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GROWTH RESPONSES OF LARVAL AND POSTMETAMORPHIC
RANA PIPIENS TO PROLACTIN
AND GROWTH HORMONE

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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ABSTRACT

GROWTH RESPONSES OF LARVAL AND POSTMETAMORPHIC RANA PIPIENS TO PROLACTIN AND GROWTH HORMONE

by

Patricia Stocking Brown

Chairman: B.E. Frye

Mammalian prolactin at doses of 1-50 $\mu\text{g}/\text{day}$ promoted growth (increased wet weight, dry weight and body length) in Rana pipiens tadpoles. Mammalian GH promoted growth only at the higher doses (50 $\mu\text{g}/\text{day}$ and above) and was not as effective as prolactin. But in thyroidectomized tadpoles, lower doses of GH (5-25 $\mu\text{g}/\text{day}$) promoted growth.

Prolactin also inhibited metamorphosis (at doses of 5 $\mu\text{g}/\text{day}$ and above). Inhibition of metamorphosis did not appear to be due to prolactin rendering the tissues insensitive to thyroxine since prolactin treated tadpoles metamorphosed in response to exogenously administered thyroxine (4-100 $\mu\text{g}/\text{l}$) to the same extent as controls. This suggested that prolactin must lower the active thyroid hormone level (either by altering secretion rates via the pituitary or thyroid or by inactivating the thyroid hormone in the circulatory system).

Prolactin promoted further growth in tadpoles in which the thyroid hormone level had been reduced with thiouracil or thyroidectomy. This suggested that the two actions of prolactin are at least partially independent--or that the growth which prolactin promotes is not just the result of thyroid inhibition.

In postmetamorphic frogs, hypophysectomy reduced growth.

Growth hormone (at doses of 10-50 $\mu\text{g/day}$) promoted growth in young frogs. Prolactin (at doses of 5-50 $\mu\text{g/day}$) did not promote growth. However, prolactin did have an effect on skin color. Prolactin-treated frogs became a bright yellow-green.

These studies suggest that the pituitary regulation of growth is different in larval and postmetamorphic stages of Rana pipiens.

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INTRODUCTION

The pituitary hormone prolactin (lactogenic hormone, mammotrophin, galactin, lactogen, luteotrophin or LTH) can elicit a number of diverse effects in vertebrates. Prolactin or a prolactin-like hormone has been implicated in the control of osmoregulation in teleosts, breeding behavior in birds and fish, lactation in mammals, luteotrophic activity in a few mammalian species and crop production of milk in pigeons and doves (Riddle, 1963; Meites and Nicoll, 1966). In amphibians, prolactin is thought to be important in the control of water-drive in land stages of newts (Chadwick, 1940; Grant and Grant, 1958) and in the regulation of growth.

The pituitary gland of amphibians has been known to be necessary for normal growth in anuran tadpoles and for regeneration in urodeles since the early work of Allen (1916) and Smith (1916) with hypophysectomized tadpoles and of Schotté (1926) with hypophysectomized newts. The suggestion that prolactin is the pituitary hormone necessary for normal growth (Etkin and Lehrer, 1960) and regeneration (Niwelinski, 1958) in amphibians is recent.

Stimulated by the work of Etkin and Lehrer (1960) who found that pituitaries removed from their hypothalamic connections promoted growth in tadpoles, Berman et al. (1964), Nicoll et al. (1965), Etkin and Gona (1967) and Remy and Bounhiol (1965, 1966) found that treatment of anuran tadpoles with mammalian prolactin preparations

promoted growth. Licht (1967) and Licht and Jones (1967) found that prolactin also stimulates growth in lizards.

However, growth hormone (somatotrophin) has also been reported to cause an increased growth in tadpoles (Bounhiol et al., 1959; Remy, 1964; Remy and Bounhiol, 1966; Etkin and Gona, 1967) as well as to promote regeneration in newts (Niwelinski, 1958; Wilkerson, 1963). Thus it appears that amphibian growth is sensitive to both growth hormone and prolactin rather than to only one or the other.

Recently prolactin has been reported to inhibit metamorphosis as well as to promote growth in tadpoles (Nicoll et al., 1965; Etkin and Gona, 1967). The thyroid gland is known to be important in the regulation of amphibian metamorphosis (see reviews by Allen, 1929; 1938 and Etkin 1955; 1963; 1964). The question is thus raised: At what level is prolactin interfering with the pituitary-thyroid-tissue axis when metamorphosis is inhibited?

Studies have shown that in addition to inhibition of metamorphosis, growth is stimulated when tadpoles are thyroidectomized or treated with thyroid inhibitors (Allen, 1918; Hoskins and Hoskins, 1919; Steinmetz, 1952; 1954). Since prolactin also inhibits metamorphosis and promotes growth in tadpoles the question is raised as to whether or not prolactin acts as a thyroid inhibitor.

While several investigators have examined the pituitary control of tadpole growth, no work has been done on the pituitary regulation of growth in frogs or toads after

metamorphosis.

The problems to be examined in this study include:

- (1) the comparative effects of low doses of prolactin and growth hormone in promoting growth in intact tadpoles;
- (2) the possibility that prolactin renders the peripheral tissues insensitive to thyroxine in its inhibition of metamorphosis;
- (3) the growth promoting characteristics of prolactin and growth hormone in thyroidectomized tadpoles or tadpoles treated with thiouracil;
- (4) the effects of hypophysectomy on the growth rate of postmetamorphic frogs;
- and (5) the comparative growth responses of postmetamorphic frogs treated with growth hormone and prolactin.

MATERIALS AND METHODS

Tadpoles

Rana pipiens tadpoles were reared from eggs obtained by induced ovulation and artificial insemination, using the method of Wright and Flathers (1961). The adults used for the fertilizations were obtained mainly from the Lemberger Company, Oshkosh, Wisconsin although several batches were from C.H. Mumley, a Vermont supplier. All of the tadpoles used in any one experiment were from the same fertilization, and in some cases tadpoles for several experiments came from a single batch. Until the tadpoles were used in an experiment they were reared at room temperature in shallow enamel pans (12" x 17" x 2½") in aerated tap water or well water. They were fed all cooked washed spinach leaves. At the beginning of each experiment tadpoles were sorted according to size and metamorphic stage so that animals in any one experiment were uniform. In order to keep individual records each animal was kept separately in a 4-inch finger bowl in approximately 200 ml of water during an experiment. They were fed and cleaned daily.

During the course of an experiment the tadpoles were kept at 20°C and on a 12-hr day when possible. When facilities were not available to control light and temperature rigidly the ranges were generally 20-23°C and 10-14 hrs daylight. The hormone responses described in these experiments were consistent whether the experiment had been run under rigidly controlled light and temperature conditions

or whether a small amount of fluctuation had occurred. Since all of the groups in one experiment were subjected to identical light and temperature conditions there is no reason to think that variation in temperature or photoperiod in itself accounts for the different responses of the control and hormone-treated animals. However the differences in light and temperature conditions could be a factor in comparing the absolute weight and length changes from one experiment to another.

In most experiments each group started with at least 10 animals. Some of the animals died and a few others were injured. Data from animals that did not stand up well to the daily treatments, as evidenced by injuries or loss of appetite, were not used. Consequently a few of the groups were very small at the end of an experiment.

To rank the tadpoles with respect to their metamorphic state, Taylor-Kollros (1946) stages for Rana pipiens were used. An effort was made in each experiment to use tadpoles that were initially at similar metamorphic stages. No tadpoles were used in any experiment that were in stages earlier than 6 or later than 16 at the beginning of the experiment.

Weight and length measurements were made every week in most experiments, and sometimes more frequently. Total body length including the tail was used for the length measure. The tadpoles were blotted on a damp cotton towel prior to weighing. Hind leg length was measured in several

experiments.

Injectons were made intraperitoneally by inserting a 30 gauge syringe needle through the opercular opening and into the body cavity, ventral to the gut. In all of the experiments the volume of hormone solution or saline that each animal received was 0.01 ml per day.

Thyroidectomy of tadpoles was done using the method of Dent (1959) on tadpoles between stage 6 and 14. Tadpoles healed in approximately 1 week after surgery at which time their food consumption approached normal. In experiments in which nonthyroidectomized controls were used, the thyroid gland was exposed but not removed. No histological examinations for thyroid remnants were done at the end of the experiments. If the tadpoles continued to metamorphose they were considered incompletely thyroidectomized.

Dry weight measurements were made on tadpoles in Experiment 3. The tadpoles were dried in a vacuum oven and weighed at 24-hour intervals until the weights were stationary.

Frogs

The postmetamorphic Rana pipiens used were collected in Kalkaska County and in Oakland County, Michigan in September and October, 1967. Many had probably metamorphosed the same summer. They were maintained on flies and crickets at room temperature until they were used in an experiment. (See Nace (in press) for details on care of postmetamorphic frogs.)

The method of Frye (in press) was used to hypophysectomize the frogs and is described below. An incision was made in 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 bone and expose the pituitary. Both the anterior and posterior lobes of the pituitary were 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. However, histological examinations were not done. 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 surgery 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. This problem was probably due to salt imbalance resulting from the lack of ACTH

stimulation of the adrenal gland after hypophysectomy.

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 would not well tolerate daily injections and handling. The volume of hormone or saline solution that each animal received was 0.05 ml per injection.

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. The frogs were all fed equal quantities of food.

Measurements were made of hypophysectomized frogs at least once a month and hormone-treated frogs were measured every two weeks. The size of the frogs used in these experiments ranged from 34-47 mm and 4-10 gm at the beginning of the experiments. Length was determined by using a pair of calipers to measure snout-urostyle length. Length rather than weight was considered a more meaningful measure of growth in frogs. Weight varies considerably during a short period and is highly dependent on water uptake. Consequently, for shorter periods of time (2 weeks or even a month) the weight data was too variable to be meaningful.

Hormones and drugs

The pituitary hormones used were National Institutes

of Health (NIH) bovine prolactin (B-2; 19.9 IU/mg), Mann ovine prolactin (approximately 20 IU/mg), NIH bovine growth hormone (GH) (B-12; 0.97 USP units/mg) and NIH porcine GH (1.6 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. Since the bovine GH preparation had a greater amount of thyrotropin (TSH) contamination than the prolactin preparations it was desirable to do an experiment using a purer preparation of GH. A porcine GH preparation which was thought to have an extremely low TSH contamination was obtained from Dr. A.E. Wilhelmi (NIH). This was used in Experiment 3.

When thyroid hormone or thiouracil solutions were used the tadpoles were immersed in, rather than injected with, the solutions. Both thyroid hormone and thiouracil solutions were made up by dissolving the powder in a small amount of dilute NaOH solution. When this was diluted to the appropriate concentration with either aerated tap water or well water the pH was approximately 7. The animals in these solutions, like the corresponding control animals, were changed daily. The thyroid hormone used was l-sodium thyroxine pentahydrate. Both the thyroxine and the thiouracil came from Nutritional Biochemicals Corporation.

The tadpoles and frogs were always anesthetized with MS222 (tricaine methanesulphonate, Sandoz) prior to surgery. The tadpoles were also anesthetized prior to hormone injection or measurement. In most experiments hormone injections began the day after (day 1) measurements were initially recorded (day 0).

Calculation and statistics

The raw data are listed separately for each experiment and are tabulated in the appendix. Only the mean changes per group and their standard errors and the number of animals per group are included in the tables in the text. Because of some initial variation in size and metamorphic stage, changes in weight and length rather than absolute weight and length were the quantities compared between groups.

Student's t test was most frequently used to compare the means of two groups (controls and experimentals). In Experiment 1, where a number of hormone-treated groups were compared with a control, Dunnett's (1955) test was used. In this case the number of animals in the lower dose prolactin groups was too small to make a meaningful dose-response curve. In cases where two doses of hormones were used and the change elicited by the smaller dose was in the same direction as that of the larger dose but too small to show a significant difference from controls, a regression of weight or length against hormone dose was calculated.

The large variance of the absolute values of the dry weight data necessitated using the Wilcoxon signed rank test (1945, 1947) in which pairs of experimental and control tadpoles were compared.

As used here, a significant difference indicates a p value of 0.05 or less.

RESULTS

Prolactin and growth hormone treatment of intact tadpoles.

Experiment 1

In order to determine the effects of various doses of prolactin and growth hormone on the body length and weight of intact tadpoles, nine groups of tadpoles were measured and weighed. Treatments consisted of daily injections of the following substances for the duration indicated:

- Group A - 0.7% NaCl for 3 weeks
- Group B - 0.5 μ g bovine prolactin for 2 weeks
- Group C - 1.0 μ g bovine prolactin for 2 weeks
- Group D - 5.0 μ g bovine prolactin for 3 weeks
- Group E - 50.0 μ g bovine prolactin for 3 weeks
- Group F - 0.5 μ g bovine GH for 2 weeks
- Group G - 1.0 μ g bovine GH for 2 weeks
- Group H - 5.0 μ g bovine GH for 2 weeks
- Group I - 50.0 μ g bovine GH for 3 weeks

The weight and length changes for a 2 week period for all nine groups are shown in Table 1 and Figures 1 and 2.

The mean weight changes in the GH-treated groups did not differ significantly from the control group. Using Dunnett's (1955) method for comparing several treatments with one control, the mean weight changes of the high prolactin groups (D and E) differed significantly from controls ($p < .05$). Although groups B and C clearly followed the same trend as D and E, using the Dunnett test, they are not significantly different. If the Student t test is used, the mean weight change in group C did differ significantly from A ($p < .05$).

If the Dunnett test is used to compare the mean length

changes of the hormone groups with the controls, only the highest prolactin group (E) is significantly different ($p < .05$). The other prolactin-treated groups obviously changed in the same direction. Again, using the Student t test, group C differed from A ($p < .05$) and the probabilities that the other groups were the same as the controls were low. Of the GH-treated tadpoles, only group I appeared likely to have differed from A ($p < .10$) although the differences were not statistically significant.

Figures 3 and 4 show the results of 3 weeks of treatment on weight and length in groups A, D, E and I. The weight changes in groups A and I closely paralleled each other. Both groups D and E increased for the first 2 weeks but D started to level off and decrease during the third week while E still increased. The length changes in I more closely paralleled D for the first 2 weeks but decreased more rapidly during the third week. Only E continued to increase throughout the 3 weeks whereas A, I and D all decreased the third week. The changes in weight and length that occurred during the third week are shown in Table 2.

When the tadpoles were staged after 2 weeks it was found that D and E tadpoles were metamorphosing slightly more slowly than the controls (A) while group I was slightly faster. This same relationship held after the third week. For instance, after the third week, group D and E tadpoles were still at stages 16-17 while A tadpoles were at 18-19 and I at 19-20. After stage 18 tadpoles begin to lose

Table 1. Weight and length changes in tadpoles during the first 2 weeks of hormone treatment. P = prolactin.
n = number of animals. SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
A	10	0.7% NaCl	1.09	$\pm .20$	0.79	$\pm .14$
B	4	0.5 μ g P	1.72	.23	1.32	.20
C	9	1.0 μ g P	1.71	.16	1.21	.13
D	8	5.0 μ g P	1.95	.18	1.15	.10
E	5	50.0 μ g P	2.26	.33	1.40	.06
F	12	0.5 μ g GH	1.26	.18	0.75	.12
G	8	1.0 μ g GH	1.09	.25	0.79	.22
H	11	5.0 μ g GH	1.11	.19	0.80	.11
I	10	50.0 μ g GH	1.00	.14	1.14	.13

Table 2. Weight and length changes during the third week of hormone treatment. P = prolactin. n = number of animals.
SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
A	10	0.7% NaCl	-.61	$\pm .17$	-.56	$\pm .18$
D	8	5 μ g P	-.03	.10	-.17	.12
E	5	50 μ g P	.48	.17	.20	.10
I	10	50 μ g GH	-.55	.23	-.44	.14

Fig. 1. Weight changes in control, prolactin and GH-treated tadpoles in 14 days. Means and standard errors represent data in Table 1.

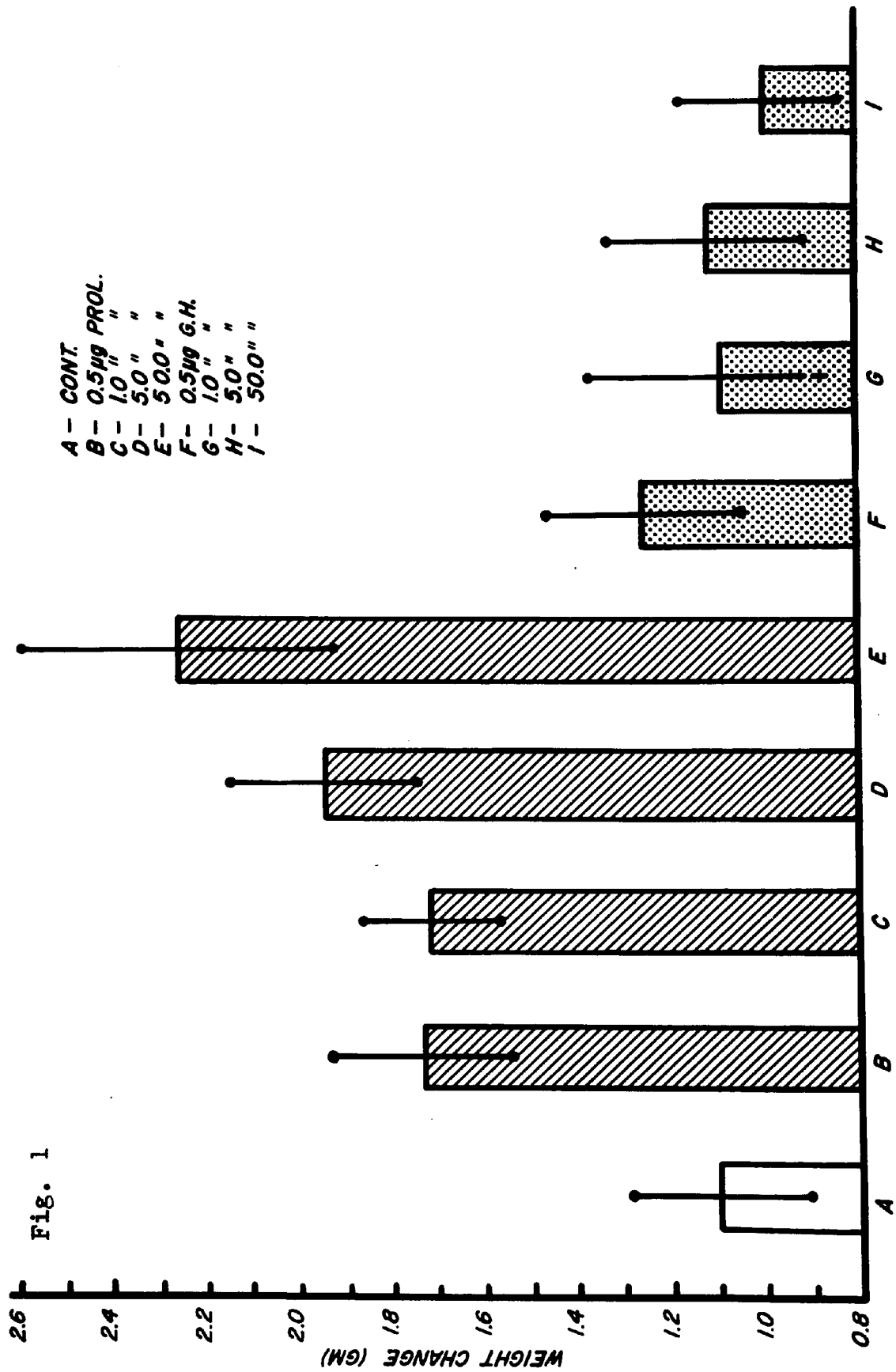


Fig. 2. Length changes in control, prolactin and GH-treated tadpoles in 14 days. Means and standard errors represent data in Table 1.

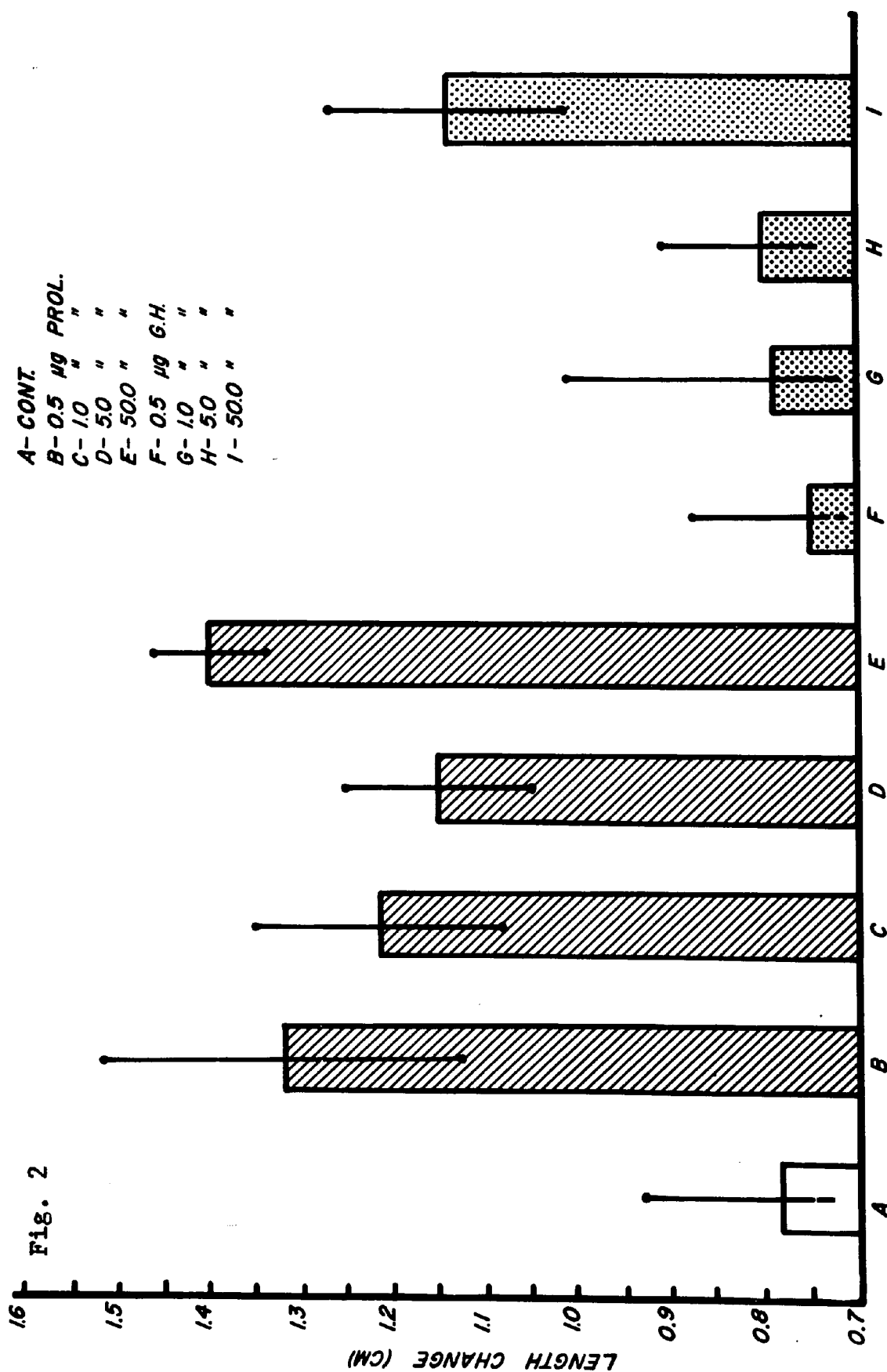


Fig. 3. Weight changes in control, prolactin and GH-treated tadpoles in 21 days. Means represent data in Tables 1 and 2.

