Significance of the Neurohypophysis in Regulation of Fluid Balance in the Frog.* (20089)

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It has been known since 1921(1) that neurohypophyseal extracts cause a temporary weight increase in frogs. It is now well established that this "water-balance effect" is due to an increased rate of water uptake through the skin(2). Heller(3) states, however, that there is insufficient evidence on which to base a theory as to the physiological significance of the water-balance effect. In this paper it is demonstrated that the frog neurohypophysis releases its hormone in response to dehydration, and that this hormone can increase the rate at which dehydrated frogs regain water loss. These facts are presented as evidence for the physiological importance of the neurohypophysis in the regulation of the water economy of the frog.

Methods. Male *Rana pipiens* averaging about 30 g were used. The technics of handling, weighing, hypophysectomy, and statistical treatment have been described previously (4). Contraction of the melanophores of the skin with subsequent inability to expand in response to dark adaptation was used as the criterion of successful total hypophysectomy. The frogs were dehydrated by placing them in individual, dry, wire-covered beakers in a tightly closed closet. To measure rehydration, 100 ml of water was added to each beaker. Neurohypophyses were removed with a generous amount of adjacent nervous tissue and kept frozen until assayed. Individual glands were homogenized and extracted by boiling in 1 ml 0.25% acetic acid for 5 minutes, centrifuged, and the supernatant used for assay. Oxytocic activity was determined by a modified Holton rat uterus method(5). The accuracy of this method is approximately $\pm 10\%$.

Results. A. *Response of neurohypophysis to dehydration.* We have attempted to determine whether the neurohypophyseal hormone is released in response to the need for water conservation. To this end we have assayed the hormone content of the hypophyses of dehydrated and control frogs. Direct estimation of the water-balance activity requires a large number of frogs and is not practical for the assay of individual frog pituitaries. Since the frog water-balance activity resides in the oxytocic fraction of mammalian neurohypophyseal extracts(6) we have used an oxytocic assay. Frogs were rapidly dehydrated under an electric fan until they had lost about 25% of their original body weight. They were then maintained at this weight for 48 hours in a saturated atmosphere over water. Control frogs were kept in water throughout the experiment.

TABLE I. Effect of Dehydration on Hormone Content of Neurohypophysis.

	Hormone in mu/gland	No. of frogs	Dehydration in % wt lost
Control	40 ± 7.3	13	—
Dehydrated	9.7 ± 2.0	12	26.3

It can be seen that the activity of the pituitaries of dehydrated frogs is only about one-fourth as great as that of control glands (Table I). In spite of the great variability a significant difference exists ($P < 0.01$). It is clear, therefore, that the frog neurohypophysis releases its hormone in response to the need for water conservation.

B. *Function of the neurohypophysis in dehydration.* In order to determine the possible physiologic role of the neurohypophysis in states of water deprivation we have compared the rates of dehydration and rehydration in hypophysectomized and intact frogs. The experiments on hypophysectomized frogs were always carried out the day following operation. Thus interference by the decrease in water-balance response, which appears only several days after hypophysectomy(4), could be avoided. Frogs were dehydrated for 24 hours, and then returned to water. Rates

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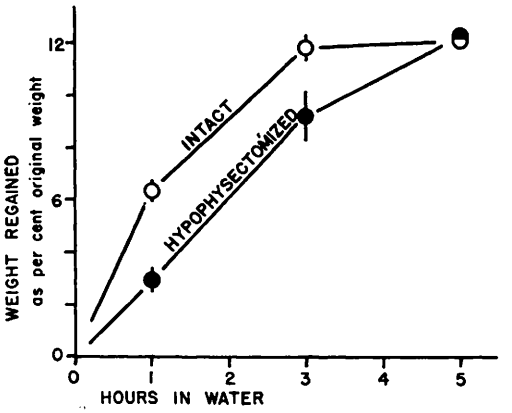

FIG. 1. Return to initial wt after dehydration of normal and hypophysectomized frogs placed in water. Means are represented by points, and stand. errors of the means by vertical lines through the points.

of dehydration and rehydration were determined by noting weight changes every two hours. The results represent pooled averages from three identical experiments, using a total of 31 hypophysectomized and 36 control frogs.

There is no significant difference in the rates at which hypophysectomized and control frogs lose weight during dehydration. Hypophysectomized frogs are not more prone to dehydration than control frogs. However, the rate of rehydration of intact frogs is considerably more rapid than that of the hypophysectomized frogs (Fig. 1). The differences in weight regained at one and at three hours are statistically significant ($P < 0.01$). Normal frogs regain the lost water within three hours while hypophysectomized frogs do not regain full weight until five hours after the return to water. It therefore seems clear that, although the neurohypophysis does not alter the rate of dehydration of the frog, its presence speeds the rate of rehydration when water becomes available.

