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Effects of Hypophysectomy, Prolactin, and Growth Hormone on Growth of Postmetamorphic Frogs[1]

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Hypophysectomy of juvenile postmetamorphic frogs (*Rana pipiens*) reduced growth (wet weight and length) compared to sham hypophysectomized controls. Mammalian growth hormone at doses of 10–50 μg/day promoted growth in intact frogs during a 2-month period. However, 5 μg/day of GH for 1 month did not promote growth. Mammalian prolactin did not promote growth in postmetamorphic frogs over a 2-month period at doses between 5 and 50 μg/day.

The hormonal regulation of growth in amphibian larvae has recently received considerable attention (Berman *et al.*, 1964; Etkin and Gona, 1967;Bern *et al.*, 1967; Remy and Bounhiol, 1965, 1966). The view has emerged that in the tadpole stages of development a prolactin-like pituitary hormone is involved in promoting growth as well as in inhibiting metamorphosis. The evidence relating to this view is discussed in a previous paper (Brown and Frye, 1969).

Little work has been done, however, on the regulation of growth by pituitary hormones in the postmetamorphic stages of amphibians, and there is no indication whether a situation similar to that in larvae might exist. Moreover, there have been no experiments with hypophysectomized animals indicating whether the pituitary is in fact involved in the regulation of somatic growth in postmetamorphic amphibians. However, Epple *et al.* (1966) reported that starved hypophysectomized toads lost weight faster than the starved controls. It has been demonstrated that hypophysectomy arrests regeneration (Hall and Schotté, 1951) and that both prolactin and growth hormone promote regeneration (Niwelinski, 1958; Wilkerson, 1963) in adult urodeles. But the relevance of this information to the problem of growth control is not certain in view of the possibility of basic differences in the cellular processes of growth and regeneration, or in the mechanisms by which hormones might affect each of these processes. The prevalent situation in higher vertebrates would lead one to expect growth hormone to be the primary growth-promoting agent in the adult, but scattered instances of growth stimulation by prolactin are known (see reviews of Riddle, 1963, and Meites and Nicoll, 1966), and the work of Licht (1967) and Licht and Jones (1967) with lizards opens up the possibility that prolactin is an important growth regulator in some groups.

The purpose of this report is to describe the effects of hypophysectomy, prolactin, and growth hormone on growth of juvenile postmetamorphic frogs, *Rana pipiens*.

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MATERIALS AND METHODS

Frogs

The postmetamorphic *Rana pipiens* used were collected in Kalkaska and Oakland Counties, Michigan, in September and October, 1967. They were small juveniles which had probably metamorphosed the same summer. They were maintained on flies and crickets at room temperatures until they were used in an experiment.

The method of Frye (1969) was used to hypophysectomize the frogs and is briefly described below. The frogs were anesthetized with MS 222 (Sandoz). An incision was made in the skin of the roof of the mouth of an anesthetized frog with a sharp scalpel. A dental drill was used to drill a hole in the parasphenoid and expose the pituitary. The entire pituitary was removed with either fine forceps or a mouth pipette. Using a dissecting microscope, many of the animals were checked at the end of the experiment for pituitary remnants and none were found. Sham hypophysectomies were done on control animals in which the pituitary gland was exposed but not removed.

A week after hypophysectomy the frogs were separated into two groups; (1) those eating normally, which were then put into a group that were fed live flies and crickets and (2) those feeding subnormally, which were then force-fed on liver and mealworms. Each of these groups had a corresponding control, sham-hypophysectomized group. All of the groups were fed two or three times per week.

Several weeks after operation many of the frogs became sensitive to slight changes in the environment and would react with spasms. This was corrected by keeping the frogs in a 0.1–0.3% sodium chloride solution. The concentration necessary to prevent convulsions increased the longer the frogs had been hypophysectomized.