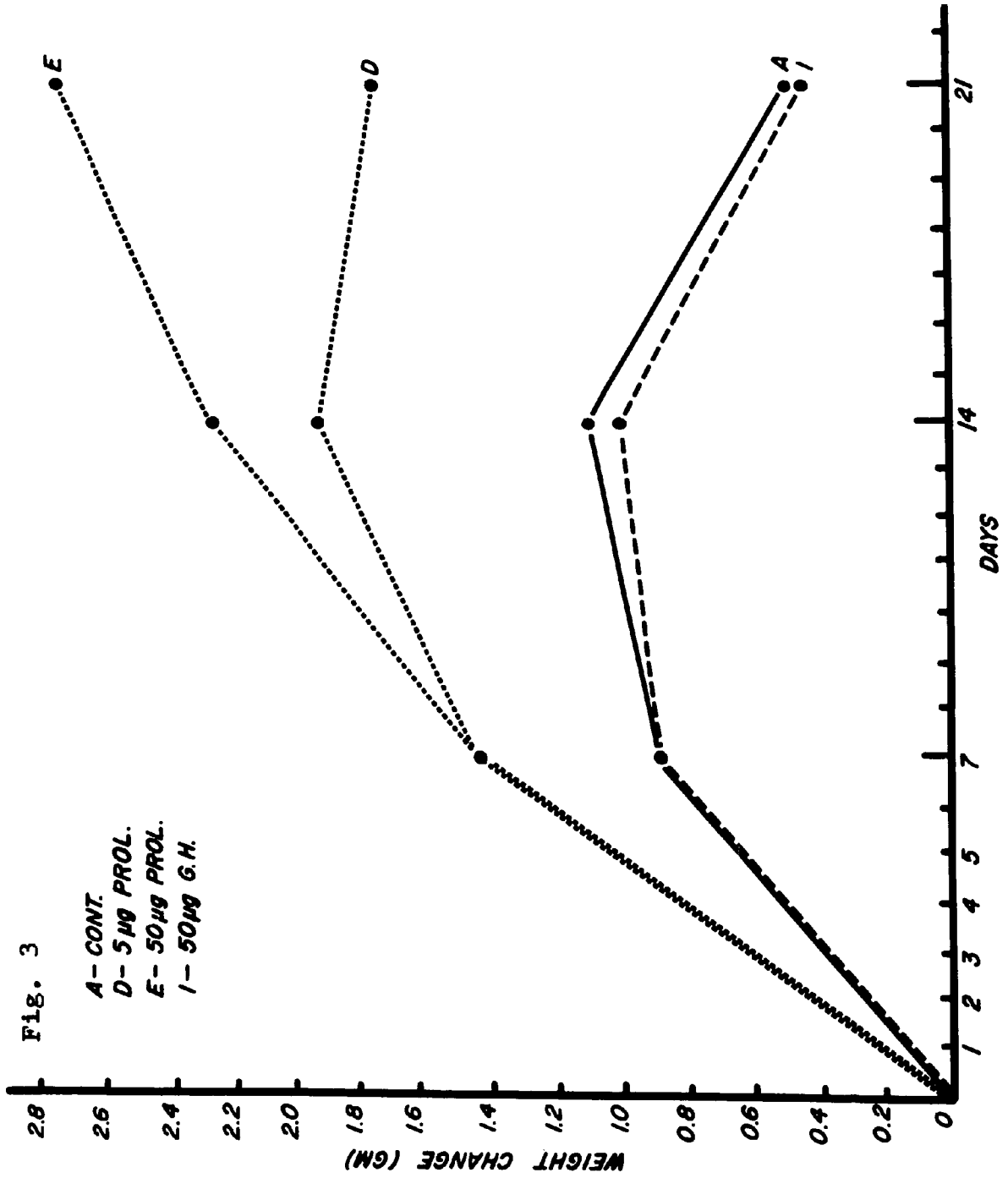
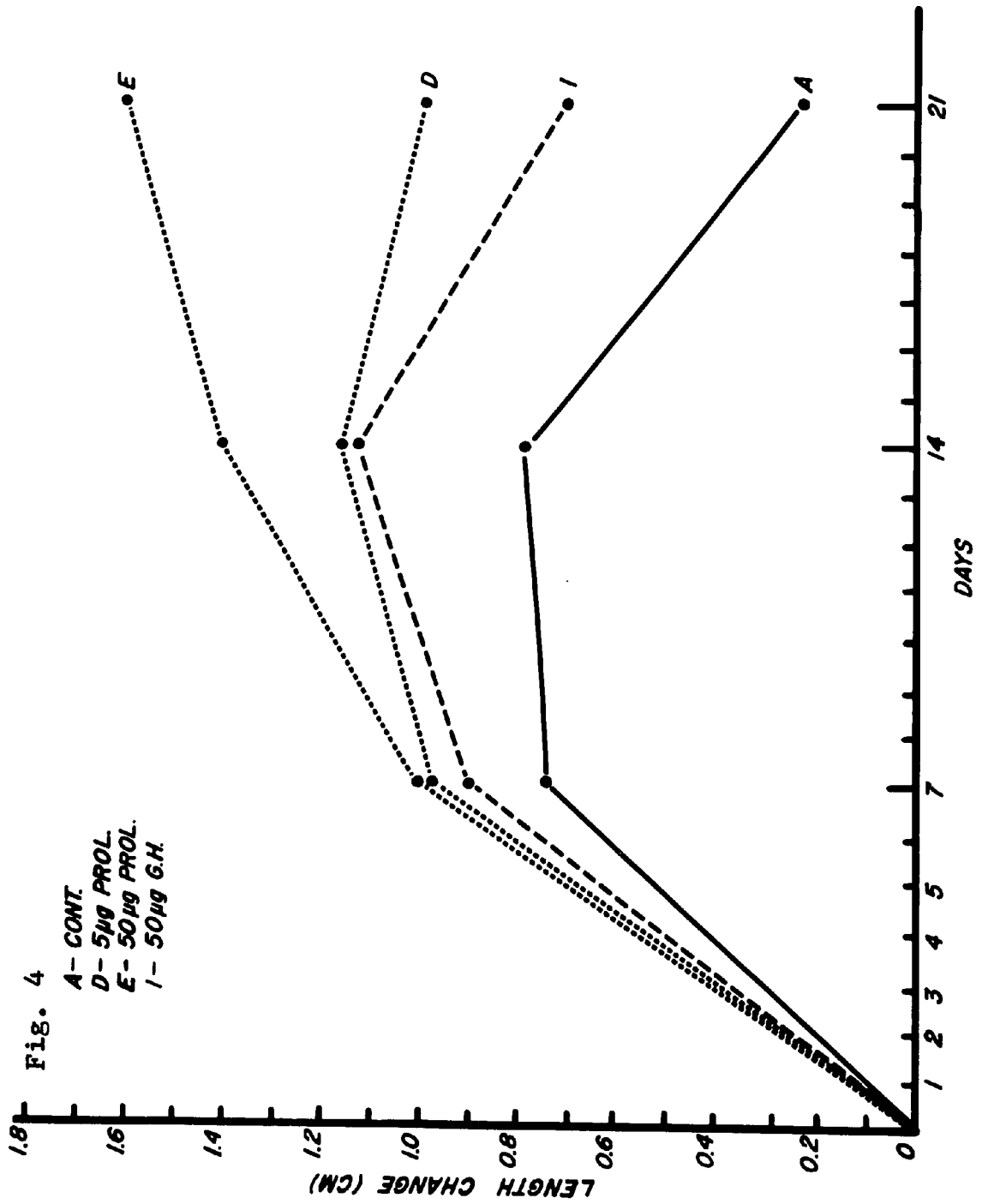


Fig. 4. Length changes in control, prolactin and GH-treated tadpoles in 21 days. Means represent data in Tables 1 and 2.



weight as tail resorption begins. It was possible that TSH contamination of the GH preparation accounted for the faster rate of metamorphosis in group I.

This experiment indicated that: (1) prolactin treatment produced an increase in both weight and length in tadpoles while (2) GH had no effect at these doses (although there is some possibility it stimulated growth in length at the highest dose). The slower metamorphic rate in the groups getting high doses of prolactin suggest that prolactin may be interfering with thyroid hormone production or activity at some level.

Experiment 2

In the previous experiment, 5 and 50 $\mu\text{g}/\text{day}$ of prolactin significantly stimulated growth, whereas 0.5 and 1.0 $\mu\text{g}/\text{day}$ did not, even though the overall trend seemed to suggest an effect. In a further attempt to determine the minimum effective prolactin dose, the two lowest doses used in Experiment 1 were repeated. Three groups of tadpoles were treated for 2 weeks with daily injections of the following:

- Group A - 0.7% NaCl
- Group B - 0.5 μg bovine prolactin
- Group C - 1.0 μg bovine prolactin

The mean weight and length changes are shown in Table 3 and Figures 5 and 6.

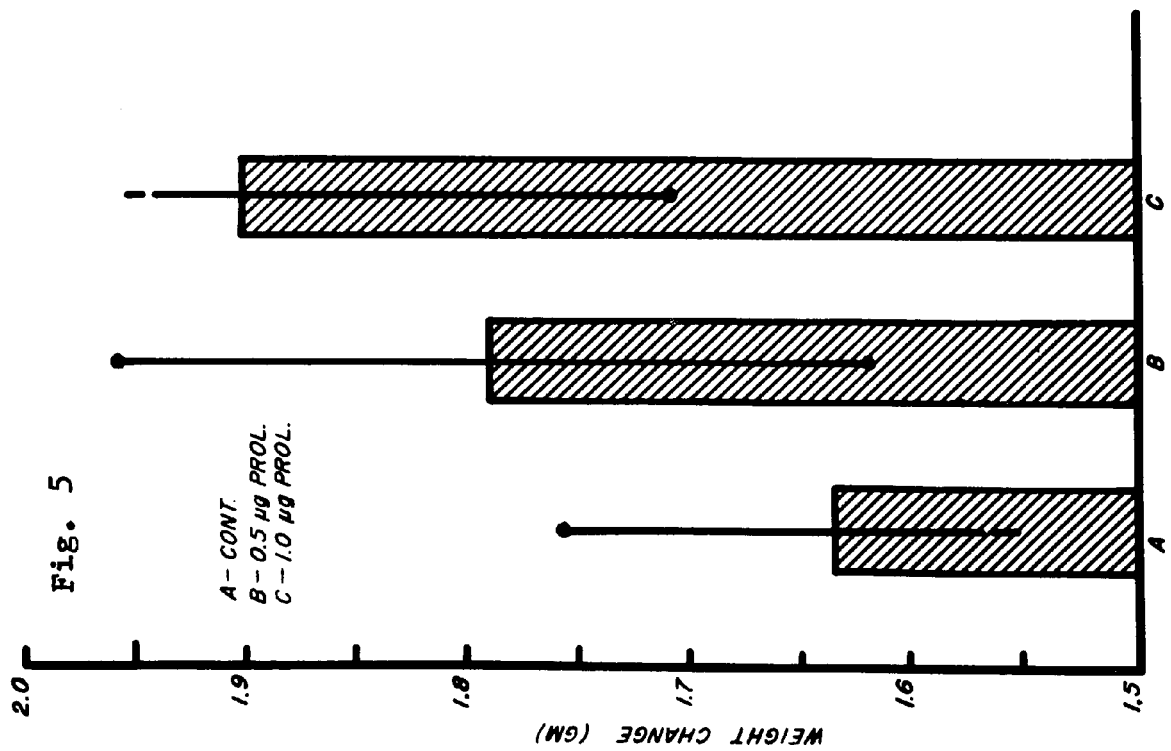
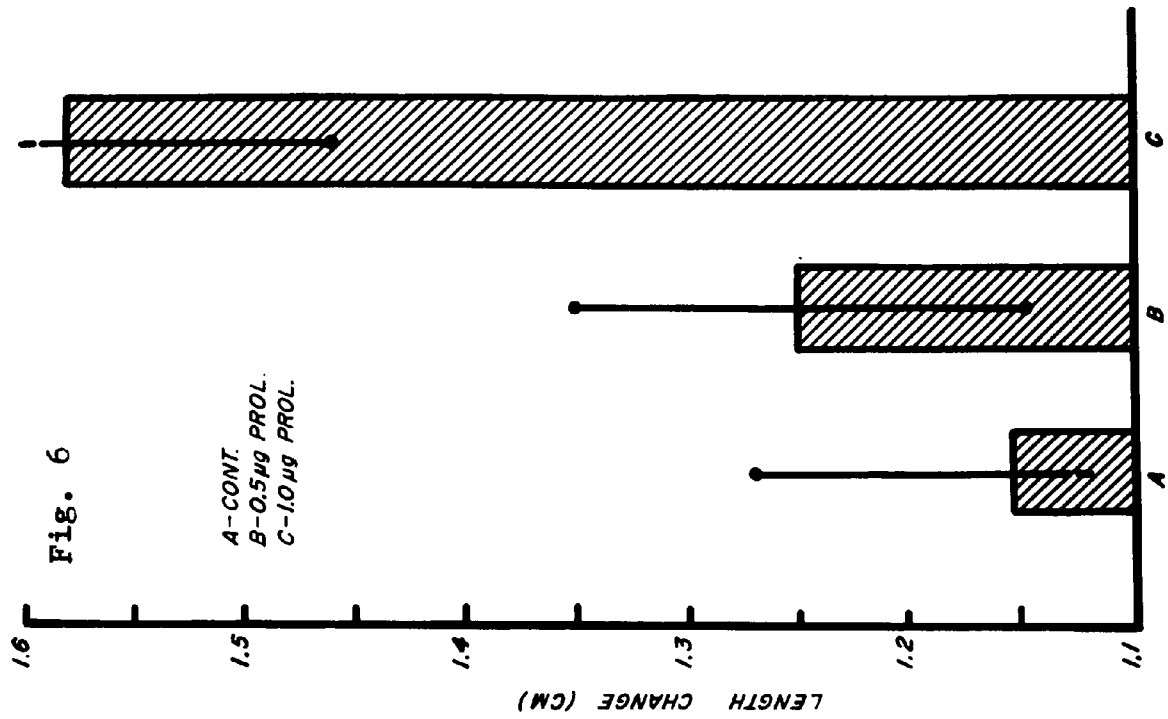
Although the mean weight and length increased with increasing prolactin doses, the weight change of prolactin-treated animals was not significantly greater than controls

Table 3. Weight and length changes in control and prolactin treated tadpoles in 2 weeks. P = prolactin. n = number of animals. SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
A	12	0.7% NaCl	1.63	$\pm .13$	1.16	$\pm .11$
B	12	0.5 μ g P	1.79	.17	1.25	.09
C	12	1.0 μ g P	1.90	.19	1.58	.12

Fig. 5. Weight changes in control and prolactin-treated tadpoles in 14 days. Means and standard errors represent data in Table 3.

Fig. 6. Length changes in control and prolactin-treated tadpoles in 14 days. Means and standard errors represent data in Table 3.



using the Student t comparison of means. Neither does a regression of weight versus dose give a significant slope. However, a regression of length versus dose gives a slope of 0.417 which is significant ($p < .025$). Comparison of the mean lengths shows C to be greater than A ($p < .025$). At the beginning of this experiment the tadpoles were at stages 10-14. At the end of the 2 weeks they were at stages 15-19. The low prolactin doses used in this experiment did not appear to **affect** the rate of metamorphosis.

- The minimum effective dose of prolactin that promotes growth is approximately 1.0 μg per day according to this experiment.

Experiment 3

Experiment 3 was done to determine: (1) if the effect of a high GH dose found in Experiment 1 (group I) on length and metamorphic rate was repeatable and (2) if the prolactin effect on wet weight was also reflected in dry weight. Porcine GH was used because it was thought to have considerably less TSH contamination than the bovine GH preparation and therefore any effect of GH on metamorphosis could be more clearly interpreted.

Tadpoles were divided into three groups and treated for 2 weeks with daily injections of the following:

- Group A - 0.7% NaCl
- Group B - 50 μg porcine GH
- Group C - 10 μg bovine prolactin

Table 4 and Figures 7 and 8 show the mean changes in length and weight of the three groups for the 15 day period.

Table 4. Weight and length changes in tadpoles after 2 weeks of daily treatment. P = prolactin; n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
A	12	0.7% NaCl	1.02	±.08	1.20	±.06
B	11	50 µg GH	1.21	.08	1.55	.11
C	12	10 µg P	1.58	.09	1.90	.08

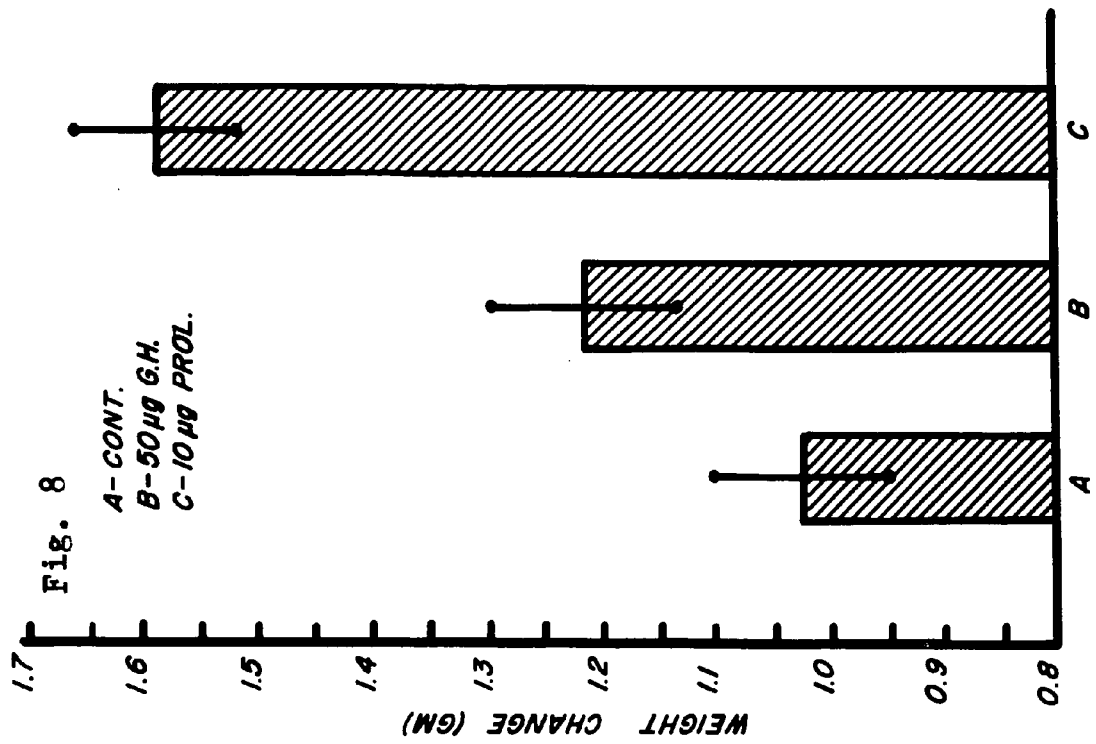
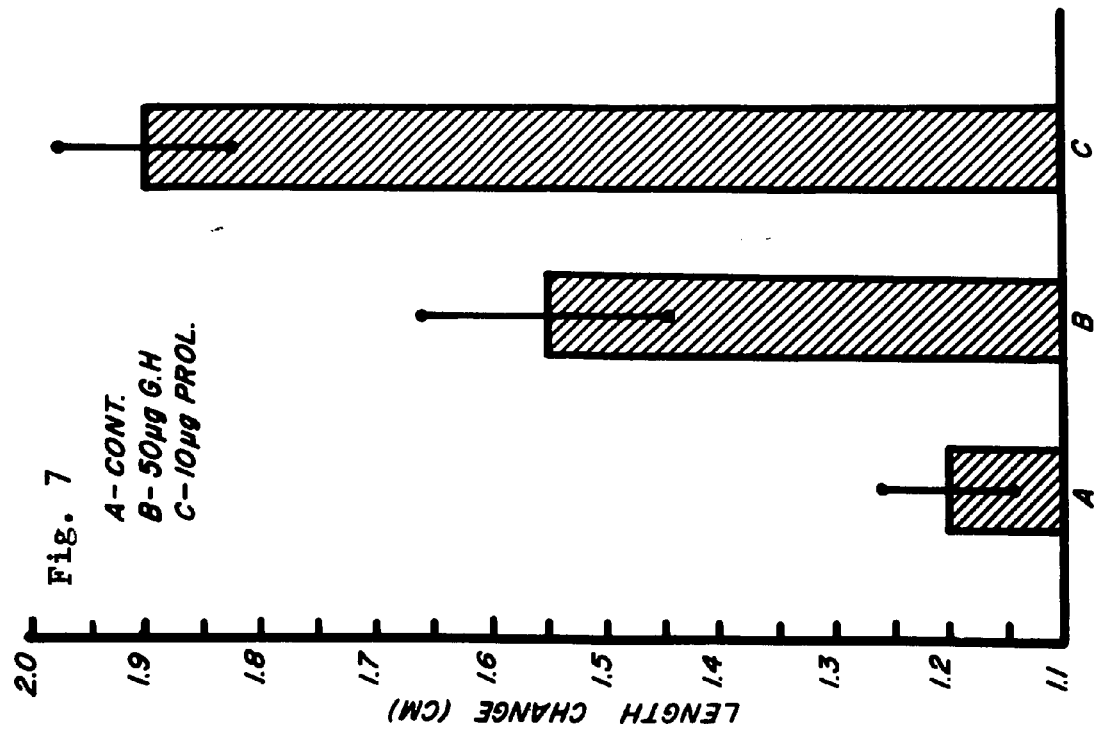
Table 5. Dry weight comparison between controls (A) and prolactin treated tadpoles (C) using the Wilcoxon signed rank test.

Final Wet Weight		Final Dry Weight		d_i	Rank
A	C	A	C		
2.2gm	2.7gm	.188gm	.252gm	+.64	12
2.2	2.9	.200	.214	+.14	6
2.3	2.9	.208	.217	+.09	5
2.3	2.9	.215	.246	+.31	10
2.3	2.9	.220	.237	+.17	8
2.4	3.0	.222	.220	-.02	-1
2.5	3.0	.232	.240	+.08	4
2.5	3.1	.195	.240	+.45	11
2.6	3.1	.238	.222	-.16	-7
2.8	3.1	.212	.230	+.18	9
2.8	3.4	.245	.242	-.03	-2
3.2	3.5	.280	.274	-.06	-3

Number of pairs = 12. T = 13. Dry weights of A and C are significantly different ($p < .025$).

Fig. 7. Length changes in control, prolactin- and GH-treated tadpoles in 14 days. Means and standard errors represent data in Table 4.

Fig. 8. Weight changes in control, prolactin- and GH-treated tadpoles in 14 days. Means and standard errors represent data in Table 4.



As before, both length and weight changes of prolactin-treated tadpoles were significantly greater than controls ($p < .001$). The GH-treated tadpoles had mean weight and length changes that were greater than controls but the weight change was not significant ($p < .10$). However the length changes between A and D were significantly different ($p < .01$). As judged by staging the tadpoles, neither GH- nor prolactin-treated tadpoles differed to any measureable extent in the rate of metamorphosis from the control tadpoles. In this experiment the initial stages of the tadpoles were 8-10. By the termination of the experiment the tadpoles were at stages 14-17.

At the beginning of the experiment the mean wet weights of groups A and C were almost identical. Dry weights were taken of the tadpoles in groups A and C after the 2 weeks of treatment. Tadpoles in both groups were ranked with respect to their final wet weight and paired accordingly. Using the nonparametric Wilcoxon's signed rank test it was found that the dry weights of A and C were different ($p < .025$). The details of the Wilcoxon test are shown in Table 5.

The results of this experiment indicate that dry weight as well as wet weight of prolactin-treated tadpoles increased and that a high dose of GH increased the growth of intact tadpoles as shown by the increased length. However, no effect of GH or prolactin on the rate of metamorphosis was found.

Prolactin and thyroxine treatment of tadpoles.

Experiment 4

Experiment 4 was done to determine whether or not prolactin would sustain body weight and inhibit metamorphosis when thyroxine was administered exogenously. This question relates both to (1) whether prolactin inhibits metamorphosis by rendering the tissues insensitive to thyroxine and (2) whether or not the prolactin stimulation of growth can occur in the presence of a high concentration of thyroxine.

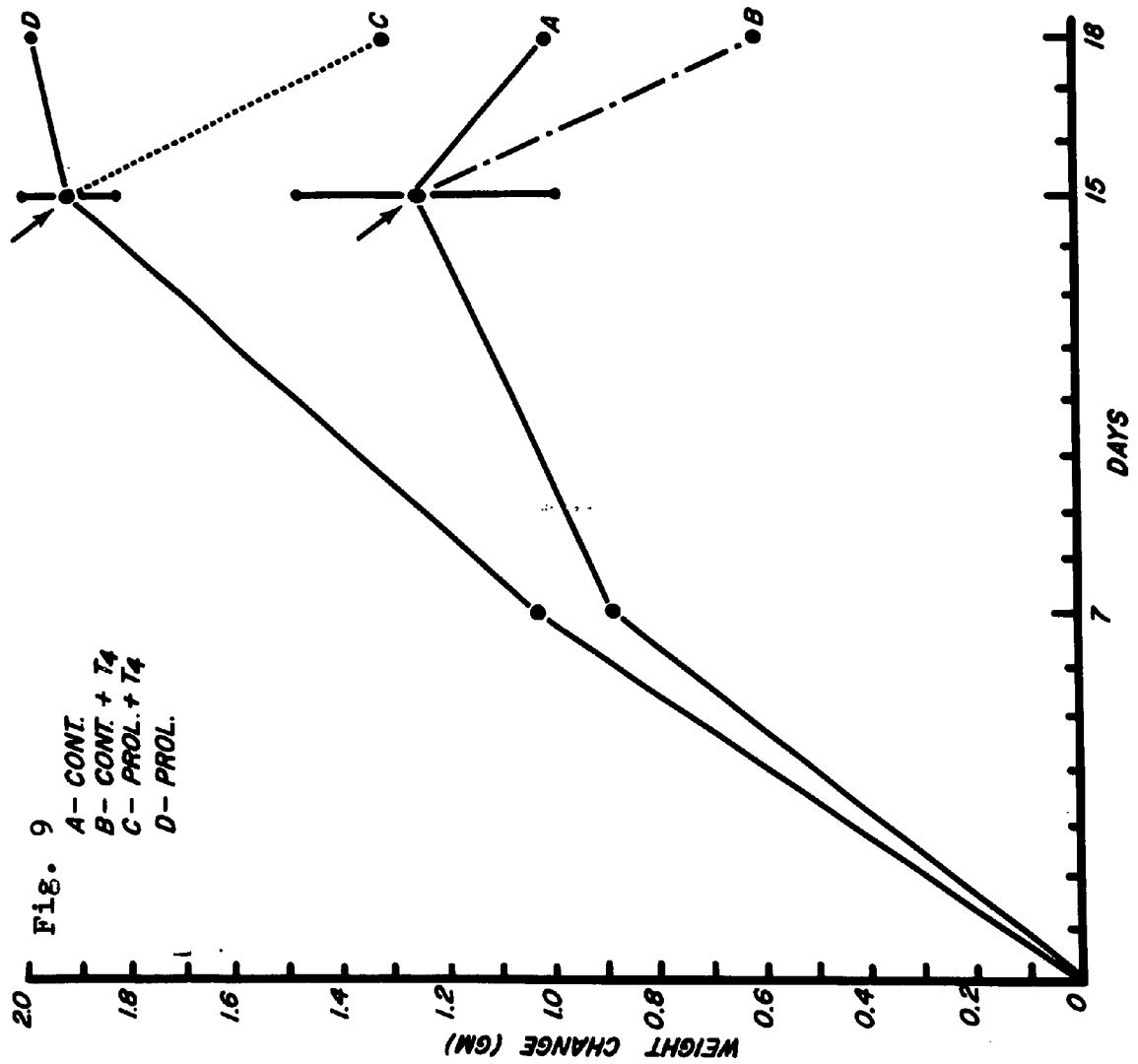
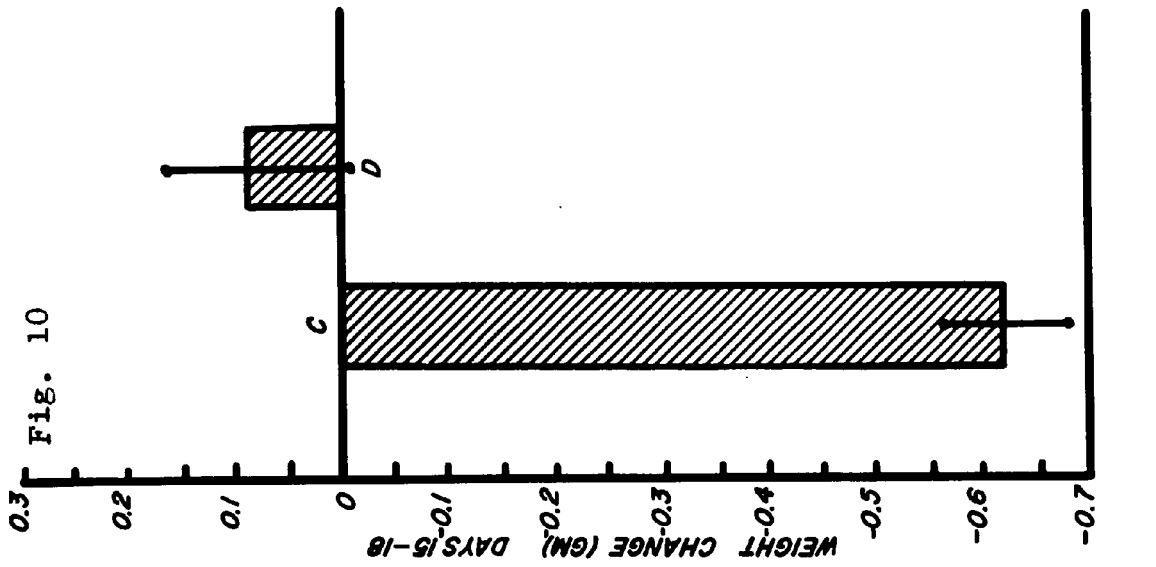
Tadpoles were measured, staged and divided into two groups. Group AB received daily injections of saline. Group CD received 5.0 µg/day of bovine prolactin. On day 15 groups AB and CD were divided into groups A and B, and C and D respectively. Groups B and C were then kept in a solution of 100 µg/l thyroxine. (This dose of thyroxine is known to be effective in stimulating tadpole metamorphosis.) Both C and D continued to get daily injections of prolactin and A and B of saline. Table 6 and Figures 9 and 10 show the effect of prolactin and thyroxine on weight. The weight differences between AB and CD at 15 days were significantly different ($p < .01$). The control group A began to lose weight after day 15 but the thyroxine-treated control (B) lost weight much faster. Prolactin-treated group D continued to gain weight but the thyroxine-treated prolactin group (C) began to lose weight. The weight change between groups C and D from days 15-18 is signifi-

Table 6. Weight changes during an 18-day period in tadpoles treated with prolactin (P) and thyroxine (T₄).
 Group AB = saline; Group CD = 5 µg P; Group A = saline;
 Group B = saline + T₄; Group C = P + T₄; and Group D = P;
 n = number of animals; SE = standard error.