Discussion. The function of the neurohypophysis in the regulation of fluid balance in mammals has been clearly defined experimentally. A similar function for the presumably homologous amphibian neurohypophyseal hormone has not been demonstrated as clearly. Thus, for example, Simon(7) showed that the pituitaries of dehydrated rats contain significantly less pressor and oxytocic activity than those of control rats. This has been interpreted as signifying that the neurohypophysis of the rat responds to the need for water conservation by releasing stored hormone. In the case of the frog Heller(3) stated that "no significant difference between the water-balance activity of glands of normal and dehydrated frogs" could be demonstrated in a small series assayed. Such results would seem to suggest that the hormone of the frog neurohypophysis is not functionally homologous to the mammalian antidiuretic hormone with respect to water conservation by the organism. The results presented in this paper, however, indicate that a definite difference in the hormone content of the glands of normal and dehydrated frogs does exist. In view of the large variability in our series it is understandable that the differences might not be apparent in a smaller series, particularly when assayed by the water-balance response of frogs, which in itself is highly variable. In any case, our results seem to establish that the frog neurohypophysis, like that of the mammal, releases its hormone in response to the need for water conservation. This is in harmony with Hild's(8) observations on the depletion of neurosecretory material, presumably representing the hormone, from the neurohypophyses of dehydrated frogs.

Granting that water-balance hormone is released in the frog in situations of water deprivation, the physiological utility of the hormone to the organism has been questioned. It has been shown that large doses of pituitrin can decrease the weight loss of frogs kept in a dry environment(9). Heller, however, has found, and we have confirmed, that hypophysectomized frogs are not more prone to dehydration than normal frogs. From these observations it would appear that the amount of hormone released physiologically is not sufficient to help the organism conserve water. From the data presented it does appear, however, that physiologically released hormone can substantially increase the rate at which dehydrated frogs can regain lost body water. We have previously demonstrated that relatively small doses (1.0 unit/100 g) of pitocin can increase the rate of entry of water through frog skin(6). This effect

probably accounts for the observation that normal frogs can regain lost body water faster than hypophysectomized frogs. In any case, this observation suggests strongly that the release of hormone from the neurohypophysis can serve a physiologically useful purpose in the frog. In the toad, which is much more sensitive to the antidiuretic effect of pituitrin(10), it would appear likely that the neurohypophysis could exert a significant effect on the rate of water loss as well.

Summary. 1. The hormone content of the frog neurohypophysis is depleted by dehydration of the animal. 2. Normal and hypophysectomized frogs become dehydrated at the same rate. Normal frogs, however, regain the body water lost by dehydration more rapidly than do hypophysectomized frogs. 3. These observations suggest that the neurohypophysis plays a physiologically significant role in the regulation of the water balance of the frog.

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In vitro Effects of Cortisone on Multiplication of Influenza B Virus.* (20090)

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Infections with a wide variety of organisms including protozoa(1), fungi(2), bacteria (3,4), and viruses(5,6) may be increased in severity by the administration of cortisone to the host. This virtually universal effect remains unexplained despite evidence that cortisone suppresses such physiological host reactions as inflammation(7) and antibody formation(8), depresses granulocytosis(9), and induces lymphocytolysis and the dissolution of lymphoid tissue(10). Although many studies of cortisone and infection have been concerned only with host mortality and disease, some have demonstrated increased concentrations of the infecting agent(3,4,11). It appeared of obvious importance to establish whether an increase in an infecting agent might be induced by cortisone in host tissue isolated from the influence of collateral cortisone effects on phagocytic infiltration and antibody reaction. Accordingly, the present study was undertaken of the multiplication of Influenza B virus in isolated sections of chick chorioallantoic membrane.

Materials and methods. Virus. Influenza B virus (Lee strain) was used. Aliquots of the same seed pot, stored in glass at —65°C were used throughout the study. 0.1 ml of $10^{7.9}$ or $10^{6.9}$ E.I.D.$_{50}$ of virus solution (undiluted and 10-fold diluted allantoic fluid) was inoculated into 2 ml of the following medium to give initial virus concentrations in the nutrient fluid of $10^{6.6}$ and $10^{5.6}$ E.I.D.$_{50}$ per ml. *Culture medium.* Equal volumes of modified glucosol solution as described by Fulton and Armitage(12) and phosphate buffer adjusted to 7.3 pH were mixed immediately before use and penicillin and streptomycin were added to yield final concentrations of 10 units and 40 mg/ml as described by Tamm *et al.*(13). This solution was distributed in test tubes (18 × 150 mm) in amounts of 2 ml. Tubes were stoppered to prevent evaporation. *Tissue*

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