In the experiments in which hormones were administered, intact frogs were used. Due to their increased mortality and susceptibility to infection, it was not feasible to use hypophysectomized frogs which did not tolerate well the daily injections and handling. The volume of hormone or saline solution that each animal received was 0.05 ml per injection. Injections were made intraperitoneally.

The hormone-treated frogs were kept in individual containers and fed three or four times per week on a diet consisting of flies, crickets mealworms, and liver. Each frog received the same quantity of food material at each feeding.

Measurements were made of hypophysectomized frogs at least once a month and hormone-treated frogs were measured every 2 weeks. The size of the frogs used in these experiments ranged from 34–47 mm and 4–10 g at the beginning of the experiments. Length was determined by using a pair of calipers to measure length from the snout to the tip of the urostyle. In all of the experiments the frogs were kept at temperatures of 20–23°C and light conditions of 12–14 hr of light per day.

Hormones

The pituitary hormones used were Mann ovine prolactin (approximately 20 IU/mg and NIH bovine growth hormone (GH) (B-12; 0.97 USP units/mg). The hormone solutions were made up by dissolving the powdered hormone in 0.7% NaCl made basic with dilute NaOH. The solution was subsequently brought to pH 8 using dilute HCl in saline. A solution of 0.7% NaCl was used to bring the hormone solution to the desired concentration. The hormone solutions were made up every 4 days and kept at 4°C when not in use.

RESULTS

Effects of Hypophysectomy of Postmetamorphic Rana pipiens

To determine if the pituitary gland is necessary for normal growth in young frogs, two experiments were done comparing length and weight changes in hypophysectomized frogs with those of sham-hypophysectomized frogs.

In the experiment illustrated in Fig. 1 the frogs were hypophysectomized or sham-hypophysectomized within a 3-day period. Feeding began approximately a week after operation. Both control and hypophysectomized frogs were force-fed, primarily on beef liver and mealworms. Many of the hypophysectomized frogs appeared to have a decreased appetite and even the quantity of food they could ingest when force fed was lower than controls. Consequently all of the frogs were fed an amount equal to the maximum capacity of the hypophysectomized animals. The maximum capacity was assessed by the maximum quantity of force-fed food they would swallow—beyond this they would eject food within a few minutes.