Days 0-15				Days 15-18			
Group	n	Weight change(gm)		Group	n	Weight change(gm)	
		mean	SE			mean	SE
AB	9	1.22	±.25	A	6	-.17	±.11
				B	8	-.59	.10
CD	24	1.90	.10	C	12	-.62	.06
				D	12	.08	.08

Fig. 9. Weight changes in tadpoles in an 18-day period. Group AB was treated with daily saline injections and group CD with 5 μ g/day prolactin. At day 15 groups AB and CD were divided into A and B, and C and D respectively. Both C and D continued to receive 5 μ g/day prolactin. Groups B and C were kept in 100 μ g/l thyroxine solution from days 15-18. Arrows indicate the initiation of thyroxine treatment. Means and standard errors represent data in Table 6.

Fig. 10. Weight changes in groups C and D between days 15 and 18. Means and standard errors represent data in Table 6. See Fig. 9 for details of the treatments.



cantly different ($p < .001$)(Figure 9).

Staging of the tadpoles showed that by day 15, AB was several stages ahead of CD (AB, 16-18; CD, 14-17). In order to be able to compare the results of thyroxine treatment on groups B and C, tadpoles were added to groups A and B at day 15 which more closely corresponded to the stages of C and D. Both groups B and C began to progress rapidly toward metamorphic climax after they were treated with thyroxine. By day 18 tadpoles in groups B and C had reached stages 18 and 19 while many in groups A and D were still at stages 16 and 17.

To recapitulate, tadpoles that received 2 weeks of daily treatment with 5 μ g prolactin showed both an increased weight and a retardation of metamorphosis compared to controls. Continued treatment with prolactin had the same effect but not in animals that were kept in a solution with a high concentration of thyroxine. Those animals lost weight and began to metamorphose as rapidly as thyroxine-treated tadpoles that were not receiving prolactin treatment. Thus it appears that prolactin treated tadpoles were still capable of responding to thyroxine.

Experiment 5

Experiment 5 was done to find out if the results of the previous experiment were repeatable using lower doses of thyroxine and measuring body length and hind leg length in addition to weight. Tadpoles were measured, staged and divided into two groups. Group CD received saline and

Group FG 10 $\mu\text{g/day}$ bovine prolactin. The prolactin dose was increased at day 12 to 20 $\mu\text{g/day}$ and at day 17 to 25 $\mu\text{g/day}$. It was kept at 25 μg for the rest of the experiment. At day 17 groups CD and FG were divided into C and D, and F and G respectively. At day 17 tadpoles were added to groups C and D that more closely corresponded to F and G tadpoles in stage. Groups D and G were kept in a 4 $\mu\text{g/l}$ thyroxine solution from days 17 to 24 when the thyroxine concentration was increased to 10 $\mu\text{g/l}$. On day 27 the thyroxine concentration was increased to 75 $\mu\text{g/l}$.

Body weight, body length and hind leg length were measured and the metamorphic stages recorded at days 0, 6, 11, 17, 24, 27 and 29. The results are shown in Tables 7-9 and Figures 11-16.

Table 7 and Figure 11 show the change in body weight for the 29 day period. By day 17 there was a significant weight difference between the two groups ($p < .001$). The 4 $\mu\text{g/l}$ dose of thyroid hormone did not affect the weight change in either prolactin (G) or non-prolactin (D) groups. However, when the dose was increased to 10 $\mu\text{g/l}$, groups D and G started to lose weight; and they lost weight even faster when the dose was increased to 75 $\mu\text{g/l}$.

The change in length of prolactin and non-prolactin groups is shown in Table 8 and Figure 12. By day 17 the difference in length between the two groups was considerable ($p < .001$). When group G was exposed to the low thyroxine concentration, instead of a decrease in length

as one would have expected, the combined treatment appeared to stimulate a further increase in length. Although the difference between F and G for days 17-24 is significant ($p < .05$), this should certainly be repeated to see if a very low thyroid hormone level is stimulatory after prolonged treatment with prolactin. Length began to decrease in G after the administration of 10 $\mu\text{g/l}$ thyroxine but in group D, length did not start decreasing until the animals were exposed to the 75 $\mu\text{g/l}$ thyroxine dose.

Hind leg length was slower than weight or length to be affected by the prolactin treatment in the first 17 days (Table 9 and Figures 13 and 14). There was no difference in the rate of hind limb growth between CD and FG until day 11. But from days 11-17 the prolactin-treated group had a decreased hind leg growth rate ($p < .025$).

Whereas the low thyroid hormone dose did not cause a decrease in weight and length in the first 7 days of treatment (days 17-24), it was effective in promoting growth of the hind legs in G compared to F ($p < .05$) as well as in D compared to C (Figure 15). The higher doses of thyroxine continued to cause accelerated hind leg growth. Data on metamorphic stage reflected the same trend as the hind leg length data. By day 27 prolactin-treated tadpoles receiving thyroxine had an average stage of 15 while tadpoles receiving only prolactin had an average stage of 14.

In this experiment a very low dose of thyroxine did not affect weight and length changes in prolactin-treated

tadpoles but did accelerate hind leg growth that the prolactin treatment had markedly decreased. Higher doses of thyroxine caused a loss of weight and length in both prolactin and non-prolactin-treated tadpoles and thus was in agreement with the results of the previous experiment.

Table 7. Weight changes in tadpoles treated with prolactin (P) and thyroxine (T4). See Fig. 11 and text for details of the experiment. n = number of animals; SE = standard error; S = saline.

Days		0-17				17-24				27-29			
		Treat-		Wt. change(gm)		Treat-		Wt. change(gm)					
Group	ment	n	mean	SE	Group	ment	n	mean	SE	mean	SE	mean	SE
CD	S	10	-.07	±.08	C	S	6	.07	±.08	.15	±.02		
					D	T4	7	.03	.09	-.11	.07		
FG	P	27	.59	.09	F	P	13	-.03	.05	.02	.04		
					G	P+T4	14	-.10	.05	-.21	.04		

Table 8. Length changes in tadpoles treated with prolactin (P) and thyroxine (T4). See Fig. 12 and text for details of the experiment. n=number of animals; SE = standard error; S = saline.

Days		0-17				17-24			
		Treat-		Length change(cm)		Treat-		Length change(cm)	
Group	ment	n	mean	SE	Group	ment	n	mean	SE
CD	S	10	-.01	±.09	C	S	6	.16	±.07
					D	T4	7	.04	.09
FG	P	27	.52	.07	F	P	13	.10	.04
					G	P+T4	14	.23	.04

Table 9. Hind leg length changes in tadpoles treated with prolactin (P) and thyroxine (T4). See Fig. 13 and text for details of experiment. n = number of animals; SE = standard error; S = saline.

Days		11-17				17-24			
		Treat-		Hind leg length(mm)		Treat-		Hind leg length(mm)	
Group	ment	n	mean	SE	Group	ment	n	mean	SE
CD	S	10	1.4	±.4	C	S	6	2.5	±.7
					D	T4	7	3.7	.7
FG	P	27	0.6	.1	F	P	13	0.6	.4
					G	P+T4	14	1.7	.2

Fig. 11. Weight changes in tadpoles injected with saline (CD) and prolactin (FG) for 29 days. Groups CD and FG were both divided at day 17 and D and G were then kept in a 4 $\mu\text{g/l}$ thyroxine solution. The thyroxine dose was increased to 10 $\mu\text{g/l}$ on day 24 and to 75 $\mu\text{g/l}$ on day 27. Arrows indicate the thyroxine dose changes. Means and standard errors represent data in Table 7.

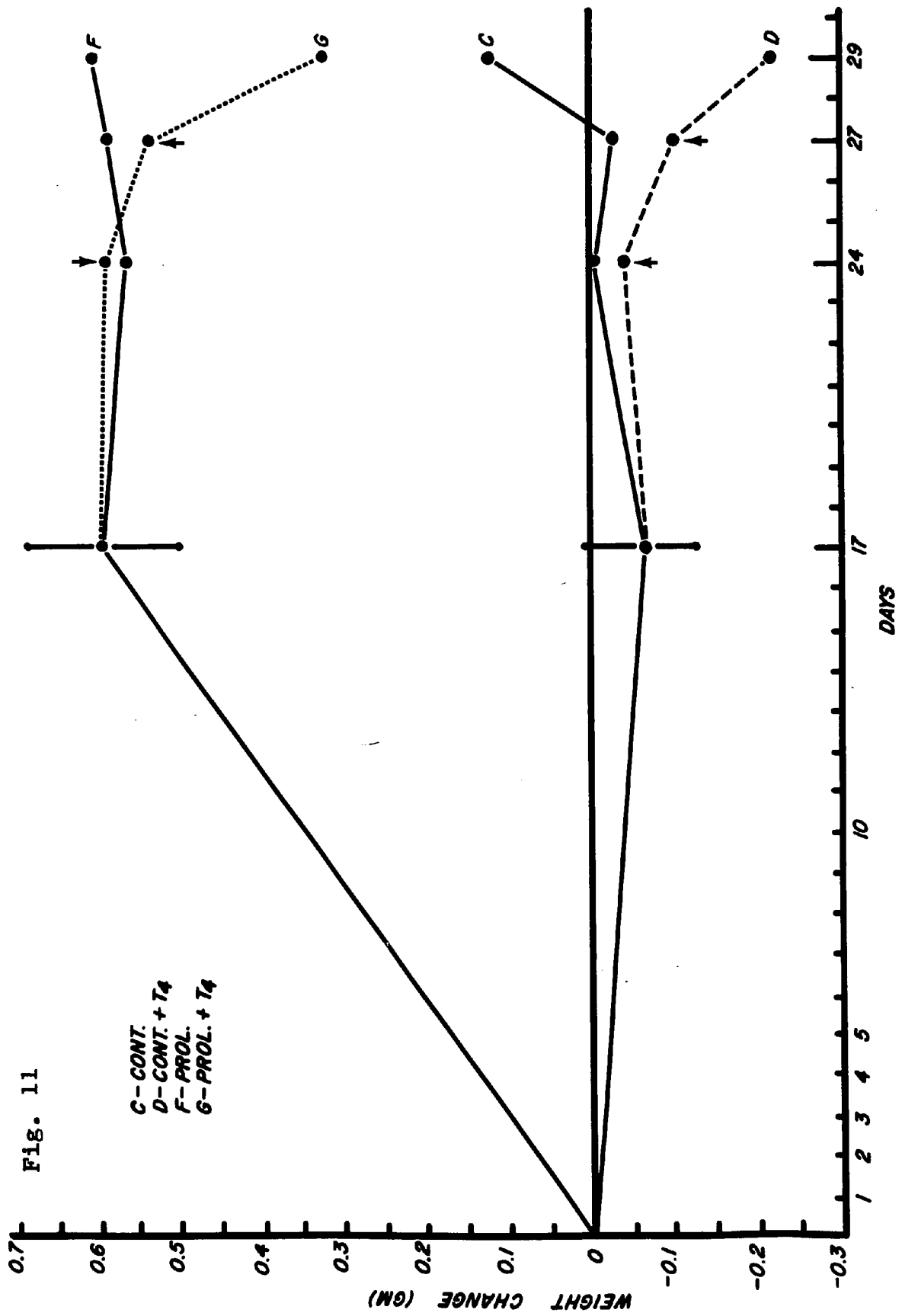


Fig. 12. Length changes in tadpoles injected with saline (CD) and prolactin (FG) for 29 days. Groups CD and FG were both divided at day 17 and D and G were then kept in a 4 $\mu\text{g/l}$ thyroxine solution. The thyroxine dose was increased to 10 $\mu\text{g/l}$ on day 24 and to 75 $\mu\text{g/l}$ on day 27. Arrows indicate the thyroxine dose changes. Means and standard errors represent data in Table 8.

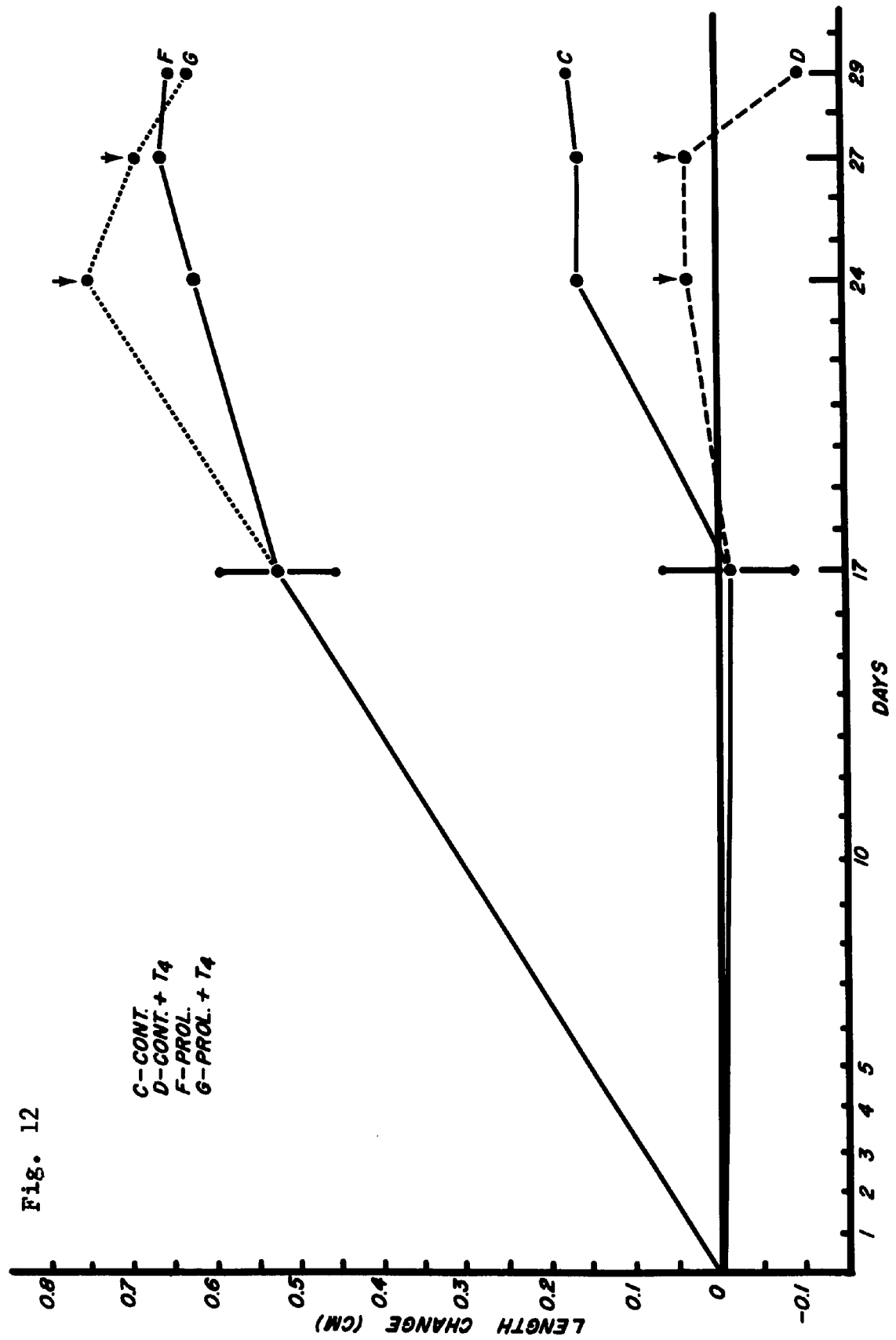
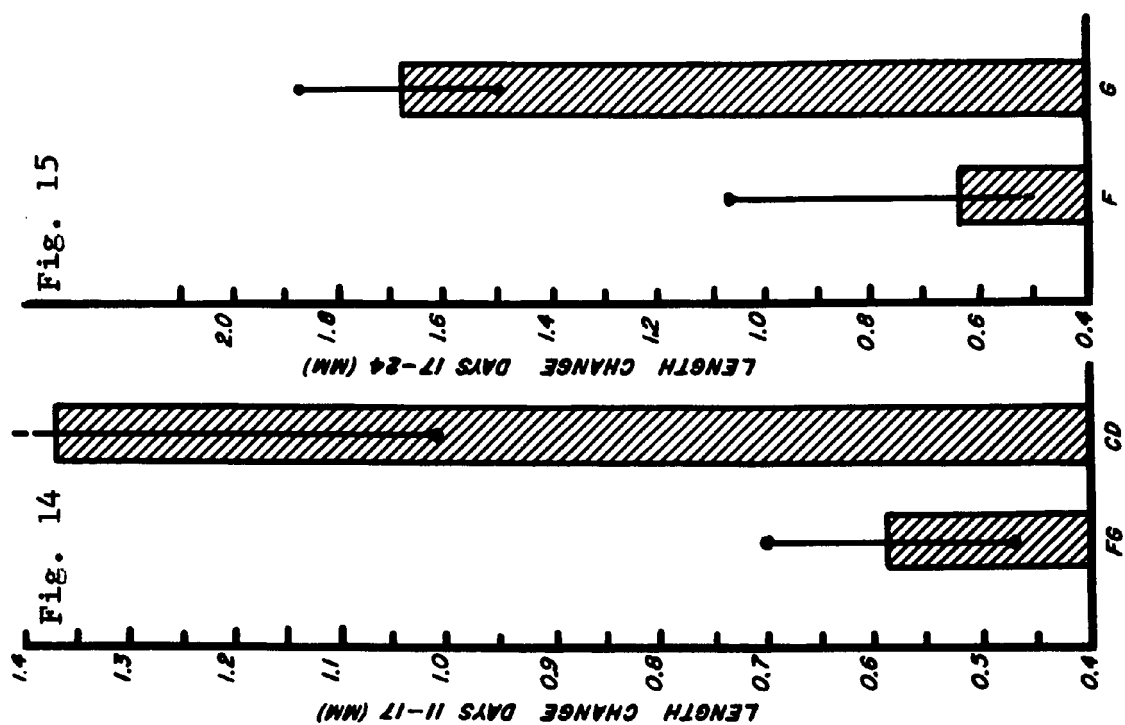
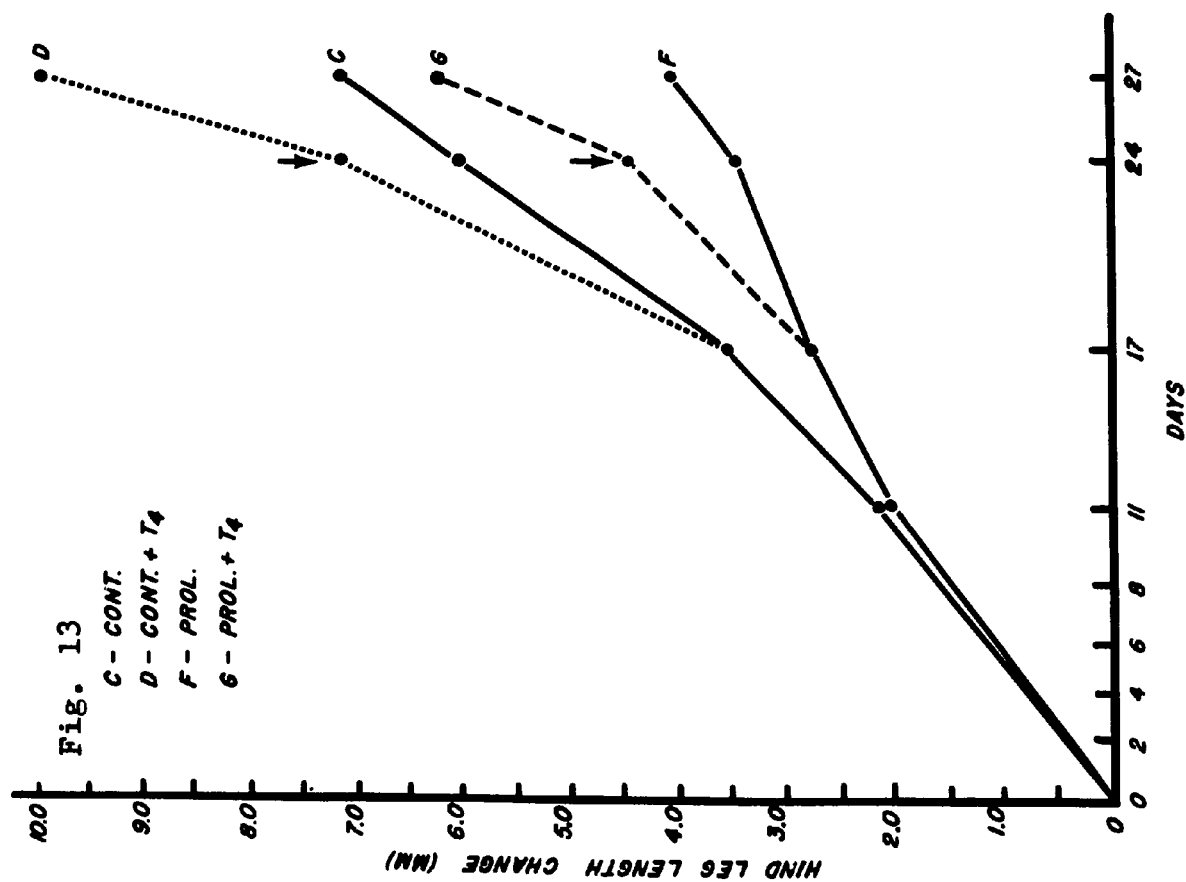


Fig. 13. Hind leg length changes in tadpoles injected with saline (CD) and prolactin (FG) for 27 days. Groups CD and FG were divided at day 17 and D and G were kept in a 4 $\mu\text{g}/\text{l}$ thyroxine solution. On day 24 the thyroxine dose was increased to 10 $\mu\text{g}/\text{l}$ which is indicated by arrows. Means represent data in Table 9.

Fig. 14. Hind leg length changes between days 11 and 17. Means and standard errors represent data in Table 9. See Fig. 13 for details of the treatments.

Fig. 15. Hind leg length changes between days 17 and 24. Means and standard errors represent data in Table 9. See Fig. 13 for details of the treatments.



Prolactin treatment of thiouracil treated and thyroidectomized tadpoles.

Because of the interference of prolactin treatment with metamorphosis, it was important to determine if additional growth could be promoted with prolactin using tadpoles that had had their thyroid activity reduced previously by treatment with a thyroid inhibitor or by thyroidectomy.

Experiment 6

In Experiment 6 thiouracil treatment was used to prevent metamorphosis and prolactin was administered subsequently.

Tadpoles were kept in a 0.05% thiouracil solution (a concentration known to inhibit metamorphosis). After 11 days of thiouracil treatment the tadpoles were divided into three groups. Group C was removed from the thiouracil. Groups A and B were kept in thiouracil for **another 2 weeks.** Groups A and C received daily saline injections and Group B received 10 µg/day bovine prolactin. Weight and length changes were recorded for the 2 weeks (days 0-15) of hormone or saline treatment (days 11-26 or thiouracil treatment). The results of the prolactin and thiouracil treatment are shown in Table 10 and Figures 16-19.

The effect of thiouracil in preventing metamorphosis can be seen from the data comparing the weight and length changes of groups A and C (Figures 16 and 18) and from the data on metamorphic stage (see appendix).

Table 10. Length and weight changes for a 7-day period of tadpoles treated with thiouracil (TU) and prolactin (P).
 n = number of animals; SE = standard error.

