linnaeus)) and rock bass (*Ambloplites rupestris* (Rafinesque)). These were obtained in the spring and summer months from their natural habitat in Lake Ontario and the Cataraqui and St Lawrence Rivers and the species identified by Mr G. C. Toner, who has made an extensive study of the fish in the waters of this district [Toner, 1937, 1938]. The fish were kept in aquaria with aerated, cold, running water and fed with minced meat; experiments on any one group of fish were completed within at least 3 weeks after their capture.

The general procedure consisted in attempting to measure changes in the body water of fish by noting any change in their weight over a short period of time during which any such change in weight could be considered as change in body water. In weighing the fish, the operator first moistened his hands to avoid removing the protective slime which covers the fish and is their first defence chiefly against fungus infection. The fish was lifted from its aquarium, placed in a 4 l. beaker containing sufficient water to cover it and the whole was weighed on a trip scale balance to the nearest 0·1 g. The fish was then lifted just above the water and held within the beaker until excess water had been thrown or drained off, after which it was placed in its aquarium and the beaker and water weighed. The difference in weight gave the weight of the fish. With experience and practice, a uniform procedure of weighing was worked out which yielded data of sufficient accuracy. For example, in fifty-six consecutive weighings on ten shiners and rock bass, the value of Pearson's coefficient of variation [Davenport & Ekas, 1936] per mean weight of each fish varied from 0·8 to 2·3 with an average coefficient of 1·2.

Pituitrin was injected intraperitoneally through the ventral belly wall in amounts corresponding to those effective in frogs, i.e. from 0·5 to 2·0 I.U. per 10 g. body weight. In a total of seventy-three experiments in which the fish were weighed every ½ hr. or at longer intervals up to 48 hr. it was impossible to demonstrate that pituitrin had any effect upon the total water content of the body of these fish. These results, in conjunction with those of Burgess, Harvey & Marshall [1933], who found that pituitary extracts did not affect the volume of the urine in the catfish (number of experiments not stated), would suggest that the neurohypophysis may not exercise much control over the body water of fish.

However, the pituitary gland of the same fish contained a substance which produced the same effect as pituitrin when injected into frogs in water, i.e. it produced an uptake of water [Boyd & Brown, 1938]. Acetic acid extracts of fish pituitaries were made by the method described in *The British Pharmacopoeia*, 1932. A total of twenty-four experiments invariably showed the presence in such extracts of a water-balance principle as effective in frogs as similar extracts prepared from fresh human, beef, guinea-pig or cockerel hypophyses, which latter extracts were made for comparative purposes. The fish hypophysis thus contains a water-balance principle, but up to the present time its control, if any, over the water metabolism of fish themselves has not been demonstrated.

Experiments on reptiles

Similar experiments were performed upon the following reptiles: thirty painted terrapins (*Chrysemys picta*), two common snapping turtles (*Chelydra serpentina*), two water snakes (*Zamenis constrictor*) and six young alligators (*Alligator mississippiensis*). All these reptiles were obtained from their natural habitat in Eastern Ontario, except the alligators which were secured from an alligator farm.

Doses of pituitrin varying from 0·02 to 2·0 I.U. per 10 g. body weight were injected subcutaneously into the terrapins and a total of 127 such injections were made, including the controls which received an equivalent volume of 0·25 % acetic acid. The animals were kept in tap water in aquaria and dried and weighed every 2 hr. or at longer intervals. Doses of