The frogs were measured several times during an 11-week period but it was not

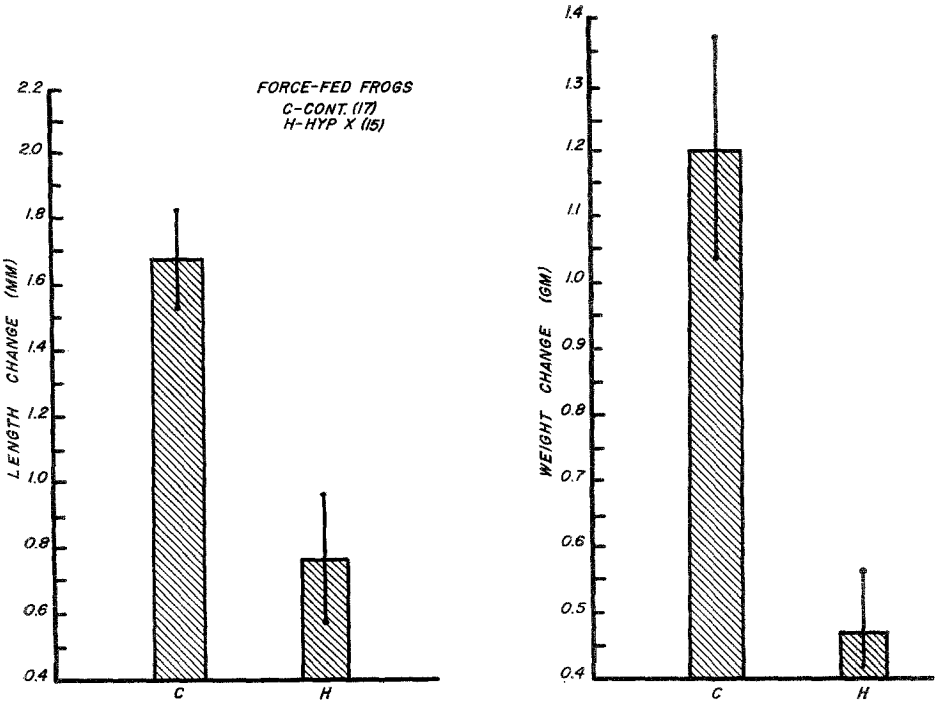

FIG. 1. Mean length and weight changes ± standard errors of sham-operated controls (C) and hypophysectomized (H) postmetamorphic *R. pipiens* force-fed on liver and mealworms for 11 weeks.

until after 6–8 weeks that the controls had grown sufficiently to be measurably different from the hypophysectomized animals. The length and weight changes in the two groups for an 11-week period are shown in Fig. 1. The total changes were small but the means of the two groups differed significantly in length ($p < .005$) and weight ($p < .001$).

The second experiment (Fig. 2) was similar to the first except that the frogs were kept in individual containers and fed live flies and crickets two or three times a week. The data from frogs whose appetites decreased (i.e., those which refused to eat the standard food allocation) during the course of the experiment were not used.

The weight and length changes of the hypophysectomized and control groups for the 7-week period are shown in Fig. 2. Both length and weight of the hypophysectomized frogs decreased to a small extent while length and weight of the controls increased. The means of the two groups differed for both parameters ($p > .05$, length; $p > .001$, weight).

Effect of Prolactin and GH Treatment on Postmetamorphic Frogs

To determine if either GH or prolactin could affect growth in postmetamorphic *R. pipiens*, intact small frogs were weighed and measured and divided into five groups. The first 30 days they were treated as follows:

Group A, 0.7% NaCl; Group B, 5 μg ovine prolactin/day; Group C, 25 μg ovine prolactin/day; Group D, 5 μg bovine GH/day; and Group E, 25 μg bovine GH/day. Injections were given daily. After 30 days the hormone doses were doubled in groups B, C, D, and E. The experiment was continued for another 26 days at the higher doses.

The length changes for the five groups for the first and second months of treatment are shown in Table 1. In the first month only the high GH-treated group (E) differed significantly from controls (A) ($p < .001$). When the doses were doubled during the second month only group D differed significantly from con-