Group	n	Treatment	Length change(cm)		Weight change(gm)	
			mean	SE	mean	SE
A	9	0.7% NaCl; TU	0.37	$\pm .06$	0.50	$\pm .14$
B	12	P; TU	0.62	.08	0.95	.09
C	10	0.7% NaCl	-3.11	.57	-1.27	.20

Fig. 16. Weight changes in thiouracil-treated tadpoles during a 15-day period. Groups A and B were kept in thiouracil and group B received 10 $\mu\text{g/day}$ prolactin.

Fig. 17. Weight changes in thiouracil-treated tadpoles between days 7 and 15. Group A received saline injections and B received 10 $\mu\text{g/day}$ prolactin. Means and standard errors represent data in Table 10.

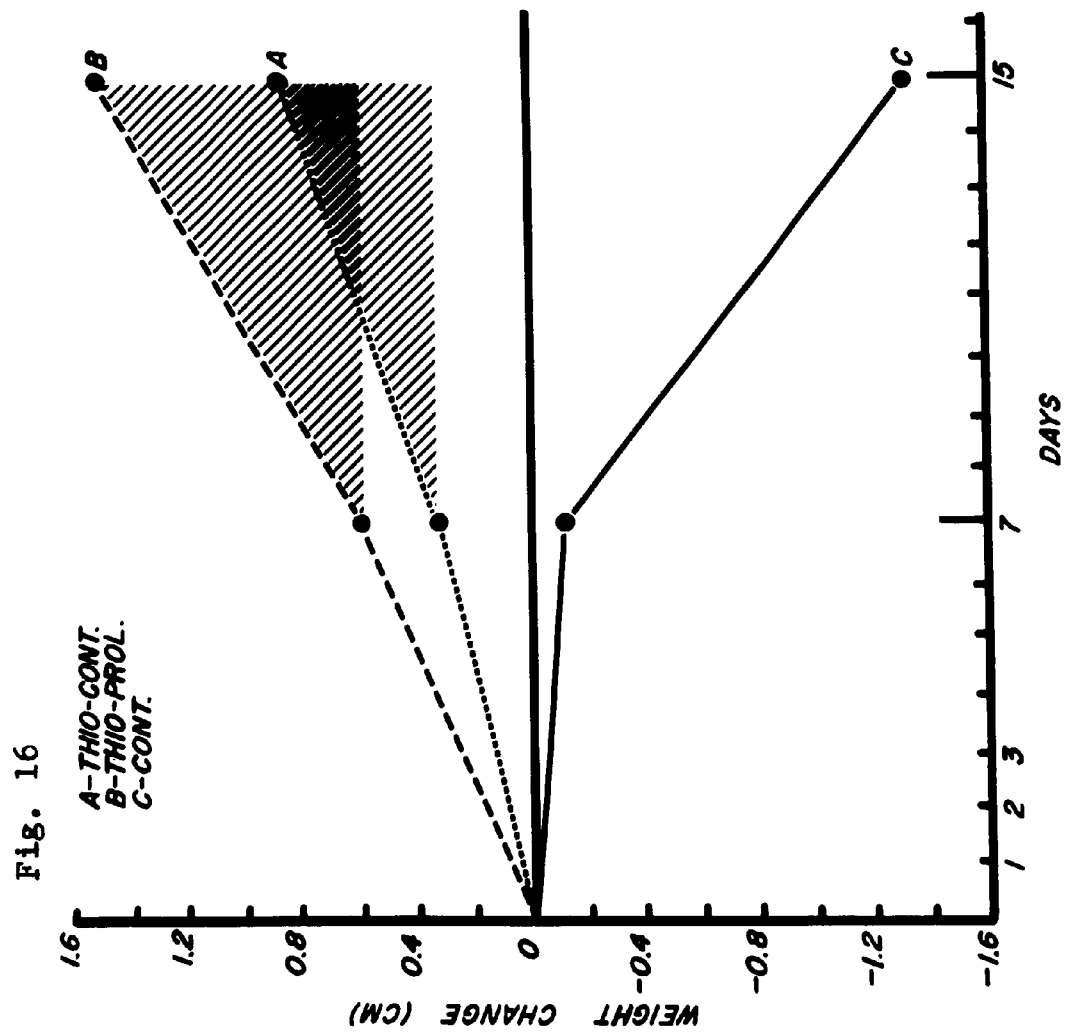
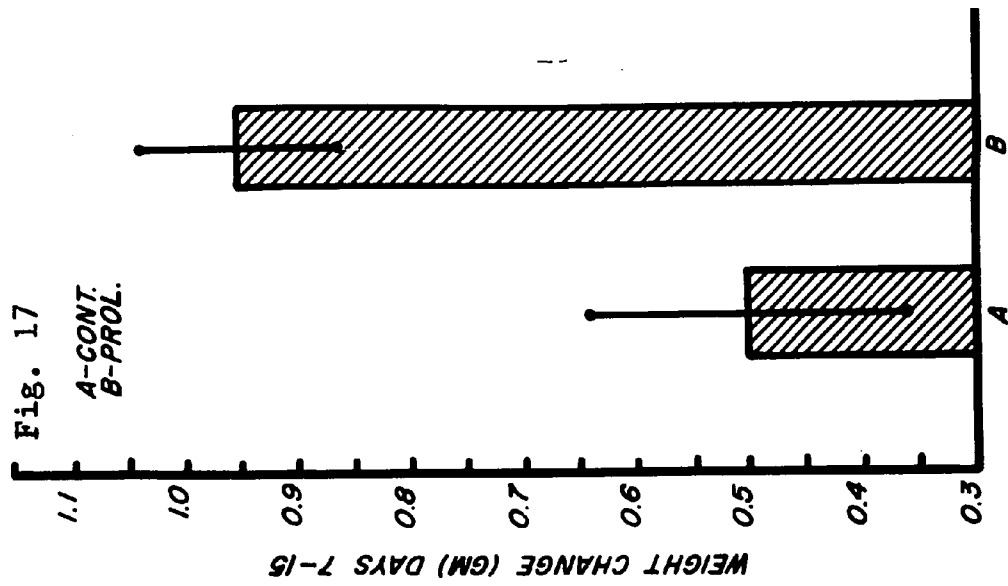


Fig. 18. Length changes in thiouracil treated tadpoles during a 15-day period. Groups A and B were kept in thiouracil and group B received 10 $\mu\text{g/day}$ prolactin.

Fig. 19. Length changes in thiouracil treated tadpoles between days 7 and 15. Group A received saline injections and B received 10 $\mu\text{g/day}$ prolactin. Means and standard errors represent data in Table 10.

Fig. 19
A-CONT.
B-PROL.

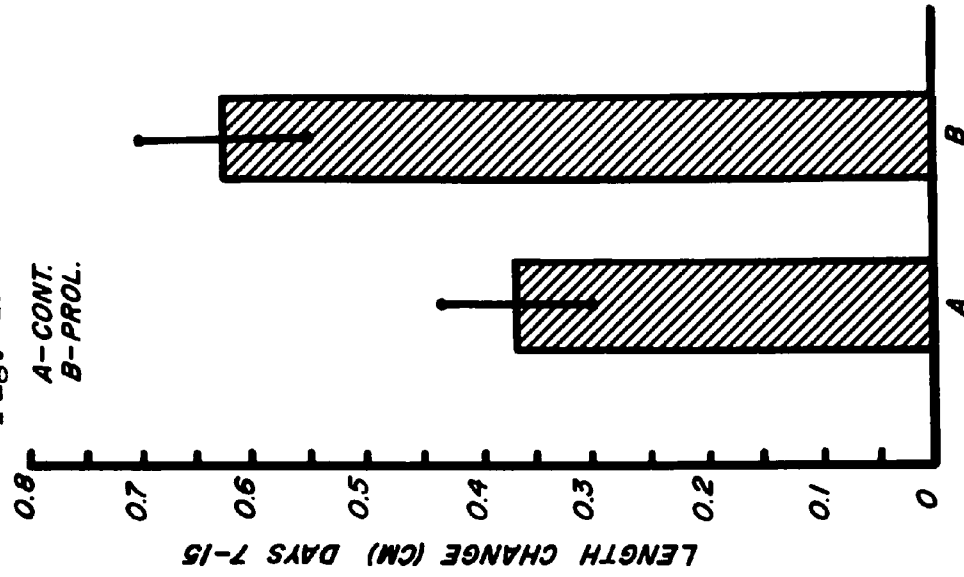
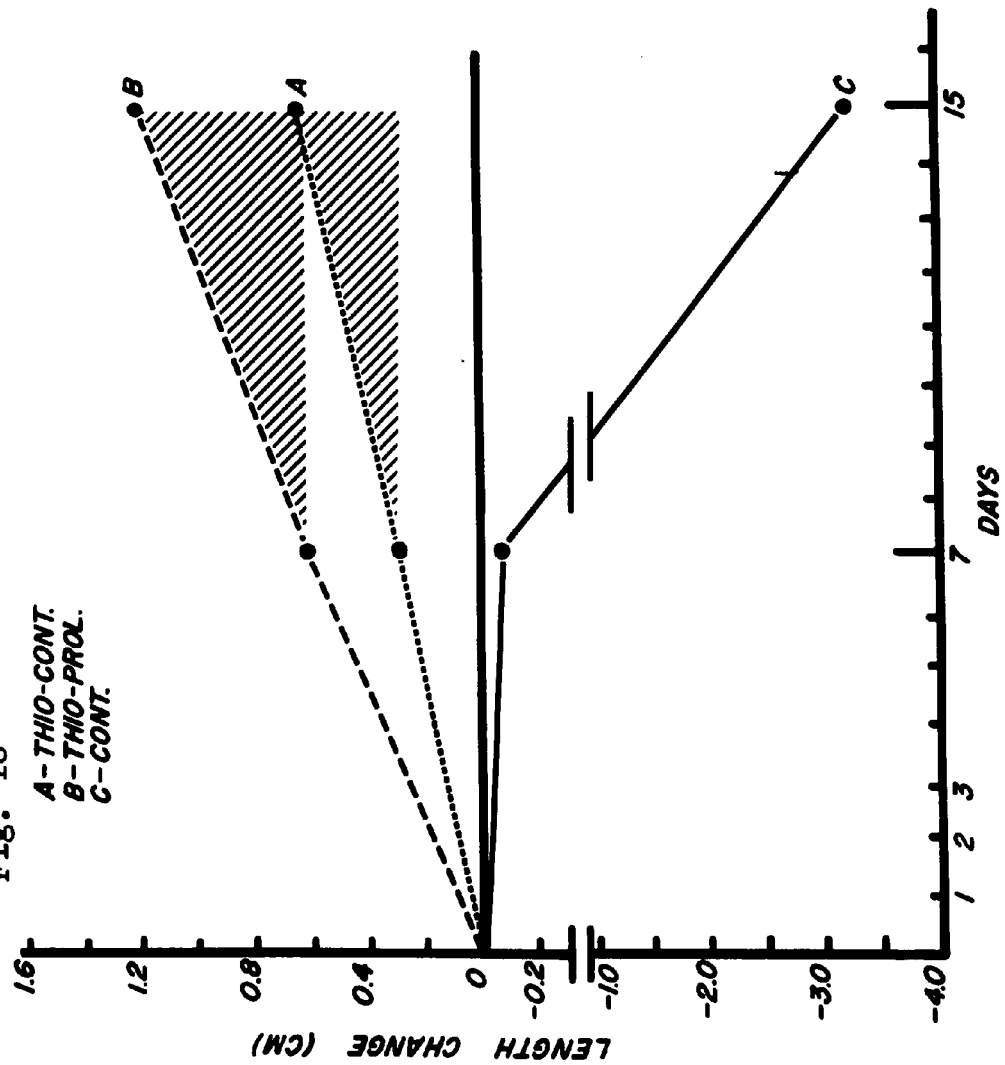


Fig. 18
A-THIO-CONT.
B-THIO-PROL.
C-CONT.



Since it appeared from staging the tadpoles that metamorphosis had not been completely arrested in A and B until day 7, weight and length data were calculated from days 7-15. Weight and length changes of groups A and B were significantly different from each other ($p < .025$, weight; $p < .05$, length).

In Experiment 6 an increase in weight and length occurred in thiouracil-treated tadpoles (A) beyond that of tadpoles that were removed from thiouracil after 11 days (C) indicating that an inhibition of thyroid activity led to increased size. Prolactin treatment evoked a further increase in length beyond that resulting from thyroid depression.

Experiment 7

Experiment 7 was similar to Experiment 6 except that thyroidectomy was substituted for thiouracil treatment. Tadpoles were thyroidectomized or sham thyroidectomized 1 week prior to hormone treatment. Four groups of tadpoles were treated for 2 weeks as follows:

- Group A - sham thyroidectomy, 0.7% NaCl
- Group B - thyroidectomy, 0.7% NaCl
- Group C - thyroidectomy, 5 μ g/day bovine prolactin
- Group D - thyroidectomy, 15 μ g/day bovine prolactin

The weight and length changes for the 2-week period are shown in Table 11 and Figures 20 and 21.

There was no significant difference between the length changes in the two control groups (A and B). Both prolactin groups responded with an increased length compared to B

($p < .02$, C; $p < .01$, D). However, group D, which received the higher prolactin dose, had approximately the same change in length as C.

Group B increased in weight compared to A ($p < .05$). Although the mean weight changes in C and D were greater than B, only the difference between B and C was statistically significant ($p < .05$). The mean weight change in D did not differ significantly from either B or C. The weight and length differences between the two control groups, A and B, were not as dramatic as between the two similar groups, A and C, in the thiouracil experiment.

It became obvious during the experiment, using metamorphic stages as criteria, that not all of the thyroidectomized tadpoles were totally thyroidless. While their rate of metamorphosis had been definitely retarded compared to group A, they had not completely stopped changing. Certainly some of the tadpoles were completely thyroidectomized since a random group was kept 5 months or more and some never metamorphosed. But instead of considering these tadpoles completely thyroidectomized it is more accurate to consider them as having had a reduced thyroid hormone level. This also applies to the tadpoles used in Experiments 9 and 10.

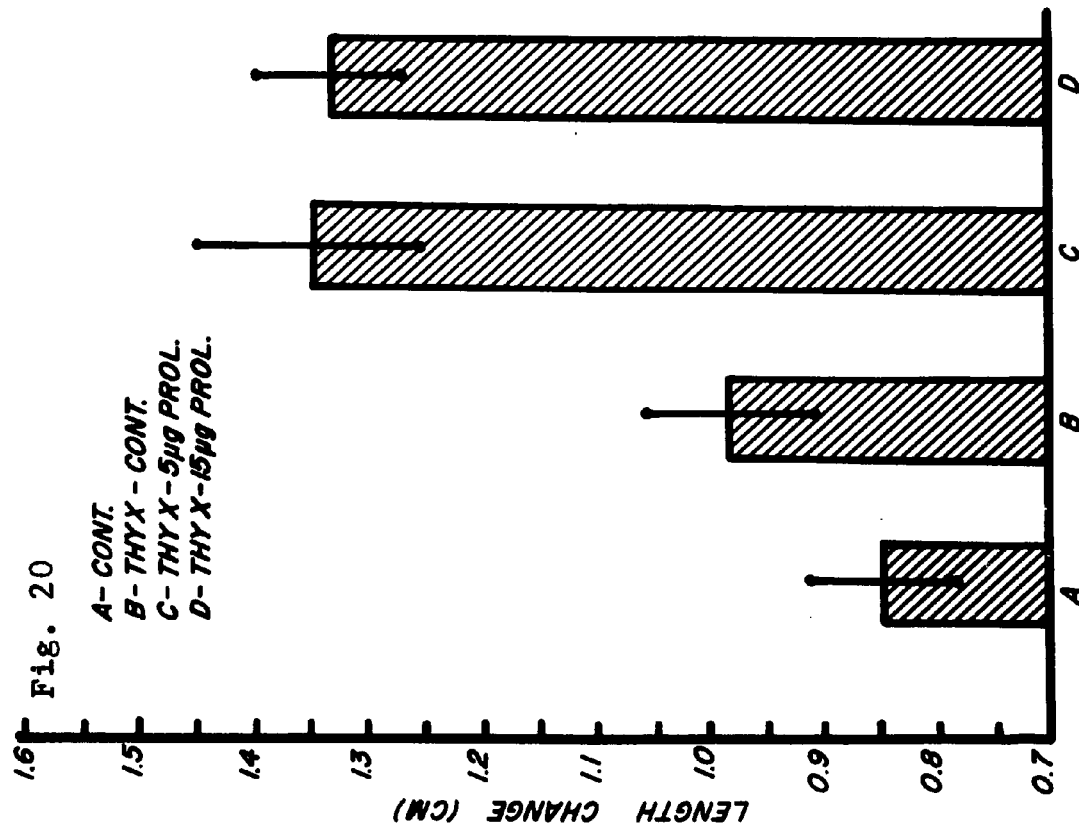
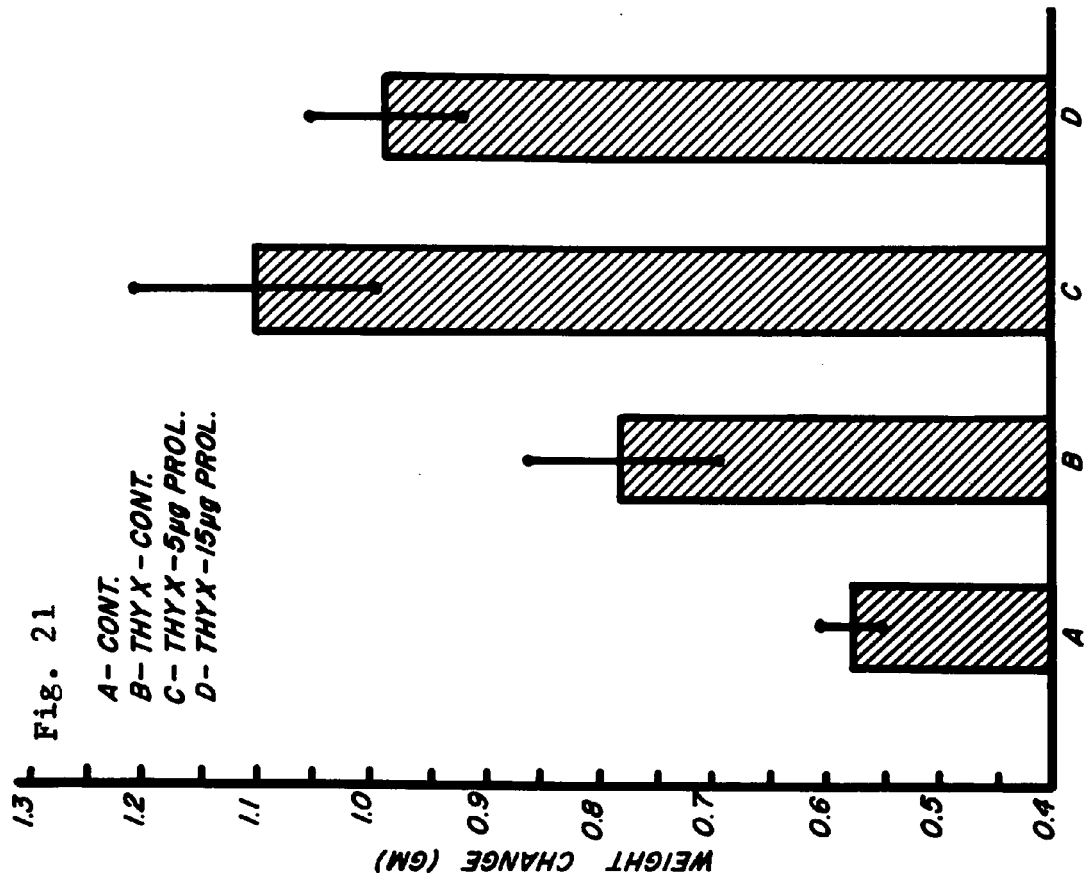
Experiment 7 indicates that tadpoles that had a reduced thyroid hormone level responded to prolactin by an increased weight and length compared to controls. Thyroidectomy alone however, did not significantly accelerate growth.

Table 11. Length and weight changes during a 2-week period in sham operated and thyroidectomized (TX) tadpoles treated with prolactin with saline and prolactin (P).
 n = number of animals; SE = standard error.

Group	n	Treatment	Length change(cm)		Weight change(gm)	
			mean	SE	mean	SE
A	11	0.7% NaCl	0.85	$\pm .06$	0.57	$\pm .03$
B	13	TX; 0.7% NaCl	0.98	.08	0.78	.09
C	13	TX; 5 μ g P	1.35	.10	1.10	.10
D	14	TX; 15 μ g P	1.33	.07	0.98	.07

Fig. 20. Length changes in tadpoles treated with saline and prolactin for 2 weeks. Means and standard errors represent data in Table 11. Groups B, C and D were thyroidectomized.

Fig. 21. Weight changes in tadpoles treated with saline and prolactin for 2 weeks. Means and standard errors represent data in Table 11. Groups B, C and D were thyroidectomized.



Experiment 8

Experiment 8 was basically a repetition of Experiment 7 but an attempt was made to use only tadpoles that were completely thyroidectomized. The tadpoles were thyroidectomized and 13 days later their length, hind leg length and stage were recorded. After 4 more days the same parameters plus weight were recorded. Tadpoles which showed a large change in stage or hind leg length were considered incompletely thyroidectomized and eliminated from the experiment. The tadpoles that had a relatively stationary hind leg length were divided into two groups, R and V. The control group, R, received daily saline injections. Group V was treated with 10 µg/day bovine prolactin for 12 days. The results are shown in Table 12 and in Figures 22-24.

Figure 22 compares the hind leg growth of R + V combined with a non-thyroidectomized control group (C) from Experiment 5. Group C was being treated with daily saline injections and subjected to similar experimental conditions at the same time that Experiment 8 was being done. While these groups are not strictly comparable, they do allow a rough comparison of "normal" and thyroidectomized hind leg growth and illustrate that the leg growth was severely reduced in groups R and V.

Figure 23 illustrates that the length change in the prolactin-treated group was significantly greater than in the control group ($p < .001$). Although the mean weight

change in V was greater than in R, it was not significantly different ($p < .10$).

Experiments 6, 7 and 8 all suggest that prolactin can produce an increase in size in tadpoles either devoid of thyroid activity or with a reduced thyroid activity.

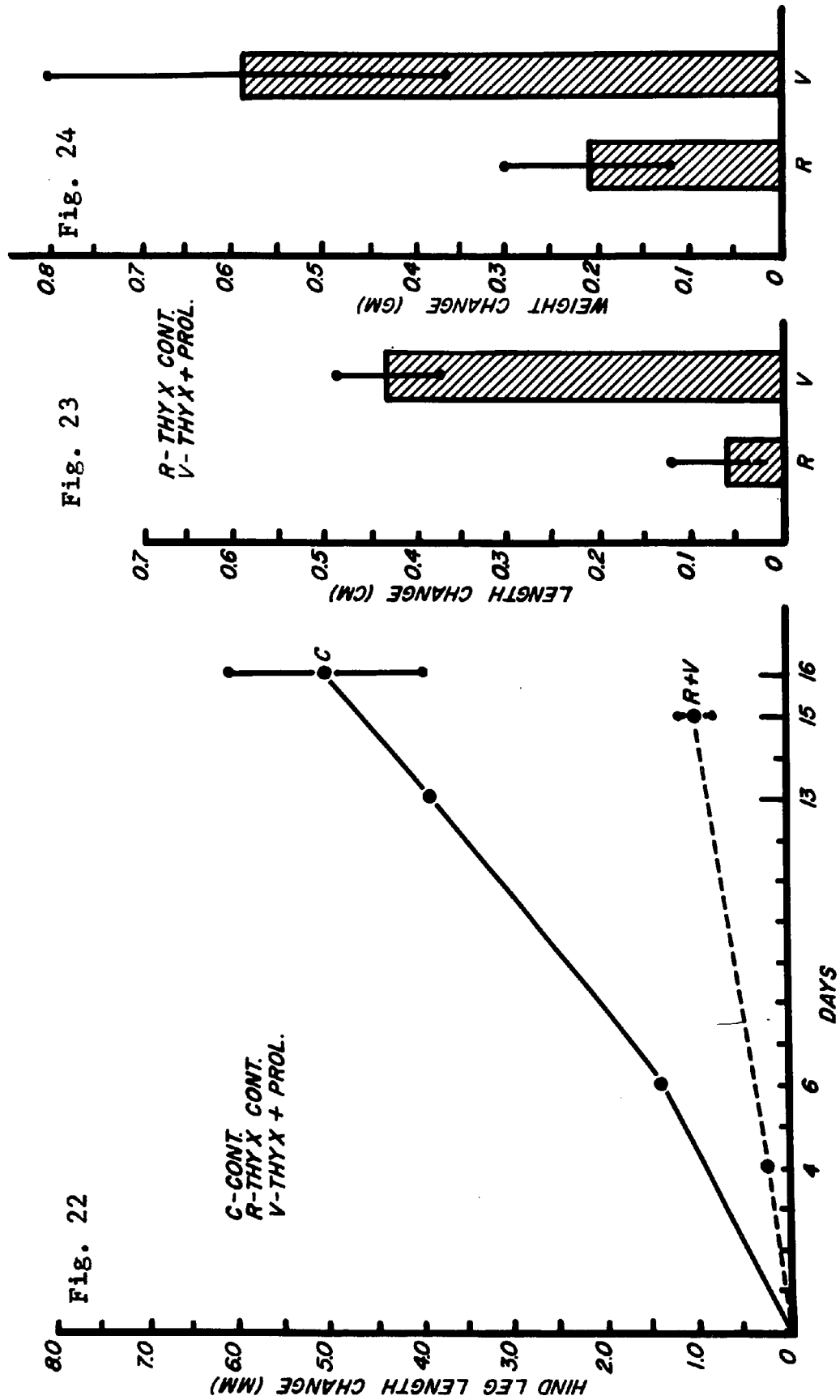
Table 12. Weight and length changes in thyroidectomized(TX) tadpoles treated with saline and prolactin (P) for 12 days. n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
R	12	TX;0.7%NaCl	0.21	± 0.09	.06	$\pm .06$
V	6	TX;10 μ g P	0.58	0.22	.43	.06

Fig. 22. Mean hind leg length changes in tadpoles that were thyroidectomized (R, V) compared to nonthyroidectomized tadpoles (C). Vertical bars at days 15 and 16 represent standard errors of mean changes from day 0.