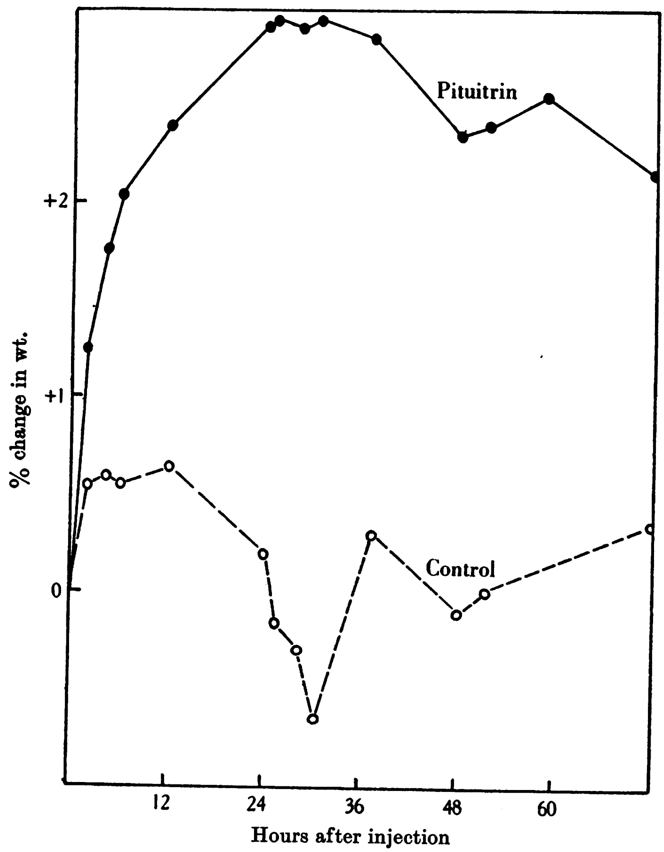

Fig. 1. Mean percentage changes in weight of ten terrapins in water and injected with 2·0 I.U. of pituitrin per 10 g. body weight as compared with ten uninjected controls.

pituitrin less than 0·5 unit seldom, and doses less than 0·1 unit never, had any significant effect upon the body water of these animals. Larger doses produced a gain in weight, presumably due to uptake of water, which in every instance reached a maximum at 6–24 hr. after injection and the extra water was slowly lost during the subsequent 2 or 3 days. A typical experiment is illustrated in Fig. 1, in which are plotted the mean percentage changes in weight of ten terrapins receiving pituitrin and ten controls. The effect of pituitrin was calculated to be statistically significant; e.g. at 24 hr. the mean difference between injected terrapins and controls was 2·83 % change in weight and the standard deviation of the mean difference was 0·91 %, which is indicative of a statistically significant difference [Davenport & Ekas, 1936]. Fewer experiments were performed on the snapping turtles, snakes and alligators, but the results were essentially similar to those obtained with the terrapins.

The response of these reptiles to pituitrin differed from that of frogs in that the uptake of water was relatively less, was more gradual in occurrence and was maintained for a longer period of time. Boyd & Whyte [1939] explained the results in frogs by showing that these amphibia could take up and lose normally about 5 % of their weight of water per hour (this was with frogs not in the hibernating season). Pituitrin was shown simply to inhibit the normal loss of water per hour for a period of 3 hr. and as a result the frogs gained about 15 % of their weight of water in this time.

Terrapins, on the other hand, were found to take up and lose much less water per hour. Those familiar with the habits of these animals will recall that if frogs are left without water over night in a warm, dry room, they will be found dried out and dead the next morning. Turtles may be kept for weeks, even months, without water before they die from dehydration. Weighing ten terrapins every 2–6 hr. after removal from water and drying the surface, it was found that they lost 3·7 % of their weight of water the first 24 hr., most of this occurring in the first 12 hr. They were then dipped in water, dried and weighed to determine how much surface water could not be removed by draining and wiping with a cloth, and they gained 0·5 % of their weight by this step. Replaced in water, they took up 1·58 % of their weight (corrected) of water in 3 hr., 1·95 in 6 hr., 2·66 in 9 hr., 3·10 in 14 hr. and 3·35 in 24 hr. The evidence suggests that terrapins in water are capable of taking up and losing about 3 % of their weight of water per 24 hr.

This is precisely the greatest amount of water which pituitrin caused to be taken up and retained in these terrapins. Could it be shown, thus, that pituitrin inhibits loss of water by terrapins, then the uptake of water might be explained on the basis of the same mechanism as in frogs. Twenty terrapins were taken out of water and dried; half of them were injected with 0·5 unit pituitrin per 10 g. body weight and half uninjected. The loss of water per hour was inhibited by pituitrin for a period of 6 hr., the rate of loss being 57 % greater in the uninjected controls in the first 2 hr., 37 % greater in the next 2 hr., 10 % greater in the 4th to 6th hr. and identical from then on. Pituitrin therefore inhibited loss of water in terrapins for a period of 6 hr. It is during this interval that pituitrin causes the greatest uptake of water by terrapins in water (Fig. 1). The latter uptake of water would appear to be largely, if not entirely, due to an inhibition of the normal water loss with probably little or no