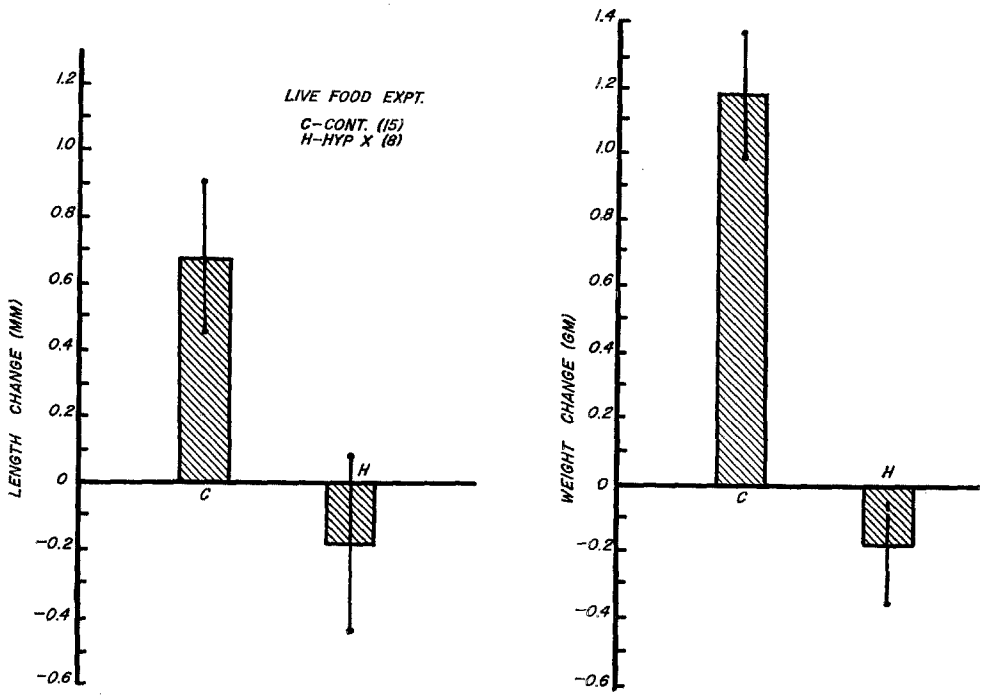

FIG. 2. Mean length and weight changes ± standard errors of sham-operated controls (C) and hypophysectomized (H) postmetamorphic *R. pipiens* fed live flies and crickets for 7 weeks.

trols ($p < .05$). The total length and weight changes for the 2-month period are shown in Fig. 3. If the length changes are combined for the 2 months, only the highest GH group (E) differed significantly from controls ($p < .01$). The weight changes of both D and E differed from the control group ($p < .01$, D; $p < .02$, E).

From this experiment it appears that postmetamorphic frogs responded by an increase in both weight and length to long-term GH treatment. The optimum GH dose appeared to be between 10–25 μg/day. When group D was treated with 5 μg/day GH no response was seen while at the same time the 25 μg/day dose was effective. But when the dose for E was increased to 50 μg, it responded to a lesser extent than D, which was receiving 10 μg. Prolactin had no significant effect upon either length

TABLE 1

LENGTH AND WEIGHT CHANGES IN FROGS TREATED WITH SALINE, PROLACTIN (P), AND GH FOR A 2-MONTH PERIOD (n = number of animals)

		Month 1		Month 2	
Group	*n*	Treatment	Length change (mm) Mean ± SE	Treatment	Length change (mm) Mean ± SE
A	11	Saline	0.32 ± .14	Saline	1.18 ± .18
B	11	5 μg P/day	0.45 ± .14	10 μg P/day	1.32 ± .19
C	14	25 μg P/day	0.61 ± .18	50 μg P/day	1.21 ± .19
D	13	5 μg GH/day	0.23 ± .15	10 μg 6H/day	1.88 ± .21[a]
E	13	25 μg GH/day	1.11 ± .14[b]	50 μg 6H/day	1.69 ± .25

[a] Differed significantly from group A; $p < .05$.
[b] Differed significantly from group A; $p < .001$.