Fig. 23. Length changes in thyroidectomized tadpoles injected with saline (R) or 25 $\mu\text{g/day}$ prolactin (V) for 12 days. Means and standard errors represent data in Table 12.

Fig. 24. Weight changes in thyroidectomized tadpoles injected with saline (R) or 25 $\mu\text{g/day}$ prolactin (V) for 12 days. Means and standard errors represent data in Table 12.



Growth hormone treatment of thyroidectomized tadpoles.

Experiment 9

Tadpoles that had been thyroidectomized 2 weeks earlier were treated for 6 days with the following:

Group B - 0.7% NaCl
Group E - 5 μ g/day bovine GH
Group F - 25 μ g/day bovine GH

The changes in length and weight are shown in Table 13 and Figures 25 and 26.

Both GH-treated groups had a greater weight and length increase than controls. Using the Student t test, the means of both length and weight changes in F are significantly greater than B ($p < .005$, length; $p < .025$, weight). A regression of dose against weight gives a slope that is significantly different from zero ($p < .005$). The slope of length against dose also is significant ($p < .005$).

As in Experiment 7, all of the tadpoles used in this experiment were not totally thyroidectomized but should be considered to have had a reduced thyroid hormone level.

Experiment 10

Considering the almost complete lack of response to GH that intact tadpoles showed in Experiment 1 (Figures 1 and 2), the results of Experiment 9 with thyroidectomized tadpoles were somewhat unexpected. It was decided to repeat the highest GH dose in another experiment.

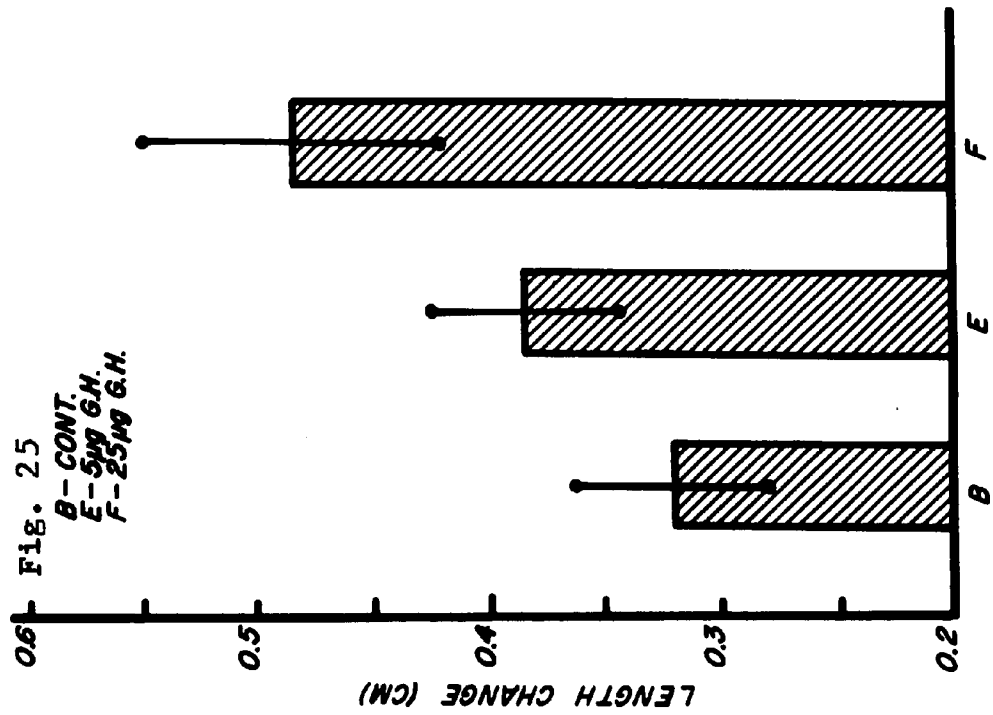
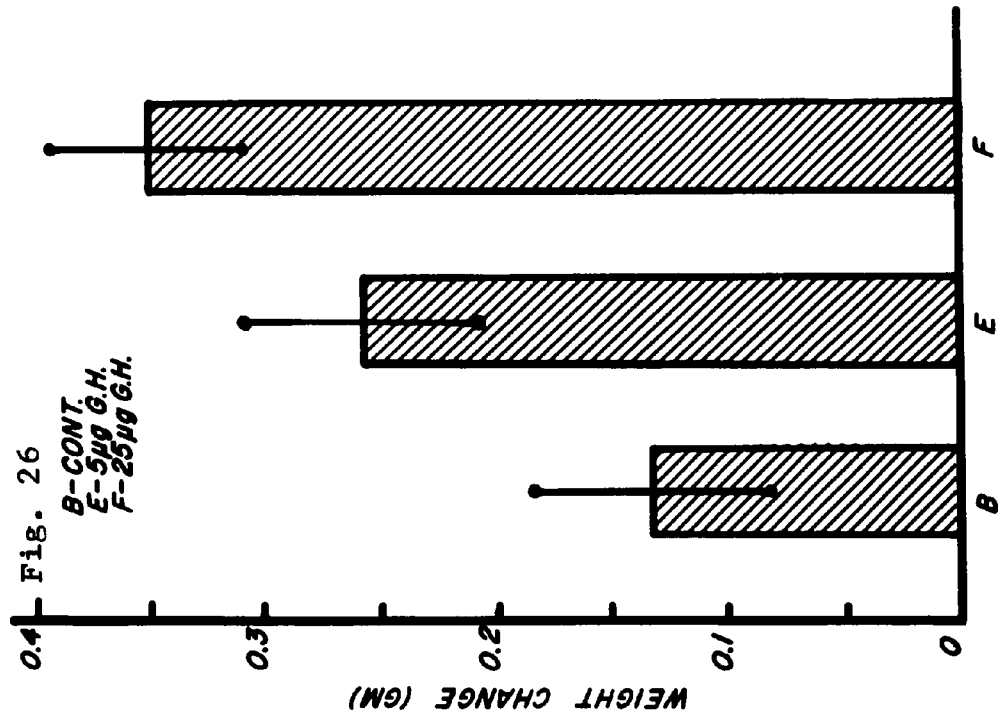
Tadpoles that had been thyroidectomized 5 weeks earlier were measured and staged and divided into two groups. Group R was given daily saline injections and

Table 13. Weight and length changes in a 6-day period in control and GH-treated thyroidectomized (TX) tadpoles. n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
B	13	TX;0.7% NaCl	.14	$\pm .05$	.32	$\pm .04$
E	14	TX; 5 μ g GH	.26	.04	.39	.04
F	14	TX;25 μ g GH	.35	.04	.49	.06

Fig. 25. Length changes in control and GH-treated thyroid-ectomized tadpoles for a 6-day period. Means and standard errors represent data in Table 13.

Fig. 26. Weight changes in control and GH-treated thyroid-ectomized tadpoles for a 6-day period. Means and standard errors represent data in Table 13.



group S was given 25 µg/day bovine GH. Hormone and saline treatment continued for 2 weeks. The changes in length and weight for the 2-week period are shown in Table 14 and Figures 27 and 28.

Both the mean length changes and mean weight changes of R and S were significantly different from each other ($p < .05$, length; $p < .025$, weight). Again these tadpoles should not be considered completely thyroidectomized but as having had a reduced thyroid hormone level. The results of this experiment support those of Experiment 9 and suggest the possibility of a somewhat lower dose of GH being effective in tadpoles that have a low level of thyroid hormone.

General remarks on thyroidectomized tadpoles

Frequently thyroidectomized tadpoles appeared to have their water balance disturbed as they became very swollen with water. Clearly this would directly effect any wet weight measurement. The tadpoles that were obviously swollen were removed from the experiment. However, the same phenomena could well have affected other tadpoles but to a lesser extent so that they did not appear swollen. Consequently length rather than weight was probably a better measure of size in thyroidectomized tadpoles.

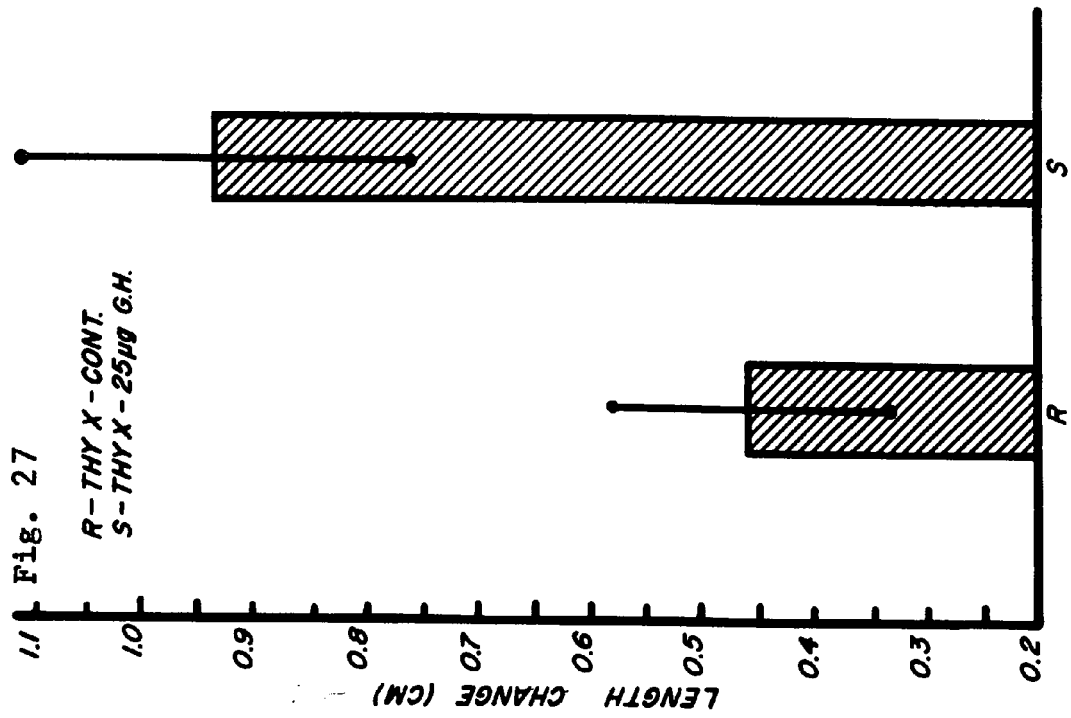
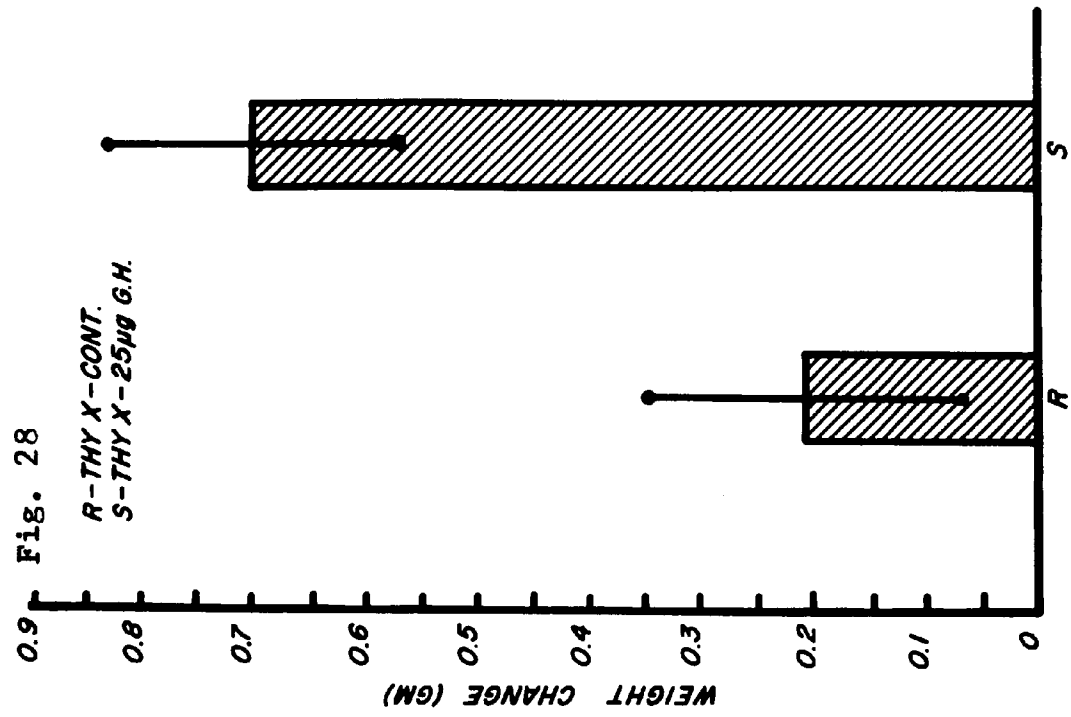
A very large percentage of the thyroidectomized tadpoles' tails appeared very red as if heavily vascularized. This never occurred to any noticeable degree in normal tadpoles.

Table 14. Length and weight changes for a 2-week period in thyroidectomized(TX) tadpoles treated with GH and saline. n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(cm)	
			mean	SE	mean	SE
R	12	TX;0.7% NaCl	0.21	$\pm .22$	0.46	$\pm .12$
S	11	TX;25 μ g GH	0.70	.13	0.93	.20

Fig. 27. Length changes in thyroidectomized tadpoles treated with saline or GH for two weeks. Means and standard errors represent data in Table 14.

Fig. 28. Weight changes in thyroidectomized tadpoles treated with saline or GH for two weeks. Means and standard errors represent data in Table 14.



Hypophysectomy of postmetamorphic *Rana pipiens*.

Experiment 11

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

The frogs in Experiment 11 were hypophysectomized or sham hypophysectomized within a 3-day period. Feeding began approximately a week after surgery. Both control and hypophysectomized frogs were always 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 frogs were measured several times during an 11-week period but it was not until after 6-8 weeks that the controls had grown sufficiently to be measurably different than the hypophysectomized animals. The length and weight changes in the two groups for an 11-week period are shown in Table 15 and Figures 29 and 30. The total changes were small but the means of the two groups differed significantly in length ($p < .005$) and weight ($p < .001$).

Experiment 12

Experiment 12 was similar to Experiment 11 except the

Table 15. Length and weight changes in 11 weeks in control and hypophysectomized (HypX) frogs force fed on liver and mealworms. n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(mm)	
			mean	SE	mean	SE
C	17	Sham HypX	1.21	±.16	1.65	±.13
H	15	HypX	0.77	.09	0.76	.20

Table 16. Length and weight changes in 7 weeks in control and hypophysectomized (HypX) frogs fed live flies and crickets. n = number of animals; SE = standard error.

Group	n	Treatment	Weight change(gm)		Length change(mm)	
			mean	SE	mean	SE
C	15	Sham HypX	1.18	±.18	0.67	±.23
H	8	HypX	-0.18	.16	-0.18	.28

Fig. 29. Length changes of sham operated controls (C) and hypophysectomized (H) postmetamorphic R. pipiens in 11 weeks. The frogs were force fed on liver and mealworms. Means and standard errors represent data in Table 15.

Fig. 30. Weight changes of sham operated controls (C) and hypophysectomized (H) postmetamorphic R. pipiens in 11 weeks. The frogs were force fed on liver and mealworms. Means and standard errors represent data in Table 15.

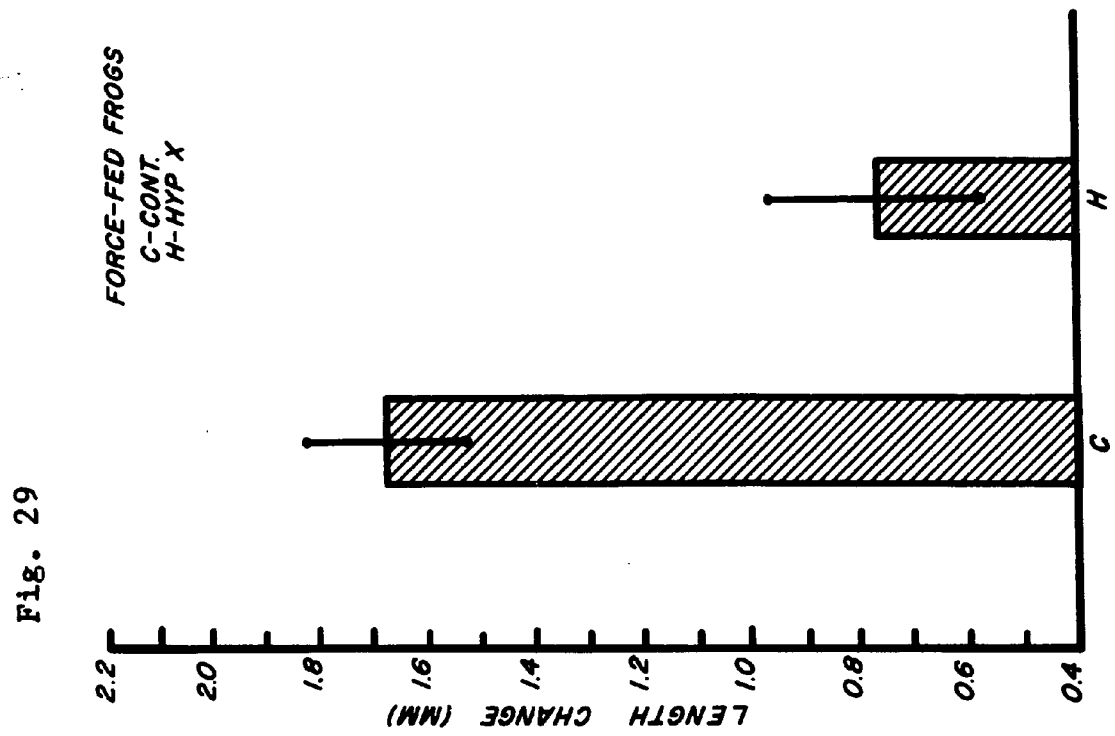
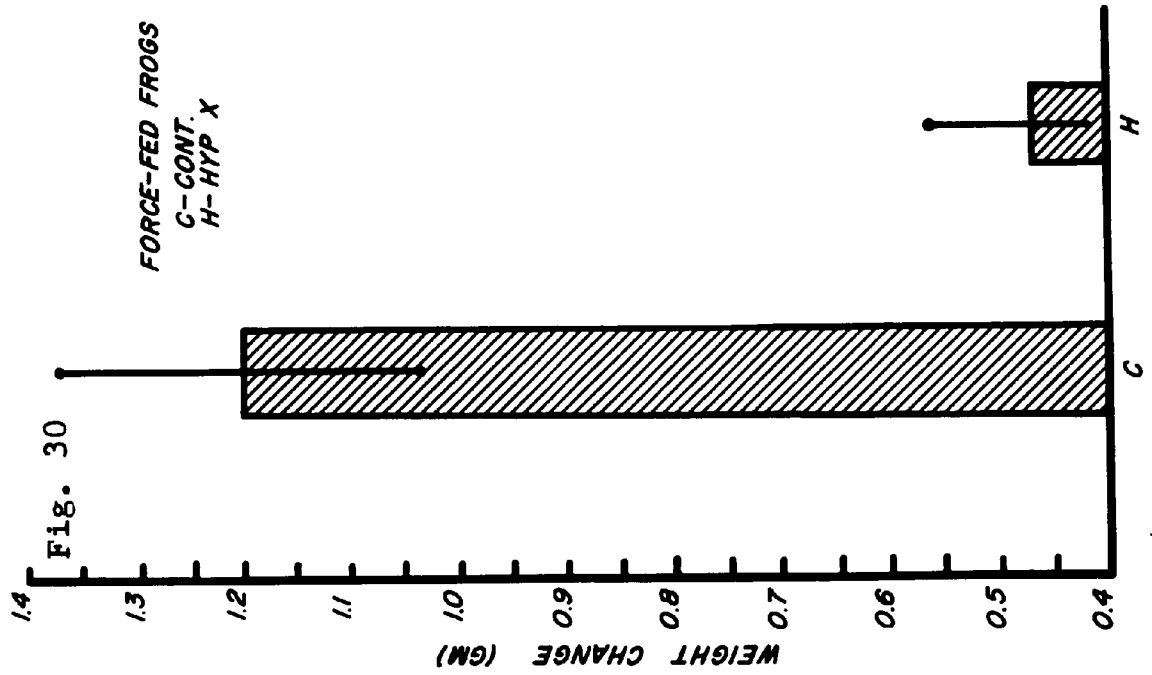
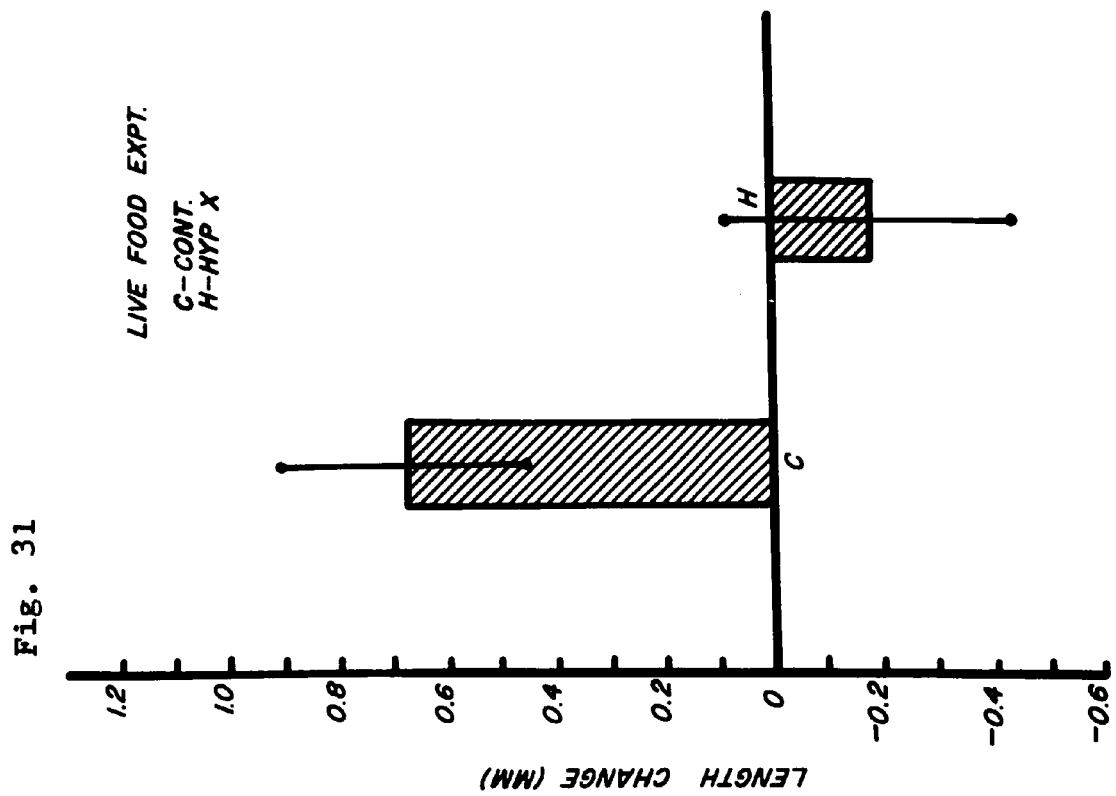
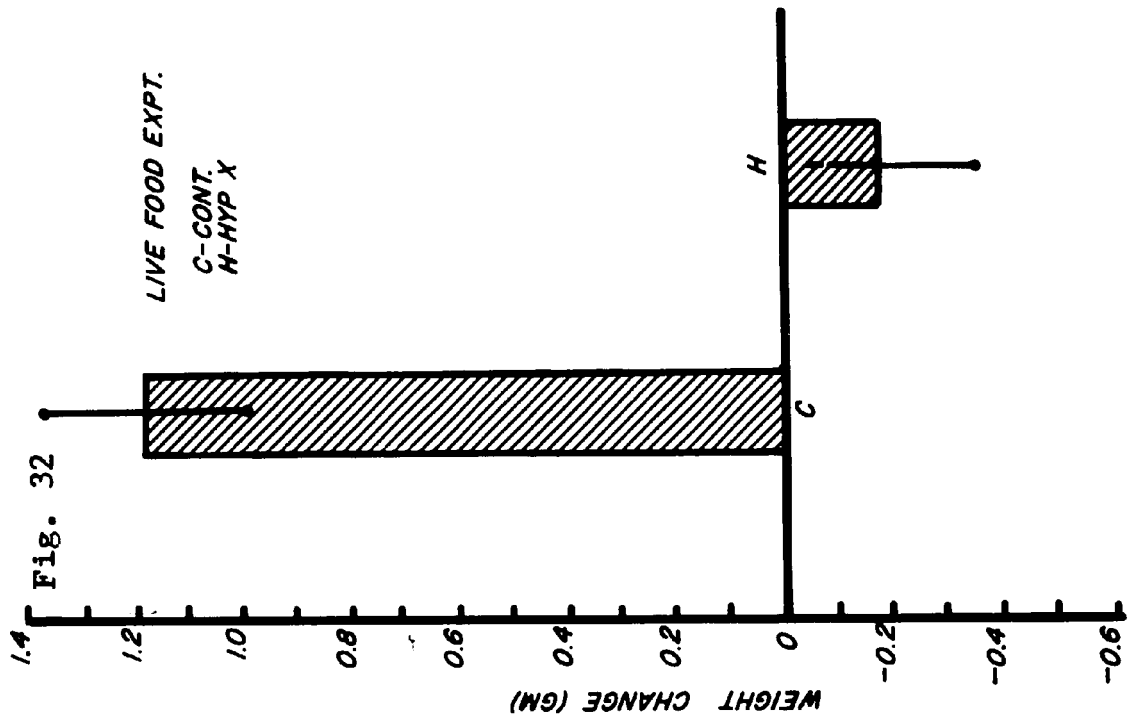


Fig. 31. Length changes of sham operated controls (C) and hypophysectomized (H) postmetamorphic R. pipiens in 7 weeks. Means and standard errors represent data in Table 16.

Fig. 32. Weight changes of sham operated controls (C) and hypophysectomized (H) postmetamorphic R. pipiens in 7 weeks. Means and standard errors represent data in Table 16.