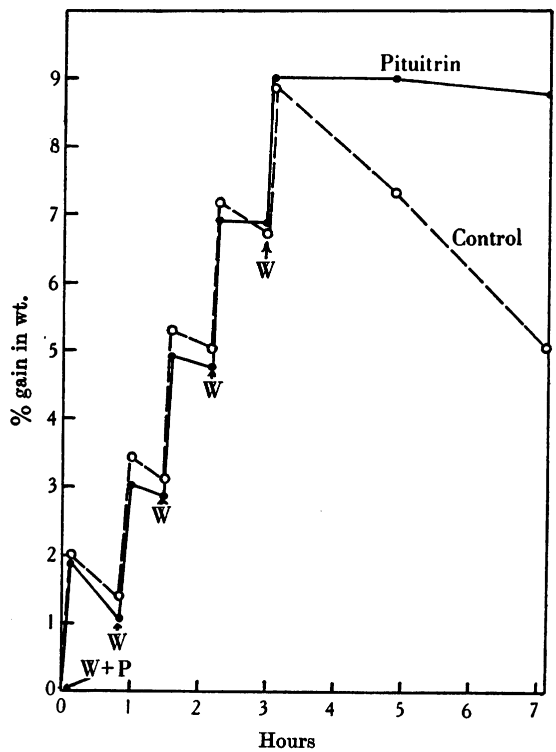

Fig. 2. The effect of simultaneous injection of pituitrin (P) and repeated administration of water (W) on the retention of extra water in terrapins.

effect on the normal intake. The results indicate that reptiles behave like amphibia in the response of the total body water to pituitrin injection, the difference in response being quantitative rather than qualitative.

In support of this conclusion, there was one further point which appeared to require establishment. It had been found that pituitrin inhibits the loss of normal body water over a period of 6 hr. But pituitrin-injected terrapins in water take up extra water during this interval; can pituitrin inhibit the loss of extra water as well as normal body water?

Extra water was added to the body of a total of forty-four terrapins by stomach tube in groups of six, giving at one time 2 % of the body weight of water and then in rotation adding another 2 % until a total of 6–10 % had been given. A water diuresis did not usually become marked until after the terrapins had received 6 % of their weight of distilled water but not more than 2 % could be held at any one time by the stomach. It required 2–3 hr. to administer 10 % of distilled water and weigh the animals regularly every ½ hr. or so. When pituitrin was injected at the same time as water administration was begun, it invariably inhibited the loss of added body water. A representative experiment with three terrapins receiving 0·5 unit pituitrin per 10 g. and three controls, all receiving a total of 10 % of their weight of administered water in five doses, is shown in Fig. 2. In other experiments the control animals lost water so rapidly during the period in which water was being administered in successive doses, that it was impossible to get their weight increased by more than 5 or 6 %, while the pituitrin-injected animals would regularly increase in weight to about 8–9 %. These results prove that pituitrin can inhibit the loss of extra water as well as the normal body water of terrapins.

When the alligators were given water by mouth, intraperitoneal injections of pituitrin inhibited the loss of water in doses of 0·001 I.U. per 10 g. body weight, had little effect at 0·005 unit and stimulated loss of water at higher doses up to 2 units. Burgess *et al.* [1933] recorded an anti-diuretic effect of pituitrin on the water diuresis of alligators using up to 0·01 pressor unit per 10 g. A stimulation of water loss following administration of water with large doses of pituitrin was found in birds and mammals by Boyd, Garand & Livesey [1939].

SUMMARY

Pituitrin, in doses from 0·5 to 2·0 I.U. per 10 g. body weight, injected into five species of freshwater fish, had no effect on the body content of water. Fish hypophyses contained a substance which produced water retention when extracted and injected into frogs.

Pituitrin injected into four species of reptiles produced an increase in body water due to an inhibition of the normal water loss with probably little or no effect on the water intake. Very large doses of pituitrin stimulated water elimination in alligators to which water had been administered.

This work was aided financially by a grant from the Banting Research Foundation.

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