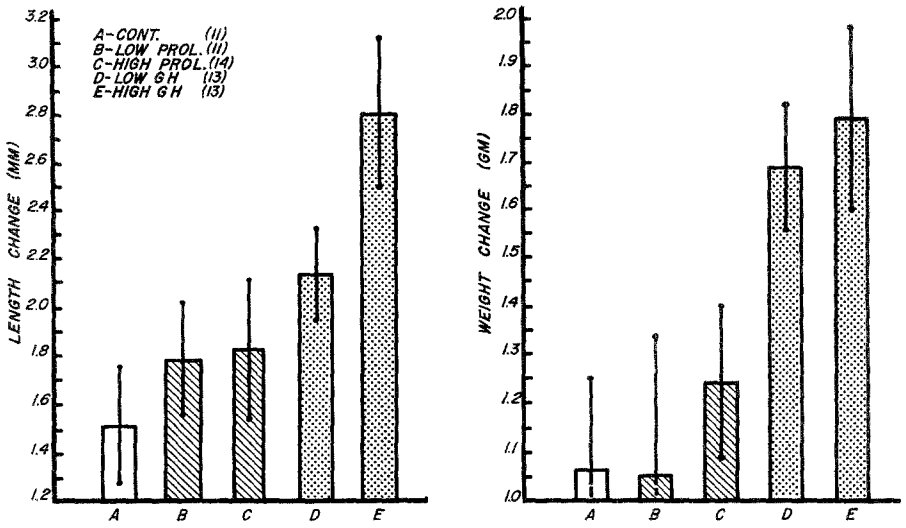

FIG. 3. Total mean length and weight changes ± standard errors of postmetamorphic *R. pipiens* treated for 2 months with saline, prolactin, and GH. Group A, 2 months of saline treatment; Group B, 5 μg/day prolactin for 1 month, 10 μg/day prolactin a second month; Group C, 25 μg/day prolactin 1 month, 50 μg/day prolactin a second month; Group D, 5 μg/day GH 1 month, 10 μg/day GH a second month; Group E, 25 μg/day GH 1 month, 50 μg/day GH a second month.

or weight in any dose used, during any interval of this experiment.

DISCUSSION

The suppression of growth in frogs by hypophysectomy was not unexpected, although it had not been previously reported. This result demonstrates that the pituitary gland plays a role in the regulation of growth in frogs, as in other vertebrates. Although it might seem reasonable to infer from this experiment that the frog pituitary produces a growth-specific hormone, as in higher groups of vertebrates, the data do not of themselves warrant this conclusion, in view of the many other consequences of hypophysectomy which might secondarily depress growth.

As was mentioned at the beginning of this paper, and in the preceding paper (Brown and Frye, 1969), prolactin has proved to be the most effective growth-promoting hormone so far tested in tadpoles. Consequently, the finding that only GH was effective in stimulating growth in frogs, whereas prolactin had no effect, was somewhat surprising. These results suggest that, if the pituitary of frogs produces a specific hormone necessary for normal growth, it may resemble mammalian GH more than it does mammalian prolactin.

The inversion in relative sensitivity to prolactin and GH between tadpole and frog stages of growth is particularly intriguing, and suggests that the hormonal mechanisms of growth regulation are different in these two stages of the life cycle. Tadpoles are in the order of 25–50 times more sensitive to prolactin than to GH (Brown and Frye, 1969) and there is room for doubt that GH-specific growth responses have been produced. Frogs, on the other hand, respond to as little as 10 μg/day of GH, but gave no growth response to up to 50 μg/day of prolactin. This difference must reflect a basic difference in the hormone-response mechanism of the target tissues, and could be due to either of two possibilities: (1) the same tissues are responding to prolactin and GH, but change their relative sensitivities to the two hormones at metamorphosis, or (2) different tissues or cell populations respond to the two hormones, and there is a change in the proportions or quantities of specifically GH- and prolactin-sensitive target tissues during metamorphosis.

This interpretation of the hormonal regulation of growth in frogs would be greatly strengthened by information on the levels of assayable growth factors in the frog pituitary, and particularly on their activity compared with mammalian GH and prolactin. Information as to whether there is a transition in the nature of or proportions of native growth factors, corresponding to the observed transition in sensitivity to exogenous prolactin and GH would be especially valuable to our understanding of the nature of growth regulation in amphibians. Bioassays of adult anurans have demonstrated the presence of both prolactin-like (Foglia, 1940; Chadwick 1966a, 1966b; Nicoll and Bern, 1965; Nicoll, Bern, and Brown, 1966) and GH-like (Solomon and Greep, 1959) activities. Muller *et al.* (1967) have found GH-releasing activity in the hypothalamus of *Rana pipiens*. However, assays of tadpole pituitary extracts have not been made, and thus the relative amounts of these two kinds of activity in tadpoles and frogs are not known. Cytological data on the presence and proportions of prolactin- and GH-secreting cell types in the pituitaries of tadpoles and frogs would be relevant to this problem. Unfortunately, although acidophils, identified with the secretion of prolactin and GH in mammals, have been described in both the frog (Ortman, 1961; Kerr, 1965) and the tadpole (Etkin and Ortman, 1960), not enough experimental work has been done in amphibians to allow correlation of cells of specific staining characteristics with specific hormones.

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