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 Table 16 and in Figures 31 and 32. 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).

Experiments 11 and 12 clearly show that although the hypophysectomized and control frogs ingested the same quantity of food, both length and weight increased more in control than in hypophysectomized frogs.

Prolactin and GH treatment of postmetamorphic frogs.

Experiment 13

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
- Group E - 25 µg bovine GH/day

Injections were given daily except for a few occasions when they were given every other day. In those cases the dose was doubled so that the animals still received the same average dose per day. 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 after one month of treatment are shown in Table 17 and Figures 33 and 37. In the first month only the high GH-treated group (E) differed significantly from controls (A) ($p < .001$). Figure 34 shows the changes for the second month (26 days). When the doses were doubled only group D differed significantly from controls ($p < .05$). The total length and weight changes for the 2-month period are shown in Table 17 and Figures 35, 36 and 37. If the length changes were 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. But there was no growth response to long-term prolactin treatment. The optimum GH dose appeared to be between 10-25 $\mu\text{g/day}$. When group D was treated with 5 $\mu\text{g/day}$ GH no response was seen while at the same time the 25 $\mu\text{g/day}$ 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 . Although the mean lengths of the prolactin-treated groups were higher than the controls, the differences were not significant.

In contrast to tadpoles, frogs receiving GH treatments increased in weight and length to a significantly greater extent than those receiving saline or prolactin treatments.

Table 17. Length and weight changes in frogs treated with saline (S), prolactin (P) and GH. n=number of animals; SE=standard error.

Group	n	Treat- ment	Month 1		Month 2		Months 1+2		Months 1+2	
			Length change(mm)		Length change(mm)		Length change(mm)		Weight change(gm)	
			mean	SE	mean	SE	mean	SE	mean	SE
A	11	S	0.32	±.14	1.18	±.18	1.50	±.23	1.06	±.19
B	11	P	0.45	.14	1.32	.19	1.77	.25	1.05	.28
C	14	P	0.61	.18	1.21	.19	1.82	.28	1.24	.16
D	13	GH	0.23	.15	1.88	.21	2.11	.21	1.69	.13
E	13	GH	1.11	.14	1.69	.25	2.81	.31	1.79	.19

Fig. 33. Length changes of postmetamorphic frogs treated one month with saline, prolactin and GH. Means and standard errors represent data in Table 17.

Fig. 34. Length changes of postmetamorphic frogs treated a second month with saline, prolactin and GH. Means and standard errors represent data in Table 17.

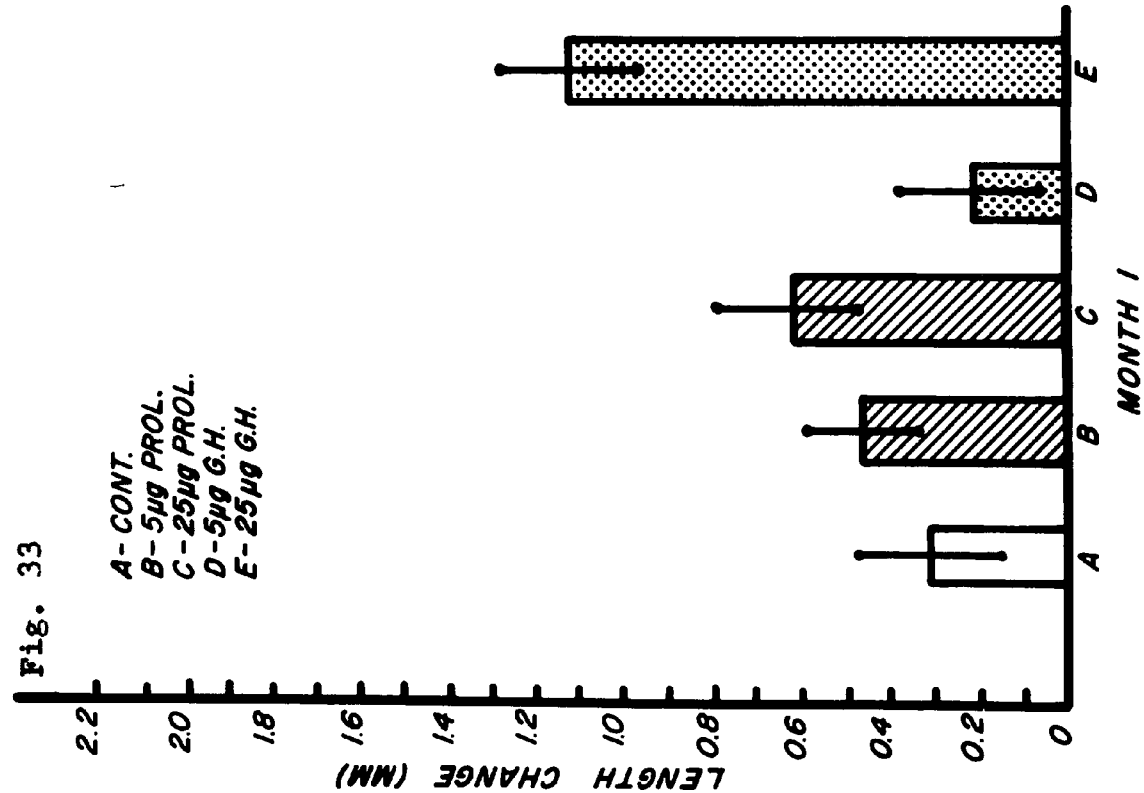
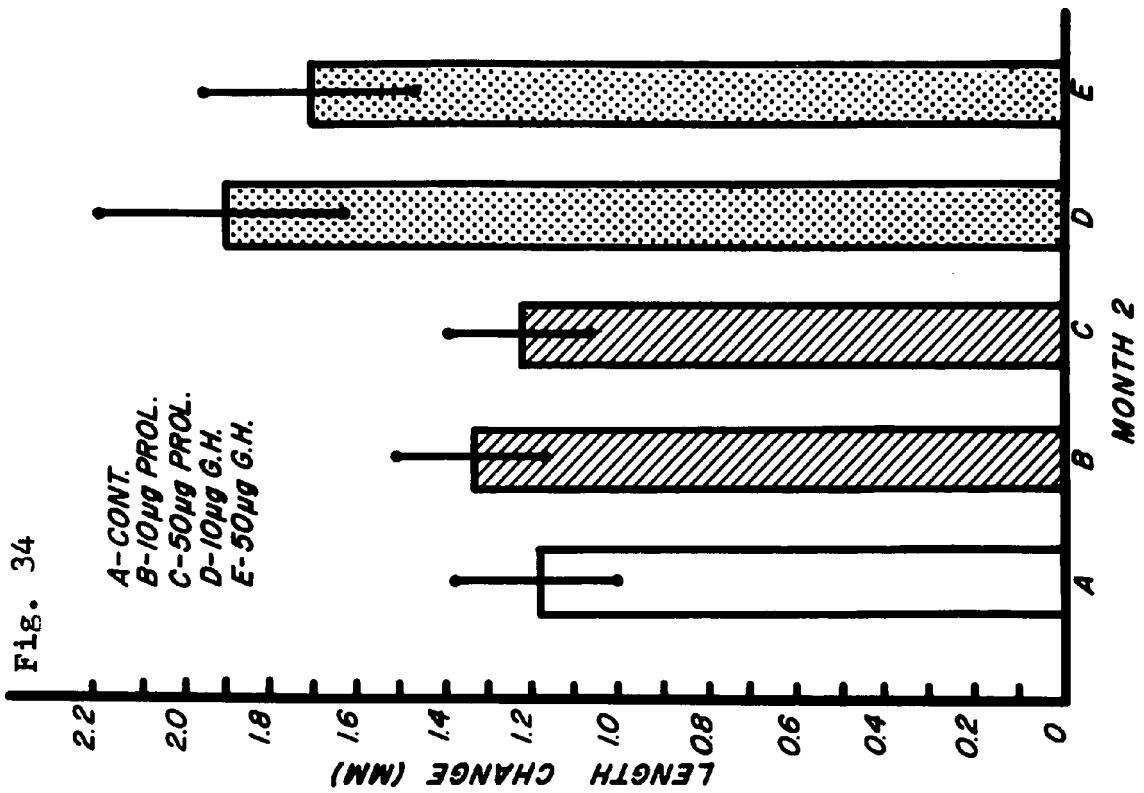


Fig. 35. Total length changes of postmetamorphic frogs treated for 2 month with saline, prolactin and GH. See text for details of the 2 month treatment. Means and standard errors represent data in Table 17.

Fig. 36. Total weight changes of postmetamorphic frogs treated for 2 month with saline, prolactin and GH. See text for details of the 2 month treatment. Means and standard errors represent data in Table 17.

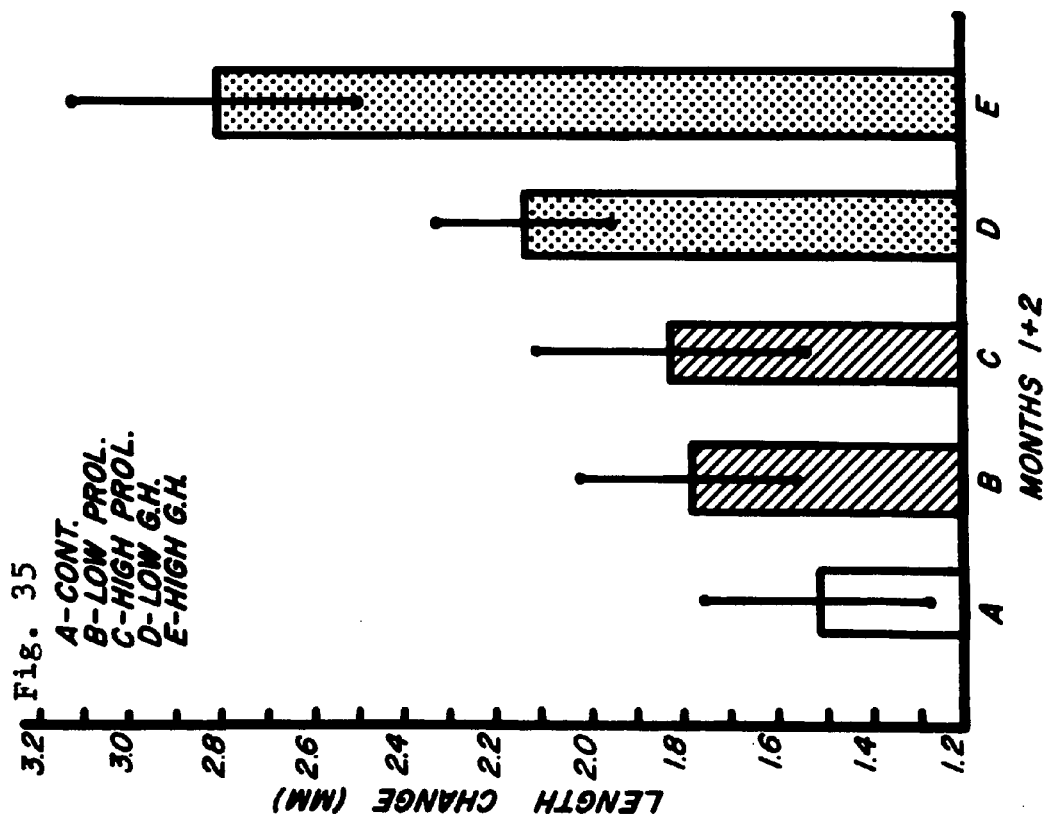
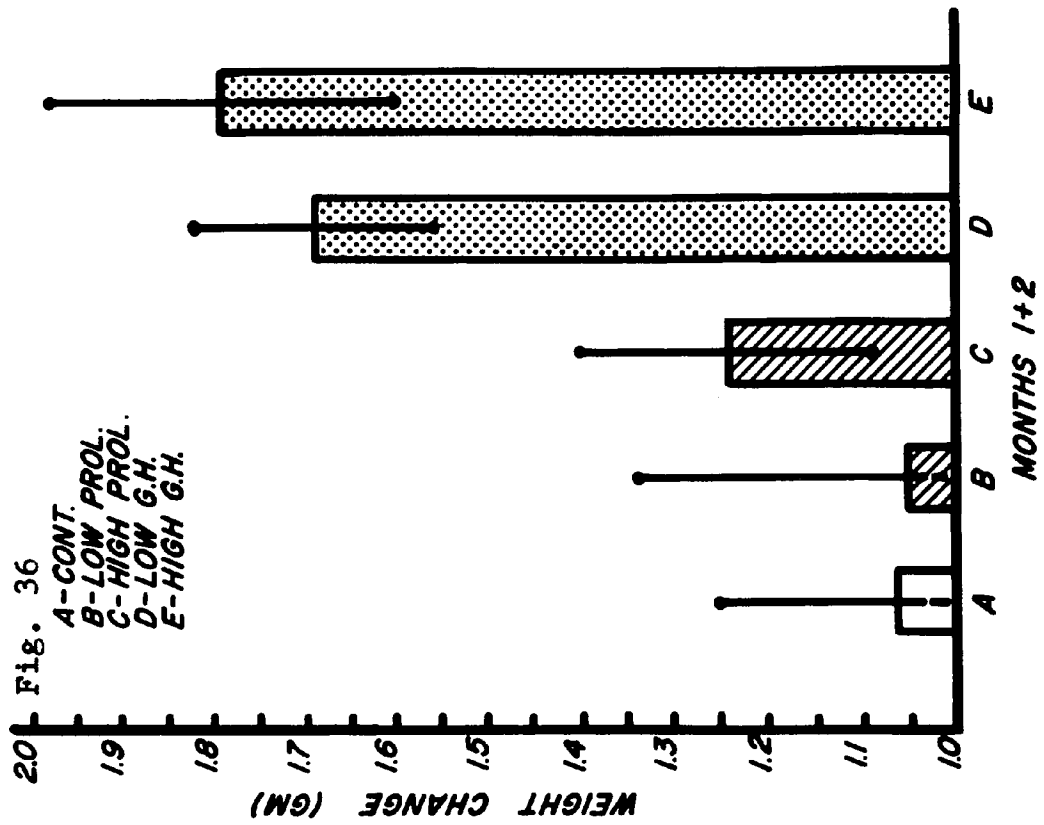
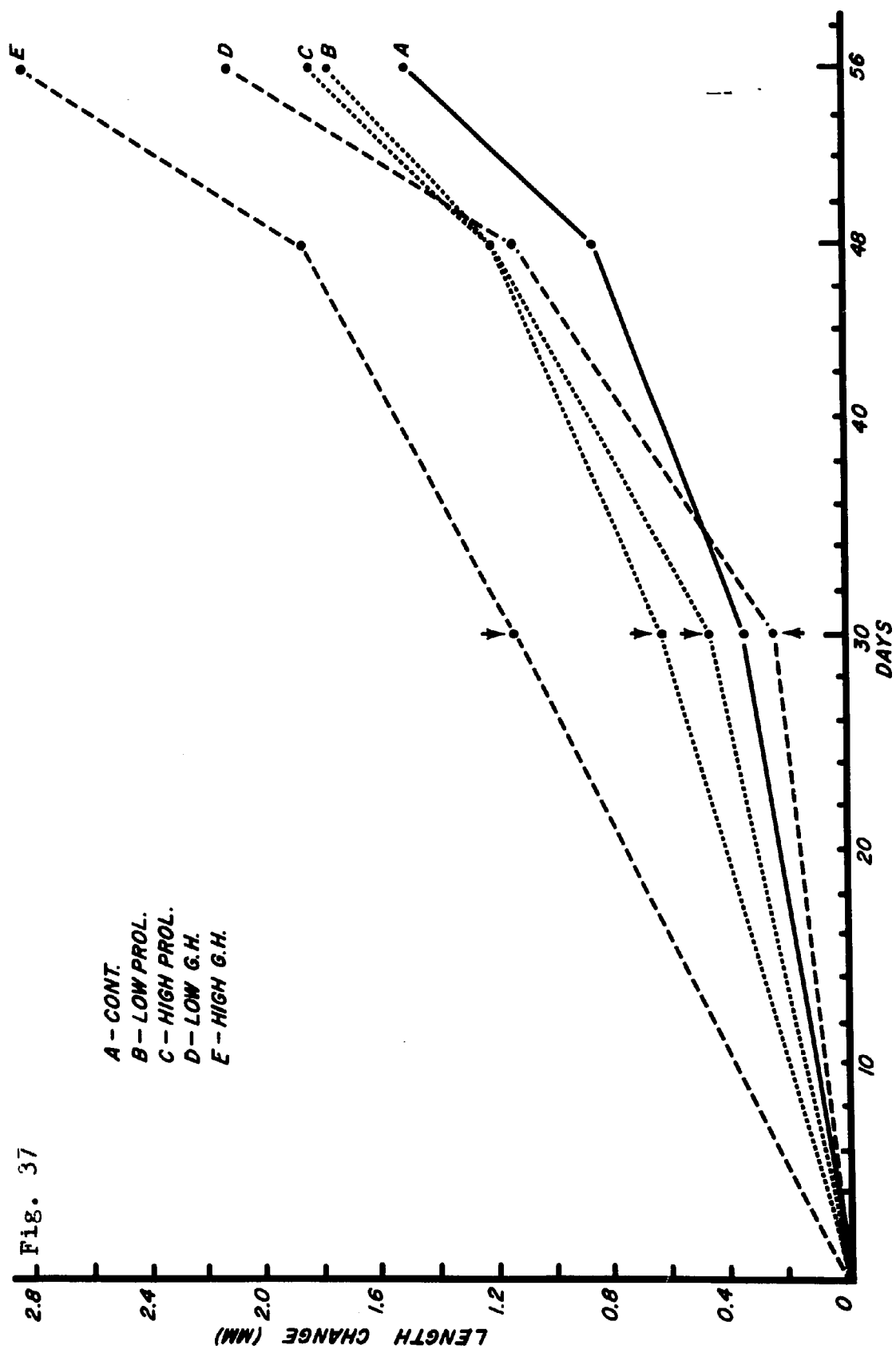


Fig. 37. Length changes of postmetamorphic frogs treated 56 days with saline, prolactin and GH. Arrows indicate the increase of the hormone doses. See text for details of the treatment.



DISCUSSION

Prolactin and growth hormone stimulation of tadpole growth.

The possibility that prolactin functions in the regulation of amphibian growth has recently received much attention. Etkin and Lehrer (1960) found that R. pipiens tadpoles that had their pituitary transplanted to the tail grew larger than controls. This suggested that the transplant might be releasing a growth factor in quantities greater than when the pituitary is in its normal location. Since mammalian pituitaries are known to release prolactin when removed from hypothalamic inhibition (Everett, 1966) it was suggested that the factor producing excessive growth in tadpoles bearing pituitary grafts might be prolactin. It has been well documented that mammalian prolactin will cause newts to migrate to water (Chadwick, 1940; Grant and Grant, 1958). Masur's (1963) study in which a transplanted pituitary in the red eft stage of the newt caused migration to water supported the possibility that a prolactin-like substance in the amphibian pituitary is released spontaneously when the pituitary is removed from its normal position.

A number of recent studies have shown that tadpoles injected with mammalian prolactin preparations grow abnormally large.¹ Publications include those of Berman

¹The only report in the literature of prolactin not producing an increase in size in amphibian larvae is from Enemar (1967) who used Rana temporaria tadpoles.

et al. (1964) and Nicoll et al. (1965) with Rana catesbiana, Etkin and Gona (1967) with R. pipiens and Remy and Bounhiol (1965, 1966) with the toad Alytes obstetricans.

The results of my experiments in which tadpoles were treated with a variety of prolactin doses (Experiments 1, 2 and 3) are in agreement with the above studies. Although the species differed, my results and those of Berman et al. (1964) and Nicoll et al. (1965) suggest that a minimum effective dose of prolactin that will produce an increase in tadpoles length is 0.5-1.0 $\mu\text{g/day}$ for 1 or 2 weeks (approximately 0.01-0.02 IU/day). Weight is a more variable measure since the weight of individual animals tends to vary within a 24 hour period to a much greater extent than length. Consequently a slightly higher dose may be necessary to elicit a significant difference in weight than to effect length significantly (Experiment 2; Nicoll et al., 1965).

Although the increase in length indicates that the growth which prolactin promotes is not simply an uptake of water, the dry weight data (Experiment 3) is further support for this conclusion. For technical reasons data was not collected on the size differences between prolactin-treated and control tadpoles after metamorphosis. However, Remy and Bounhiol (1966) have found that in the few toads that metamorphosed successfully, the prolactin-treated toads were larger than controls. In addition to the length and wet weight data that is recorded in most studies, the dry weight

data given here and the increased size after metamorphosis reported by Remy and Bounhiol (1966) indicate that a prolactin-like hormone may indeed be playing an important physiological role in controlling size in amphibia.

The effect of GH on growth in tadpoles is not as clear cut as the prolactin effect, and definite stimulation is obtained only when relatively high doses of GH are used. Etkin and Gona (1967) reported stimulation of weight gain in tadpoles after treatment for 3 or 5 weeks with GH. I found that 50 $\mu\text{g}/\text{day}$ was the lowest GH dose producing a significant increase in length in R. pipiens tadpoles treated for a 2-week period (Experiment 3). In this experiment the increase in size was less than in tadpoles treated with a smaller dose (10 $\mu\text{g}/\text{day}$) of prolactin. Nicoll et al. (1965) reported finding no effect on tail length and depth with 5 and 10 $\mu\text{g}/\text{day}$ doses of GH, using R. catesbiana tadpoles. Thus the minimum effective dose appears to be around 50-100 μg (0.05-0.08 USP units) of GH per day.

The NIH bovine growth hormone preparation used in my experiments was assayed (by NIH) for contamination by other pituitary hormones. No significant prolactin contamination was found in the given quantity assayed. From this information the maximum possible prolactin contamination of the GH was calculated to be approximately 0.02 IU/50 μg . As mentioned above, this is the minimum effective prolactin dose. Since the prolactin contamination was calculated on

the basis of no significant prolactin response, the growth response of tadpoles to GH cannot clearly be attributed to prolactin contamination but this is certainly a possible explanation.

From these experiments with prolactin and GH it appears that only prolactin unequivocally promotes growth in tadpoles. The dose of GH necessary to promote tadpole growth is approximately 50 times greater than that of prolactin.

Prolactin and GH effects on metamorphosis.

Accelerated hind leg growth and stimulation of metamorphosis in GH-treated tadpoles has been reported by Etkin and Gona (1967) , Nicoll et al. (1965) and Bounhiol et al. (1950). The same effect was found in my experiment in which 50 µg/day bovine GH was administered to one group of tadpoles (Experiment 1). Accelerated metamorphosis and hind leg growth are specific responses to rising titers of thyroxine during metamorphosis (Etkin, 1964). Since at least some of the GH preparations in the experiments mentioned above contained measurable TSH activity, it is possible that these effects were all due to TSH stimulation of thyroid activity and not to an effect of GH per se. This interpretation is substantiated by the results of Experiment 3, done with a porcine GH preparation (which was more highly purified than the bovine preparation), in which no acceleration of metamorphosis or leg growth occurred.

Prolactin doses of 5, 25 and 50 µg/day retarded metamorphosis (Experiments 1, 4 and 5). Hind leg measurements showed that hind leg growth was significantly slowed by 25 µg/day of prolactin but the metamorphic rate was not measurably reduced with doses of 0.5 and 1.0 µg/day. Thus it appears that doses of 5 µg/day or higher are necessary to interfere with metamorphosis in an obvious way. However, no effect of the 10 µg prolactin dose in Experiment 3 was seen in group C. Since these tadpoles were at considerably earlier stages at the beginning of the experiment compared

to tadpoles in Experiment 1, 4 and 5, it is possible that not only a dose of 5 $\mu\text{g}/\text{day}$ or above is necessary but also that prolactin is more clearly effective in retarding the rate of metamorphosis if the tadpoes are at stages 13 or later. This is supported by reports that tadpoles treated with thyroid inhibitors or thyroidectomized tadpoles progress to stages 12-14 before stopping (Hanaoka, 1966; Smith and Smith, 1923; and Steinmetz, 1952, 1954). Prolactin would thus appear to be most effective in the retardation of metamorphosis during the thyroxine dependent phase of metamorphosis.

Nicoll et al. (1965) reported no change in hind leg length in R. catesbiana tadpoles in 1- and 2-week periods of treatment with 5 or 10 $\mu\text{g}/\text{day}$ of prolactin. However, Etkin and Gona (1967) reported a decrease both in hind leg growth and tail resorption in prolactin-treated tadpoles, although they indicated that during the first week of treatment prolactin increased rather than decreased hind leg growth.

In Experiment 5, body weight and length increased after 1 week of prolactin treatment, but hind leg length did not respond until the second week. Contrary to what Etkin and Gona (1967) reported, no initial increase in hind leg length was found.

Since one of the actions of prolactin administered to tadpoles is clearly in inhibition of the rate of metamorphosis, as judged by staging and hind leg length, the

question concerning the level at which prolactin is acting is raised. The several possibilities include (1) prolactin renders the peripheral tissues insensitive to thyroid hormone, either partially or completely; (2) prolactin interferes with either the synthesis or secretion of TSH which in turn decreases the level of thyroid hormone; (3) prolactin acts directly on the thyroid gland, interfering with synthesis or secretion of the thyroid hormone; or (4) prolactin interacts directly with the thyroid hormone, inactivating it in the circulatory system.

One of the more easily tested hypotheses is that prolactin makes the peripheral tissues less sensitive to thyroxine. However, the evidence obtained in this work gives no indication that prolactin reduces tissue sensitivity to thyroxine. If tadpoles are injected with prolactin until their metamorphic rate has been severely reduced and then exogenous thyroxine is administered, one would expect no metamorphic response if the tissues have become insensitive or a reduced response if the tissues are less sensitive. In Experiment 4, tadpoles were put in a 100 $\mu\text{g}/\text{l}$ thyroxine solution after 2 weeks of prolactin treatment. Both prolactin-treated and control tadpoles lost weight in a 3-day period in response to the thyroxine. Because of the relatively high thyroxine dose¹ it was not possible to determine whether or not the tadpoles had a

¹The terms high and low doses of thyroxine are used with reference to doses of thyroxine known to influence amphibian metamorphosis.

reduced thyroxine sensitivity but it was clear that they had not become completely insensitive to thyroxine.

To test the possibility of a reduced thyroid hormone sensitivity, lower doses of thyroid hormone were used in Experiment 5. After 17 days of prolactin treatment, a relatively low dose of thyroxine ($4 \mu\text{g/l}$) was administered to the tadpoles for a week. In this case the thyroxine did not stimulate a reduction in weight and length in either the prolactin-treated tadpoles (G) or the controls (D). In fact, instead of stimulating a loss of body length, thyroxine at this dose appeared to increase body length in the prolactin-treated tadpoles. But this dose of thyroxine did cause an increase in hind leg length in both groups G and D. Apparently hind leg length is more sensitive to low thyroxine doses than are weight and length. Not until the thyroxine dose was increased to 10 and $75 \mu\text{g/l}$ did weight and length actually decrease. Since the tadpoles that were receiving prolactin responded to the thyroxine at either 4, 10, 75 or $100 \mu\text{g/l}$ in the same way as controls, there is no reason to think that prolactin acts to make the tissues insensitive to thyroxine.

This conclusion that prolactin is not acting to render the tissues insensitive to thyroxine agrees with that of Gona (1967). He found that when $100 \mu\text{g}$ prolactin and $10 \mu\text{g}$ thyroxine were administered simultaneously to R. catesbiana tadpoles and compared to another group that received just the thyroxine dose, both groups underwent

similar metamorphic changes. Gona concluded that prolactin does not inhibit thyroxine at the peripheral tissues and must therefore influence the secretion or action of TSH or the secretion of the thyroid hormone. In order to determine if the prolactin interference with metamorphosis is due to TSH inhibition, Gona (1967) injected tadpoles with (1) TSH alone and (2) TSH + prolactin. While TSH alone stimulated metamorphosis, TSH + prolactin inhibited it. He concluded from this and histological examination of the thyroid that prolactin interferes with metamorphosis at the level of the thyroid.

Although my results support Gona's conclusion, it should be pointed out that Nicoll et al. (1965) have data which they feel indicates that the anti-metamorphic effect of prolactin is due to reduced peripheral tissue sensitivity to thyroxine. They found that R. catesbiana tadpoles treated with 100 µg/l thyroxine responded by a reduction of tail length and width. But tadpoles treated with 1 or 10 µg/day prolactin did not respond to such doses of thyroxine. Aside from the difference in prolactin doses, Nicoll's experiments and mine should be quite comparable. The different results obtained are puzzling and perhaps warrant repeating the experiments with both species.

Interrelationship of growth and metamorphosis effects.

Considering the two effects that prolactin has on tadpoles, (1) increasing size and (2) decreasing the rate of metamorphosis, the question arises as to whether the two effects are separable or whether one is a result of the other. If one effect is a direct result of the other, one possible hypothesis is that tadpole growth is controlled by the absolute level of thyroid hormone. In this case prolactin would act to lower the effective thyroid hormone level and this in itself would stimulate growth. If the effects are separate, then while prolactin is interfering with the thyroid, it is also acting independently to cause growth.

If prolactin could cause an increase in size in tadpoles whose thyroid hormone level had already been maximally decreased, either by thyroidectomy or thiouracil, it would be an indication that its growth effect is independent of an interaction with the thyroid hormone. This was tested in Experiments 6, 7 and 8 and the data indicated that prolactin caused an increase in size of tadpoles beyond that which occurred when the level of thyroid hormone was decreased by thyroidectomy or thiouracil treatment. This further growth stimulation in animals that already had a maximally inhibited thyroid indicated that the prolactin action on growth may indeed be separable from its action on the thyroid. Consequently, a direct effect on the peripheral tissues rather than one mediated only by the thyroid hormone

is the more likely alternative. Of the many workers who have studied amphibian metamorphosis apparently only Smith and Smith (1923) have done similar experiments. They injected thyroidectomized tadpoles with anterior pituitary lobe extracts and found an increase in size compared to control thyroidectomized tadpoles.

It has been well documented that a decreased thyroid hormone level in tadpoles leads to an increase in size. The earliest reports of increased body size but decreased growth of the hind legs after thyroidectomy are those of Allen (1918, 1925) and Hoskins and Hoskins (1919). For reviews of the early papers see Allen (1929, 1938) and Etkin (1955). More recently, Steinmetz (1952, 1954) measured wet weight, dry weight and total nitrogen after tadpoles had been kept in thiouracil for various lengths of time. The tadpoles in thiouracil increased in size compared to controls while tadpoles in thyroxine decreased. Wet weight changes were also reflected by dry weight and total nitrogen measurements.

Thus it appears that the increase in size in tadpoles after thyroidectomy or treatment with a thyroid inhibitor has been demonstrated. However, it is not clear whether the decrease in the level of thyroid hormone is the direct cause of the increase in size or whether the decreased thyroid hormone allows a growth factor from the pituitary to be more effective. If a pituitary factor is involved, it is possible that the lower thyroid hormone after thiouracil

treatment or thyroidectomy allows a greater synthesis and secretion by the animals' own pituitary of a prolactin-like substance or else allows a prolactin-like substance which is secreted at the normal level to be more effective. This relationship between the pituitary and the thyroid has been suggested earlier by Steinmetz (1954), although little was known of the possible nature of the growth factor.

Although Experiments 1 and 3 indicated that intact tadpoles will respond only to relatively high doses of GH, Experiments 9 and 10 suggest the possibility that tadpoles with a lower thyroid hormone level respond to somewhat lower doses of GH. While prolactin clearly affects both size and metamorphosis, GH appears only to affect size. It would seem that the capacity of prolactin to inhibit metamorphosis facilitates its capacity to increase size. Although very small doses of thyroid hormone did not interfere with growth promotion by prolactin, larger doses did (Experiment 5). Thus it seems that both GH and prolactin depend on the lower thyroid hormone level, but a major difference between the two hormones is that prolactin can lower the thyroid hormone level itself. Of course, it is possible that the different growth-promoting capacities of prolactin and GH are a result of each hormone affecting different organs or cell populations within the tadpoles. Again, it is possible that the growth-promoting properties of GH described here are due to prolactin contamination.

Growth effects of hypophysectomy and hormone treatment in frogs.

It was not unexpected that hypophysectomy would interfere with growth in frogs although it had not been reported. Considering the hormone effects in tadpoles, it was however somewhat surprising that only GH was effective in stimulating growth in frogs while prolactin had no significant effect. The 2-month period was apparently long enough so that even the weight changes of the GH-treated frogs were significantly greater than controls or prolactin-treated animals. The length data for the 2 months suggest that a GH dose between 10 and 25 $\mu\text{g}/\text{day}$ is most effective.

In frogs, neither 25 nor 50 μg of prolactin/day for 1 month was enough, either alone or combined, to stimulate a significant growth while in tadpoles a dose of 1 μg of prolactin/day was sufficient to promote significant growth in 2 weeks. However, approximately 50 times more GH than prolactin was needed to stimulate tadpoles' growth.

Both prolactin and GH stimulate regeneration in adult newts. However, with present information there is no means of comparing this with the growth effects of these hormones in frogs in view of the possible basic differences in the processes of growth and regeneration as well as between the orders Anura and Urodela.

General Discussion and Conclusions.

In summary of what has been discussed up to this point the following statements can be made concerning the effects of pituitary hormone on tadpole growth and metamorphosis:

- (1) Hypophysectomized tadpoles do not grow as large as control tadpoles.
- (2) Tadpoles in which the pituitary anlage is transplanted to the tail region in the embryo grow larger than normal tadpoles.
- (3) Both prolactin and GH stimulate growth in tadpoles but prolactin elicits greater responses at lower doses.
- (4) Growth hormone may be more effective in promoting growth in tadpoles which have a reduced level of thyroid hormone than it is in normal tadpoles.
- (5) Thyroidectomized tadpoles show an increase in growth compared to controls.
- (6) Prolactin further increases growth in thyroidectomized tadpoles and in tadpoles treated with thiouracil.
- (7) Prolactin can act to increase size in tadpoles in the presence of low doses of exogenous thyroxine but not in the presence of high doses of thyroxine.
- (8) Prolactin inhibits metamorphosis but GH does not.
- (9) TSH does not stimulate metamorphosis in tadpoles in which metamorphosis has been inhibited with prolactin.
- (10) Prolactin-treated tadpoles metamorphose in response to exogenous thyroxine in the same way as nonprolactin-treated tadpoles.

With regard to frogs, it is known that:

- (11) Hypophysectomized frogs have a decreased growth rate.
- (12) Intact frogs increase in weight and length in response to GH but growth is unaffected by prolactin.

From this information it appears likely that the tadpole pituitary produces at least one hormone necessary for normal growth. This growth effect is most prominent when the pituitary is not in its normal position adjacent to the hypothalamus. Considering the tadpole growth response to prolactin and GH, it is likely that the tadpole pituitary growth factor is similar to either mammalian prolactin or GH. Since tadpoles are more sensitive to prolactin, it is more likely that the tadpole growth factor resembles prolactin. Since prolactin can inhibit metamorphosis it is apparently interfering with the pituitary-thyroid axis and/or the action of thyroid hormone. Prolactin does not appear to decrease tissue sensitivity to thyroxine since prolactin-treated tadpoles respond to exogenous thyroxine with about the same sensitivity as nonprolactin-treated controls. Since TSH does not stimulate metamorphosis in tadpoles inhibited with prolactin it appears that prolactin does not inhibit TSH synthesis or secretion. The alternative ways that prolactin may act to inhibit metamorphosis are (1) at the thyroid gland itself, by inhibiting synthesis or secretion of thyroid hormone or (2) interacting with thyroid hormone in the circulatory system and inactivating it. Either of these latter two actions could lead to a

stimulation of TSH secretion by the pituitary which would result in the goiterous thyroid gland described by Gona (1967) in R. catesbiana.

The growth action of prolactin on tadpoles does not appear to be due only to its action on the thyroid since prolactin can stimulate further growth in tadpoles that have had their thyroid glands inhibited either by thyroidectomy or thiouracil treatment prior to the administration of prolactin. However, it remains possible that the prolactin inhibition of the thyroid facilitates the growth-promoting action of prolactin, since prolactin cannot stimulate growth when the thyroid hormone level is high. Apparently, both prolactin and GH can promote growth more effectively in tadpoles with a low thyroid hormone level but only prolactin has the capacity to influence the effective thyroid hormone level.

During the normal development of tadpoles through metamorphosis, probably a balance of prolactin-like hormone and thyroid hormone is maintained, with the thyroid hormone level increasing at metamorphic climax. Perhaps when tadpoles are thyroidectomized or treated with thiouracil, the normal amount of growth factor from the pituitary is released but is now more effective in the low thyroid hormone environment. Substantiation of these ideas would require demonstration of a growth-stimulating/metamorphosis-inhibiting factor (prolactin-like) in the tadpoles' pituitary, and information on its normal levels and secretion rates

during the development of the tadpole.

The growth responses of postmetamorphic frogs to prolactin and GH indicate that if the pituitary of frogs produces a specific hormone necessary for normal growth it resembles mammalian GH more than it does mammalian prolactin. In any case, the differences in the growth responses of tadpoles and frogs to prolactin and GH indicate that the mechanisms of primary growth regulation are different in these two stages of the life cycle. The lack of a growth response of frogs to prolactin suggests that either (1) tissues once sensitive to prolactin have become insensitive, presumably at metamorphosis or (2) that the larval tissues of the tadpoles that were sensitive to prolactin are decreased in size or quantity so that their response is not detectable.

This interpretation of the hormonal regulation of growth would be greatly strengthened by information on the levels of assayable growth factor in the frog pituitary, and particularly its activity compared to GH and prolactin. Information as to whether there is a transition in the nature of a native growth factor, corresponding to the observed transition in sensitivity to exogenous prolactin and GH would be especially interesting and valuable in the understanding of the nature of growth regulation in this group of animals.

Although no assays of tadpole pituitaries have been done, several bioassays of adult amphibian pituitaries have

been made. Anuran pituitaries have been found to have positive pigeon crop stimulating activity (Foglia, 1940; Nicoll and Bern, 1965). as well as mammotrophic activity (Chadwick, 1966a, 1966b; Nicoll, Bern and Brown, 1966). But according to Chadwick the prolactin-like activity did not mimic mammalian prolactin preparations exactly. The amphibian preparations possessed the element responsible for growth of the mammary gland but not the element responsible for the onset of milk secretion. The only report of GH activity in the amphibian pituitary is that of Solomon and Greep (1959). They used the rat tibia test and found that amphibian pituitaries did have a measureable amount of growth-promoting activity.

A number of histochemical studies of amphibian pituitaries have been done but the greater share of them have been primarily concerned with the identification of TSH and gonadotropin producing cells. While acidophils are found, no positive identification of either prolactin or GH cells has been made. Etkin and Ortman (1960) reported well-developed acidophils in pituitary transplants of R. pipiens tadpoles. Ortman (1961) described two types of acidophils in R. pipiens adults. Kerr (1965) reports two acidophil types in the pituitary of Xenopus laevis. While acidophils have been tentatively identified with either prolactin or GH, very little experimental work has been done in amphibians which would allow a correlation of cells of specific staining characteristics with specific hormones.

A number of different approaches could be useful in further studies attempting to elucidate the role of the pituitary in the regulation of amphibian growth. These include (1) cytological studies utilizing classical histochemistry, immunofluorescent and enzyme-antibody localization and electron microscopic techniques to identify pituitary cell types during both pre- and postmetamorphic stages; (2) demonstration of factors in the amphibian pituitary that have actions in amphibians similar to those of mammalian hormone--this should ideally include isolation and purification of the amphibian hormones; and (3) examination of the response of specific organs and tissues to growth-promoting factors in an effort to determine where and how these factors are acting.

When more is known regarding the activity and structure of amphibian pituitary hormones, comparisons between them and those of other vertebrate classes could be important in understanding both functional and structural evolution at the molecular level.

A NON-GROWTH EFFECT OF PROLACTIN: SKIN COLOR IN
POSTMETAMORPHIC FROGS

Most of the studies of the endocrine control of chromatophores in amphibians have been concerned with melanophores. Bagnara's (1966) recent review on non-melanophore pigment cells indicates that little or nothing is known of the possible pituitary control of xanthophores or guaninephores (iridophores) in postmetamorphic frogs.

In the experiment in which frogs were treated with hormones (Experiment 13), prolactin was found to affect the skin color of the frogs. Both groups of prolactin-treated frogs (B and C) were distinctly more yellow-green in color than either the controls (group A) or the GH-treated frogs (groups D and E). In order to investigate this action of prolactin somewhat further, pairs of animals were matched according to color and one animal of each pair was injected with either ovine prolactin, bovine prolactin or adult *R. pipiens* pituitary gland. The control in each pair received saline injections. A discernable change in color occurred within 5-20 days after the initiation of the prolactin or pituitary injections.

Although no attempt has yet been made to establish a dose response curve, either 5 µg/day of prolactin or one adult frog pituitary gland every other day was sufficient to elicit an observable change. Prolactin gave a quicker response than whole pituitary injections, probably reflecting a relatively low content of the active principle

in the pituitaries. The frogs survived the pure prolactin preparations better than the whole pituitary injections (the pituitary injected animals usually surviving 2-4 weeks). The comparative potency of prolactin and whole pituitary extracts noted was after the same duration of treatment. When hormone treatment was discontinued, the bright yellow color gradually dissipated over a period of several weeks.

Pieces of skin from paired frogs were mounted in Kero syrup and examined to determine if a noticeable change in the chromatophores could be found. The only clear difference appeared in the xanthophores. The yellow carotenoid pigments in the skin of the controls more often were condensed into a small area within the xanthophore, while in the skin of a prolactin-treated frog the yellow pigments were more uniformly expanded into a network. This was most clearly seen in the areas of the skin which had a lower density of pigment cells--such as the sides of the body. It should be emphasized that both types of carotenoid distribution can often be seen in control as well as prolactin-treated frogs and the difference appears to be one of extent.

Although quantitative extractions have not yet been done, preliminary extractions show that the amount of yellow pigments is increased with prolactin treatment. Thus prolactin would appear to stimulate both pigment dispersion in the xanthophores and pigment synthesis. According to Morton and Rosen (1949), β -carotene and lutein

are the two most common carotenoids in frog skin.

Preliminary work has been started using acetone extracts of equal size pieces of skin and separating the pigments using the chromatographic and spectrometric methods of Green (1957), but it is not yet possible to say whether one or both of these pigments or different pigments are involved in the prolactin effect on color.

Although tadpoles have been treated for a long period with prolactin, no effects similar to these pigmentation effects found in frogs have been observed.

Some of the areas of this problem that I am interested in pursuing include the following: (1) which pigments are being affected by prolactin (this will include extraction, separation and identification); (2) to what extent both movement and synthesis of the pigment or pigments are involved; (3) the use of long term hypophysectomized frogs to determine to what extent the pituitary is responsible for the normal degree of yellow pigmentation; (4) whether the variation in color seen in natural populations is due to differences in either the tissue sensitivity or the endogenous titer of a prolactin-like hormone; (5) whether prolactin affects the carotenoids in other tissues besides the skin, such as the fat bodies or gonads; (6) how widespread this phenomena is both within the amphibians and in other vertebrate classes; and (7) the specificity of the color response for prolactin and the possibility of using this response as a prolactin :

bioassay once extraction techniques are worked out.

Perhaps the most significant aspect of these observations is the difference noted in the response of tadpole and frog chromatophores. Presuming the xanthophores of tadpoles and frogs are functionally (biochemically) identical in all other respects, here we have a specific differentiative change involving only the acquisition of responsivity to a particular hormone. Such a system could be valuable in the analysis of the fundamental problem of the molecular basis of hormone sensitivity and the mechanism of action of hormones.

SUMMARY

Mammalian prolactin at doses of 1-50 $\mu\text{g}/\text{day}$ promoted growth (increased wet weight, dry weight and body length) in Rana pipiens tadpoles. Mammalian GH promoted growth only at the higher doses (50 $\mu\text{g}/\text{day}$ and above) and was not as effective as prolactin. But in thyroidectomized tadpoles, lower doses of GH (5-25 $\mu\text{g}/\text{day}$) promoted growth.

Prolactin also inhibited metamorphosis (at doses of 5 $\mu\text{g}/\text{day}$ and above). Inhibition of metamorphosis did not appear to be due to prolactin rendering the tissues insensitive to thyroxine since prolactin treated tadpoles metamorphosed in response to exogenously administered thyroxine (4-100 $\mu\text{g}/\text{l}$) to the same extent as controls. This suggested that prolactin must lower the active thyroid hormone level (either by altering secretion rates via the pituitary or thyroid or by inactivating the thyroid hormone in the circulatory system).

Prolactin promoted further growth in tadpoles in which the thyroid hormone level had been reduced with thiouracil or thyroidectomy. This suggested that the two actions of prolactin are at least partially independent--or that the growth which prolactin promotes is not just the result of thyroid inhibition.

In postmetamorphic frogs, hypophysectomy reduced growth. Growth hormone (at doses of 10-50 $\mu\text{g}/\text{day}$) promoted growth in young frogs. Prolactin (at doses of 5-50 $\mu\text{g}/\text{day}$) did not promote growth. However, prolactin did have an

effect on skin color. Prolactin-treated frogs became a bright yellow-green.

These studies suggest that the pituitary regulation of growth is different in pre- and postmetamorphic stages of the frogs.

APPENDIX

Symbols: L = length (cm for tadpoles; mm for frogs)
W = weight (gm)
S = stage (Taylor-Kollros metamorphic stages)
H = hind leg length
T4 = thyroid hormone (thyroxine)
TX = thyroidectomy
Numerical subscripts to L, W, S and H refer to the day of the experiment on which the measurement was made.

Experiment 1 - 12 April - 3 May, 1967

A - Control
B - 0.5 ug/day prolactin
C - 1.0 ug/day prolactin
D - 5.0 ug/day prolactin
E - 50.0 ug/day prolactin
F - 0.5 ug/day GH
G - 1.0 ug/day GH
H - 5.0 ug/day GH
I - 50.0 ug/day GH

	L ₀	L ₁₄	L ₂₁	W ₀	W ₁₄	W ₂₁	S ₁₄	S ₂₁
A200	7.0	7.9	7.9	3.1	4.6	4.2	15	18
A201	7.7	8.5	8.0	4.0	4.6	3.6	17½	20
A202	7.7	8.0	6.2	4.0	4.5	3.0	18	21
A203	6.7	7.5	7.2	3.2	4.4	4.4	17	19
A204	6.8	8.1	8.0	3.1	4.1	4.1	14-15	17
A205	8.0	7.9	8.1	4.4	4.2	4.3	16-17	17½
A206	8.1	9.5	8.6	5.0	6.9	6.1	16-17	19
A208	7.4	8.4	7.5	3.3	4.6	4.0	16-17	18
A209	7.8	8.5	7.7	3.3	5.0	4.1	17½	19½
A210	7.4	8.2	7.7	3.8	5.2	4.2	16-17	19½
B219	7.0	8.2		3.1	4.7		16-17	
B220	6.2	8.1		3.1	4.9		13	
B221	6.7	7.7		2.6	3.8		14	
B222	8.3	8.3		2.8	5.1		16-17	
C225	7.0	8.5		3.8	6.2		16-17	
C226	8.0	9.8		5.0	7.2		16-17	
C229	7.2	8.3		3.5	4.9		15	
C230	7.9	8.8		4.3	5.4		17	
C231	7.8	8.8		4.1	5.2		17	
C232	8.2	9.3		5.1	7.2		16-17	
C233	7.2	8.8		3.3	5.3		16-17	
C234	7.3	8.7		3.3	5.1		16-17	
C235	6.7	7.2		2.3	3.6		15	

Experiment 1 (continued)

	L ₀	L ₁₄	L ₂₁	W ₀	W ₁₄	W ₂₁	S ₁₄	S ₂₁
D237	8.4	9.9	9.8	5.1	6.9	--	16-17	17½
D240	7.9	9.1	9.3	4.0	6.8	7.1	16-17	16-17
D241	7.5	8.6	8.8	3.5	5.4	5.7	16-17	16-17
D242	7.4	8.7	8.2	3.2	5.0	4.9	16-17	16-17
D243	7.8	8.7	8.0	4.0	5.6	5.5	16-17	16-17
D244	7.6	9.0	8.6	3.9	5.7	5.7	16-17	16-17
D245	7.7	9.0	9.0	4.2	6.8	6.4	16-17	17½
D246	7.7	8.2	8.1	3.7	5.1	4.9	15	16
E250	7.0	8.5	8.9	2.9	5.4	6.2	15-16	16-17
E251	6.9	8.5	8.5	3.5	6.5	6.6	16-17	16-17
E252	6.9	8.2	8.6	3.1	5.9	6.8	16-17	16-17
E257	7.2	8.5	8.8	4.0	5.7	6.2	16-17	16-17
E258	7.5	8.8	8.7	3.5	5.8	5.9	16-17	16-17
F260	6.8	7.7		2.8	5.2		16-17	
F261	7.4	7.7		3.8	4.2		16-17	
F262	8.4	8.8		4.7	5.6		17½	
F263	8.5	9.0		5.4	6.1		16-17	
F264	7.8	8.8		3.9	5.5		16-17	
F265	7.6	8.5		3.7	5.3		17	
F266	7.0	7.6		3.1	4.5		16-17	
F267	7.9	8.9		4.2	6.2		16-17	
F268	8.1	8.2		4.5	5.1		16-17	
F269	7.2	8.4		3.3	4.8		16-17	
F270	7.1	8.6		3.6	5.0		16	
F271	7.6	8.2		4.3	4.9		16-17	
G272	7.4	8.8		3.7	5.2		15	
G274	7.9	8.0		4.5	5.3		17½	
G275	7.5	8.5		4.1	5.8		17	
G276	8.2	8.5		5.0	5.2		16-17	
G277	7.5	9.1		3.9	5.6		16-17	
G280	7.8	8.4		4.7	5.4		17½	
G281	8.0	8.0		4.4	4.6		17½	
G283	7.2	8.5		3.5	5.4		16-17	
H284	7.9	8.3		4.7	5.8		16-17	
H285	8.2	8.9		5.2	5.6		17½-18	
H287	7.2	8.7		3.8	5.5		16	
H288	7.1	8.0		3.0	3.9		16-17	
H289	7.4	7.7		3.4	3.6		16-17	
H290	8.0	8.9		4.8	6.7		17	
H291	7.1	8.4		3.7	5.9		16-17	
H292	7.5	8.3		4.7	5.4		17	

Experiment 1 (continued)

	L_0	L_{14}	L_{21}	W_0	W_{14}	W_{21}	S_{14}	S_{21}
H293	7.0	7.6		3.3	4.0		16-17	
H294	7.5	8.3		3.3	4.5		16-17	
H295	7.8	8.4		3.7	4.9		17½	
I297	7.3	8.8	8.4	3.8	5.0	4.6	17½	19½
I298	7.2	8.1	8.0	3.2	4.5	4.2	17½	19½
I299	7.7	8.9	8.3	4.5	5.5	4.3	17½	20
I302	8.6	8.9	7.7	5.4	6.0	4.1	18-19	21
I303	7.2	9.0	8.4	3.9	5.8	5.5	17-17½	19½
I304	7.0	8.2	8.2	3.4	4.3	4.4	18½	19½
I305	7.2	8.1	8.0	3.5	3.8	3.7	18	19½
I306	6.8	8.1	7.6	3.4	4.4	4.1	17½	19

Tables 1 and 2 and Figures 1-4 are from this raw data.

Experiment 2 - 6 June-21 June, 1967

A -0.7% NaCl B-0.5 μ g/day prolactin C-1.0 μ g/day prolactin

	S ₀	S ₁₅	L ₀	L ₁₅	W ₀	W ₁₅
A400	10	16-17	6.0	7.9	2.6	4.9
A401	10-11	18	6.2	7.7	2.6	4.3
A402	10	16-17	6.1	7.5	2.7	4.4
A403	11	18	6.0	7.8	2.7	5.0
A404	12-13	18	6.4	7.4	2.8	4.1
A405	13-14	17½-18	6.3	7.2	3.0	5.1
A406	14-15	18½	6.6	7.4	3.2	4.3
A407	13-14	17	6.7	7.6	3.3	4.8
A408	11-12	19	6.7	7.6	3.3	4.2
A409	13-14	18	6.7	7.5	3.4	4.8
A410	10	14-15	6.0	7.1	3.4	5.3
A411	13-14	18	7.0	7.9	3.7	5.1
B412	11-12	15-16	6.0	7.5	2.6	4.5
B413	12-13	18	7.2	7.7	3.7	5.1
B414	10-11	15-16	6.4	7.4	2.7	5.7
B415	12	18	6.6	8.0	2.8	4.7
B416	10	14-15	5.8	7.6	2.8	5.4
B417	13	18	6.2	7.4	3.0	4.3
B418	11-12	18½	6.9	8.4	3.2	5.0
B419	13-14	18	6.6	8.0	3.3	4.9
B420	14-15	18	6.5	7.7	3.3	4.9
B421	13	18	6.6	7.8	3.4	4.4
B422	11-12	18	6.9	8.3	3.5	5.8
B423	12-13	18	6.6	7.5	3.5	4.6
C424	11-12	16	5.8	7.4	2.6	4.4
C425	10-11	15-16	6.1	8.3	2.7	5.3
C426	11-12	16-17	6.3	8.1	2.7	5.3
C427	11-12	17½	6.5	8.2	2.8	5.4
C428	11-12	15-16	6.2	7.7	3.0	5.1
C429	14-15	18	6.4	7.1	3.1	4.3
C430	11-12	18	6.8	8.5	3.2	5.4
C431	12-13	17½	6.6	7.6	3.3	4.2
C432	11	19	6.3	8.2	3.4	4.3
C433	12-13	18	6.8	8.1	3.4	5.2
C434	13-14	18	6.9	8.7	3.5	6.2
C435	12-13	18	6.5	8.2	3.5	4.9

Table 3 and Figures 5 and 6 are from this raw data.

Experiment 3 - 4 December-19 December, 1967

A - 0.7% NaCl

B - 50 μ g/day GHC - 10 μ g/day prolactin

	W_0	W_{15}	L_0	L_{15}	S_0	S_{15}	Dry Wt.
A703	1.3	2.8	5.1	6.5	9	15-16	.232 gm
A704	1.3	2.5	5.0	6.1	9 $\frac{1}{2}$	16	.212
A705	1.35	2.3	5.3	6.4	9 $\frac{1}{2}$	16-17	.208
A706	1.25	2.3	4.8	6.2	9 $\frac{1}{2}$	15-16	.215
A707	1.4	2.8	5.5	6.9	10	16-17	.245
A708	1.5	2.5	5.2	6.3	9 $\frac{1}{2}$	16-17	.195
A709	1.5	2.4	5.2	6.8	9	16-17	.222
A710	1.5	2.2	5.3	6.4	9	14	.188
A711	1.55	2.2	5.3	6.2	9 $\frac{1}{2}$	16-17	.200
A712	1.6	2.6	5.4	6.4	10	16-17	.238
A713	1.6	2.3	5.4	6.4	10	16-17	.220
A714	2.0	3.2	5.7	7.0	9	16-17	.280
B729	1.3	2.5	4.8	6.6	8	14	
B730	1.3	2.4	5.2	6.7	8	15	
B731	1.3	2.7	4.8	6.7	8	16	
B732	1.35	2.2	5.1	6.1	9	15-16	
B733	1.35	3.0	5.2	7.2	9	15-16	
B734	1.4	2.9	5.1	7.2	8	15-16	
B735	1.2	2.4	4.9	6.5	9	16	
B739	1.5	2.7	5.4	6.8	9 $\frac{1}{2}$	16-17	
B740	1.6	2.7	5.2	6.5	9 $\frac{1}{2}$	16-17	
B741	1.8	3.1	5.5	6.8	10	16-17	
B742	1.9	2.7	5.7	6.9	9 $\frac{1}{2}$	17	
C715	1.25	2.9	5.1	7.0	9	14-15	.214
C716	1.3	3.4	5.2	7.3	9	15-16	.242
C717	1.3	3.0	5.1	7.4	9	14	.220
C719	1.35	2.9	5.0	6.9	9 $\frac{1}{2}$	16	.217
C720	1.25	3.0	4.8	6.9	9	15	.240
C721	1.4	3.1	5.2	7.0	10	14-15	.240
C722	1.5	3.5	5.4	7.5	9	16	.274
C723	1.5	2.9	5.5	7.2	10	16-17	.246
C724	1.5	2.9	5.3	6.7	10	16-17	.237
C725	1.55	3.1	5.4	7.4	9	15-16	.222
C726	1.6	2.7	5.3	7.3	10	16-17	.252
C728	2.0	3.1	5.7	7.2	10	16	.230

Tables 4 and 5 and Figures 7 and 8 are from this raw data.

Experiment 4 - 31 May-18 June, 1967

A - 0.7% NaCl

C - 5 µg/day prolactin (+T4)

B - 0.7% NaCl (+T4)

D - 5 µg/day prolactin

	S ₀	S ₇	S ₁₅	S ₁₈	W ₀	W ₇	W ₁₅	W ₁₈
AB340	11-12	15-16	17½		2.6	3.2	3.3	
AB341	12-13	16-17	18		2.7	3.6	3.7	
AB346	13-14	16-17	18		3.3	3.7	3.7	
AB348	14-15	16	17½		3.5	4.6	5.3	
AB349	12-13	16-17	17½		3.5	4.1	4.1	
AB351	12-13	16-17	18		3.8	4.2	4.2	
A450	10-11	14	16	17	2.9	3.6	4.7	4.8
A451	10	13-14	16-17	18	3.1	4.8	5.4	5.4
A452	12-13	15-16	16-17	18	3.2	4.8	5.2	4.9
A453			16	16-17			3.0	2.9
A454			15	15-16			5.1	4.7
A455			16-17	16-17			4.5	4.2
B457	-----	-----	16-17	18	---	---	4.2	4.1
B458	-----	-----	16-17	18	---	---	4.6	3.5
B459	-----	-----	16-17	18½	---	---	4.5	3.6
B460	-----	-----	16	17½-18	---	---	4.5	4.4
B461	-----	-----	15	17	---	---	3.9	3.4
B462	-----	-----	17½	19	---	---	4.4	3.3
B463	-----	-----	16-17	17½-18	---	---	4.7	4.0
B464	-----	-----	14-15	17	---	---	4.0	3.8
C465	13-14	15-16	15-16	18	2.7	3.9	5.3	4.8
C466	12-13	15-16	16-17	17½	3.3	4.8	4.9	4.4
C467	14	16-17	16-17	17	3.6	4.2	5.1	4.4
C468	13-14	16-17	16-17	18	3.5	4.8	5.6	5.1
C469	14-15	16-17	16-17	16-17	3.7	4.5	4.3	3.9
C470	11-12	14-15	16-17	17	2.9	4.3	5.2	4.4
C471	11-12	16	16-17	17	3.6	4.7	6.0	5.4
C472	10-11	13-14	15-16	16	2.9	4.5	5.8	5.5
C473	10	12-13	15	17	3.1	4.1	5.2	4.3
C474	11-12	15	15	16-17	3.3	4.6	5.3	4.9
C475	14-15	16-17	17½	18½-19	2.9	3.5	4.3	3.7
C476	13-14	16-17	17	19	3.0	4.2	4.9	4.2
D477	11-12	14-15	16-17	16-17	3.2	4.4	5.6	5.7
D478	14-15	16-17	16-17	17	3.5	3.9	4.3	4.1
D479	11-12	16-17	16-17	16-17	2.9	4.1	4.7	4.3
D480	13-14	16-17	16-17	17	3.2	4.4	5.0	5.4
D481	11-12	14-15	16-17	16-17	2.9	4.0	5.4	5.5
D482	10-11	15-16	16-17	17	3.1	4.2	5.0	4.9
D483	13-14	16-17	16-17	17	3/7	5.0	6.0	6.0

Experiment 4 (continued)

	S ₀	S ₇	S ₁₅	S ₁₈	W ₀	W ₇	W ₁₅	W ₁₈
D484	10-11	14-15	16	16-17	3.0	4.2	5.0	5.4
D485	11	12-13	14-15	15-16	3.2	4.8	6.0	6.3
D486	13	15-16	15-16	16	3.5	4.1	5.4	6.0
D487	13	16-17	17	18	3.6	4.2	4.6	4.3
D488	13-14	16-17	17	18	3.4	4.2	5.0	5.0

Experiment 5- 6 March-4 April, 1968

C - 0.7% NaCl

F - prolactin

D - 0.7% NaCl + T4

G - prolactin + T4

	L ₀	L ₁₁	L ₁₇	L ₂₄	L ₂₇	L ₂₉	W ₀	W ₁₁	W ₁₇	W ₂₄	W ₂₇	W ₂₉
C1	5.9	6.3	6.2	6.4	6.4	6.5	2.2	2.3	2.3	2.3	2.3	2.5
C2	---	---	7.0	7.2	7.2	7.3	---	---	3.3	3.3	3.3	3.4
C4	---	---	6.9	7.2	7.3	7.3	---	---	3.1	3.4	3.7	3.6
C5	6.6	6.4	6.8	7.2	7.1	---	2.9	3.1	3.2	3.3	3.4	---
C6	7.1	7.1	7.2	7.2	7.2	7.2	3.2	3.0	2.9	3.0	2.8	3.1
C7	6.7	6.9	7.0	6.9	6.9	6.8	3.0	3.1	3.3	3.0	2.9	3.1
C8	7.0	6.8	6.9	7.0	7.0	7.0	2.9	2.8	2.8	3.1	2.8	3.1
D2	---	---	6.5	6.7	6.7	6.8	---	---	3.2	3.3	3.0	2.7
D3	---	7.1	7.1	7.0	7.0	6.8	---	---	3.3	3.5	3.2	2.8
D4	6.7	6.4	6.3	6.5	6.6	6.2	2.7	2.5	2.5	2.6	2.5	2.5
D5	6.9	6.8	6.6	6.9	6.9	6.9	3.1	2.9	2.9	2.8	2.8	2.8
D6	6.9	7.0	7.2	6.8	6.9	6.8	3.0	3.3	3.0	3.0	3.1	3.0
D7	6.7	6.4	6.4	6.3	6.2	6.1	2.7	2.6	2.3	1.9	1.9	1.8
D8	7.1	6.9	6.9	7.1	7.0	6.8	3.4	3.1	3.2	2.9	3.0	3.1
F1	5.9	6.2	6.4	6.6	6.5	6.5	2.3	2.6	2.6	2.6	2.5	2.5
F2	6.5	6.9	7.1	7.2	7.2	7.2	2.4	2.8	2.9	2.8	2.8	2.8
F3	6.2	7.0	7.4	7.5	7.5	7.4	2.6	3.2	3.7	3.7	3.8	3.7
F4	6.6	7.1	7.0	7.0	6.9	6.7	2.8	3.3	3.3	3.1	3.1	3.4
F5	6.3	6.9	6.9	6.9	6.9	6.7	2.7	3.3	3.2	2.9	2.8	2.9
F7	7.0	7.2	7.4	7.7	7.8	7.8	3.1	3.8	3.5	3.5	3.9	3.9
F8	6.7	7.2	7.3	7.1	7.2	7.2	3.1	3.5	3.6	3.4	3.4	3.4
F9	7.0	7.4	7.9	8.3	8.4	8.1	3.2	4.0	4.6	4.6	4.4	4.5
F10	7.4	7.7	7.2	7.5	7.6	7.5	3.7	4.4	4.1	4.2	4.2	3.9
F11	6.9	7.2	7.2	7.3	7.4	7.4	3.1	3.4	3.5	3.4	3.3	3.5
F12	7.0	7.0	7.2	7.2	7.3	7.1	3.6	4.0	3.7	3.6	3.9	4.0
F13	6.8	7.5	7.8	7.8	7.9	7.8	3.5	4.3	4.6	4.7	4.7	4.7
F14	6.9	7.0	6.9	6.9	6.9	6.9	2.6	2.9	2.5	2.9	2.8	2.7
G1	6.0	6.5	6.5	6.7	6.6	6.6	2.3	2.8	3.0	2.9	2.8	2.8
G2	6.4	6.8	6.9	7.1	7.0	7.1	2.5	2.9	2.9	2.7	2.7	2.6
G3	6.5	7.2	7.3	7.5	7.1	7.0	2.3	2.9	3.1	2.9	2.9	2.7
G4	6.5	6.9	7.3	7.2	7.3	7.3	3.1	3.7	4.1	3.8	3.6	3.5

Experiment 5 (continued)

	L ₀	L ₁₁	L ₁₇	L ₂₄	L ₂₇	L ₂₉	W ₀	W ₁₁	W ₁₇	W ₂₄	W ₂₇	W ₂₉
G5	6.6	7.0	7.4	7.7	7.8	7.6	2.9	3.4	3.6	3.6	3.5	3.1
G6	7.0	7.2	7.4	7.7	7.8	7.7	3.5	3.8	3.8	4.0	4.1	4.0
G7	7.0	6.9	7.1	7.4	7.3	7.2	3.5	3.9	3.8	3.8	3.9	3.6
G8	6.7	7.5	7.8	8.3	8.4	8.3	2.7	3.8	4.3	4.7	4.6	4.4
G9	7.0	7.3	7.7	8.0	7.9	7.9	3.7	4.7	4.6	4.5	4.6	4.2
G10	7.1	8.0	8.2	8.4	8.4	7.8	2.6	3.8	3.8	3.8	4.0	3.8
G12	6.9	7.0	7.6	7.8	7.7	7.8	3.0	3.9	4.1	4.2	4.1	4.0
G13	7.0	7.0	6.8	6.9	6.8	6.7	3.8	3.8	3.7	3.9	3.6	3.1
G14	6.9	7.2	7.3	7.5	7.3	7.3	3.5	3.1	3.4	3.3	3.3	3.1
G15	7.4	7.6	7.3	7.6	7.6	7.6	3.9	3.8	4.0	3.9	3.6	3.5

	S ₀	S ₁₁	S ₁₇	S ₂₄	S ₂₇	H ₀	H ₁₁	H ₁₇	H ₂₄	H ₂₇
C1	8-9	11	12	13-14	14	.30	.50	.58	.68	.72
C2	---	----	12-13	14-15	14-15	---	---	.58	.82	.92
C4	---	---	14	15-16	16	---	---	.76	1.15	1.30
C5	10-11	12	14	15-16	15	.42	.52	.70	.90	1.05
C6	11-12	15	15	16	16	.60	.85	.78	.94	.94
C7	11-12	15-16	16-17	16-17	17	.55	.85	1.22	1.78	2.00
C8	11	14	15	15-16	15-16	.48	.65	.82	.90	1.00

D2	---	---	13	15-16	16-17	---	---	.72	1.25	1.45
D3	---	---	14	17	17	---	---	.82	1.45	1.95
D4	9-10	13-14	14	15-16	17	.40	.68	.82	1.28	1.46
D5	10-11	13-14	14	14-15	17	.42	.64	.76	1.02	1.25
D6	10-11	15	16	16-17	17	.55	.88	1.10	1.32	1.76
D7	10	12	12-13	13-14	15-16	.42	.50	.58	.72	.95
D8	12-13	14-15	15	16-17	16-17	.72	.90	.98	1.32	1.50

F1	8-9	10	10	10-11	10	.30	.36	.42	.40	.45
F2	10	11	12	11-12	11-12	.38	.42	.45	.48	.45
F3	9-10	11-12	12-13	13-14	13-14	.38	.62	.52	.65	.70
F4	10	12	13	12-13	12-13	.36	.54	.58	.52	.54
F5	10	12	13-14	13-14	13-14	.42	.72	.60	.60	.62
F7	11	14	14-15	15	14-15	.60	.78	.82	.85	.85
F8	11-12	14-15	14-15	14-15	15	.54	.68	.76	.74	.80
F9	11-12	14	15-16	15-16	16-17	.50	.78	.95	1.30	1.50
F10	13	14-15	15-16	15	15	.84	1.05	1.05	1.10	1.05
F11	12	14-15	16	14-15	15	.62	.90	.95	.95	.95
F12	12	16-17	16-17	16-17	16-17	.65	1.10	1.20	1.20	1.24
F13	13	16	16-17	16-17	17	.72	1.35	1.50	1.95	2.32
F14	10	13	13-14	13-14	13-14	.48	.60	.64	.62	.64

G1	9-10	11	12	12-13	13-14	.32	.45	.48	.62	.80
G2	10	12	12	12	13	.45	.48	.48	.60	.75
G3	11	12	12-13	13	14	.50	.58	.62	.76	.90
G4	9-10	12	13-14	14-15	16-17	.35	.58	.66	.95	1.15
G5	11	14	13-14	14-15	15-16	.55	.70	.76	.96	1.10

Experiment 5 (continued)

	S ₀	S ₁₁	S ₁₇	S ₂₄	S ₂₇	H ₀	H ₁₁	H ₁₇	H ₂₄	H ₂₇
G6	10-11	14	14-15	14	15-16	.42	.72	.75	.92	1.02
G7	11-12	14	14-15	14-15	15-16	.54	.75	.78	1.05	1.24
G8	10-11	13-14	15	15-16	16-17	.42	.72	.78	.95	1.12
G9	12	15-16	15-16	16	16-17	.68	.98	1.02	1.24	1.45
G10	11	14-15	16	16-17	17	.60	.78	.92	1.06	1.30
G12	12-13	15-16	16-17	16	17	.65	1.10	1.28	1.34	1.62
G13	10-11	12	12	13	14	.46	.52	.57	.68	.85
G14	11	12-13	13-14	13-14	14-15	.50	.60	.64	.70	.82
G15	12	14-15	15	15-16	17	.60	.80	.95	1.20	1.45

Experiment 6 - 10 July-4 August, 1967

Group A - 0.7% NaCl (thiouracil)

Group B - 10 μ g/day prolactin (thiouracil)

Group C - 0.7 % NaCl

	S ₀	S ₇	S ₁₁	S ₁₅	L ₀	L ₇	L ₁₅	W ₀	W ₇	W ₁₅
A602	15-16	17½	17	17	7.1	7.8	8.3	2.8	3.7	4.1
A603	15-16	17	17½	17½	7.5	7.7	8.0	3.4	3.6	4.0
A604	15-16	16-17	16-17	16-17	7.5	7.9	8.1	3.6	4.0	4.6
A606	16	16-17	16-17	16-17	8.3	8.0	8.6	4.1	4.0	5.3
A607	16-17	17½	17½	17½-18	7.3	7.8	8.4	3.4	3.7	4.3
A608	16-17	18	18	18	8.1	8.2	8.4	3.9	4.2	4.6
A609	16-17	16-17	17	17	8.3	8.6	9.0	4.0	4.5	4.6
A610	16-17	17½	17½	18	8.2	8.4	8.5	4.4	4.7	4.6
A612	17	17½	17	17	8.2	8.7	9.1	4.5	4.9	5.7
B613	15	16-17	16-17	16-17	7.0	7.4	7.8	2.4	2.8	3.6
B614	15-16	17½	18	18	7.6	7.6	8.3	3.1	3.3	4.2
B615	15-16	16-17	16-17	17	7.6	8.4	9.4	3.5	4.4	5.8
B616	15-16	16-17	16-17	16-17	7.8	8.6	8.8	3.7	4.9	5.2
B617	16	16-17	16-17	16-17	7.4	8.0	8.4	3.2	3.6	4.4
B618	16	17½	17½	17½	8.1	9.0	9.8	4.2	5.3	6.2
B619	16-17	18	18	18	7.7	8.4	9.1	3.6	4.2	5.2
B620	16-17	17½	17½	17½-18	8.0	8.8	9.4	3.9	4.4	5.3
B621	16-17	17½	17½	17½-18	7.8	8.5	9.5	4.1	4.7	5.5
B622	16-17	16-17	16-17	16-17	8.2	8.7	9.5	4.4	4.9	6.2
B623	17	18	18	18	8.2	8.8	9.5	4.0	4.6	5.1
B624	17	17½	17½	17½	8.9	9.3	9.4	4.8	5.0	5.5
C637	15-16	16-17	17	18	7.8	8.1	7.8	3.3	3.7	3.4
C638	16-17	18½	20	22	8.8	8.7	6.3	4.9	4.8	3.0
C639	16-17	18	19	21	7.7	8.0	5.3	3.7	3.6	2.1
C640	17	17½	18½	20	7.5	8.1	7.4	3.4	3.3	3.4
C641	17	19	20	23	8.2	8.2	3.7	3.6	4.0	1.8
C642	17	18	18½	21	7.7	8.1	6.2	3.2	3.6	2.8
C644	18	18	20	24	7.6	7.7	2.8	3.7	3.3	1.9
C646	18	19	21	23	8.0	7.9	2.8	4.4	3.8	2.0
C647	18	18½	20	24	7.4	7.4	2.9	3.5	3.3	1.9
C648	18	20	21	23	8.3	8.0	2.9	4.3	3.1	1.9

Table 10 and Figures 16-19 are from this raw data.

Experiment 7 - December 7-21, 1967

Group A - sham TX; 0.7% NaCl Group C - TX; 5 μ g prolactin
 Group B - TX; 0.7% NaCl Group D - TX; 15 μ g prolactin

	W ₀	W ₇	W ₁₄	L ₀	L ₇	L ₁₅	S ₀	S ₁₅
A801	1.4	1.7	2.2	4.9	5.4	5.7	9½	13
A802	1.6	2.1	2.5	5.2	5.8	6.2	9	14
A803	2.0	2.4	2.3	5.5	5.9	6.2	10	15-16
A804	1.8	2.1	2.2	5.7	6.1	6.3	9	14-15
A805	1.1	1.5	1.6	4.7	5.2	5.6	9	11
A806	1.6	2.0	2.1	5.2	5.9	6.2	10	14-15
A808	1.4	2.0	2.1	5.2	5.6	6.0	9	13-14
A809	1.5	1.8	2.2	5.3	5.6	6.0	9	12
A810	1.2	1.8	2.1	4.9	5.7	6.2	9	12-13
A811	1.4	1.4	1.6	4.9	5.4	5.7	8½	12-13
A812	1.5	1.8	1.9	5.1	5.7	5.8	9	12-13
B813	1.0	1.7	2.0	4.6	5.4	5.7	7	7
B814	1.1	1.7	1.9	4.8	5.7	6.2	8	10-11
B815	1.2	1.6	1.6	4.9	5.5	5.7	8½	11
B816	1.4	1.9	2.1	5.3	5.7	6.0	9	12
B818	1.7	2.5	2.9	5.4	6.1	6.6	9½	13
B819	1.8	2.8	2.9	5.3	6.0	6.4	9	11-12
B820	2.0	2.6	2.5	5.9	6.2	6.2	10	15-16
B821	1.1	1.5	1.4	4.6	5.3	5.5	7	10
B823	1.5	2.2	2.4	5.3	6.0	6.4	8	10
B825	2.0	2.0	3.3	5.8	6.7	7.1	10	11-12
B826	1.4	1.4	2.1	4.8	5.7	6.1	9	11
B828	2.1	2.1	2.7	5.9	6.3	6.7	10	12
B829	2.2	2.2	2.8	5.7	6.3	6.5	9	13
C831	1.2	1.9	2.4	4.8	5.8	6.6	7	10
C832	1.2	1.8	2.0	4.7	5.7	6.2	7	8
C834	1.2	2.4	3.0	4.7	6.1	6.6	8	12-13
C835	1.8	2.6	2.8	5.8	6.4	6.7	10	12
C836	1.9	2.9	3.2	5.3	6.4	6.8	9	12
C837	1.0	1.6	1.8	4.4	5.3	5.5	6	7
C838	1.2	1.7	2.2	4.7	5.7	6.2	8½	11-12
C839	1.4	2.3	2.9	5.0	6.2	6.7	8½	12
C840	1.5	1.9	1.9	5.1	5.9	6.2	8	9-10
C841	1.6	2.2	2.4	5.4	5.9	6.3	9	12
C842	2.1	3.1	3.5	5.5	5.7	6.3	10	11
C843	1.4	1.9	2.4	5.1	5.7	6.2	9	10
C844	1.7	2.6	3.1	5.3	6.5	7.0	9	11

Experiment 7 (continued)

	W ₀	W ₇	W ₁₄	L ₀	L ₇	L ₁₅	S ₀	S ₁₅
D845	1.1	1.8	2.0	4.6	5.6	6.1	8	10
D846	1.2	2.2	2.6	4.6	5.9	6.4	9	11
D847	1.3	2.1	2.5	5.1	6.1	6.5	6-7	7
D848	1.4	2.0	2.3	4.8	5.7	6.4	8	10
D849	1.5	2.2	2.8	5.2	5.9	6.3	9-10	10
D850	1.8	2.5	2.7	5.6	6.2	6.7	9-10	12
D851	1.8	2.5	2.9	5.2	6.3	6.9	10	14-15
D852	1.0	1.7	1.9	4.5	5.2	5.8	7	10
D853	1.2	2.3	2.6	5.0	6.0	6.6	7	11-12
D854	1.4	1.9	2.0	4.8	5.6	6.0	6	7
D855	1.5	2.0	2.3	5.0	5.8	6.1	8½	10
D856	1.8	2.3	2.9	5.5	6.4	6.8	9½	11-12
D857	1.3	1.6	1.9	4.9	5.4	5.9	7½	8-9
D858	1.5	2.0	2.2	5.3	5.8	6.2	11	11-12

Table 11 and Figures 20 and 21 are from this raw data.

Experiment 8 - 5 March-2 April, 1968

Group R - TX; 0.7% NaCl

Group V - TX; 10 µg prolactin

	L ₀	L ₄	L ₁₀	L ₁₅	W ₄	W ₁₀	W ₁₅	H ₀	H ₄	H ₁₀	H ₁₅
R1061	6.7	6.8	6.8	6.9	3.0	3.4	3.7	.28	.28	.30	.30
R1062	6.1	6.3	6.4	6.6	2.4	2.4	2.6	.26	.29	.32	.32
R1064	6.7	7.0	7.1	7.0	3.5	3.9	3.6	.42	.42	.46	.50
R1065	6.5	6.7	6.6	6.4	3.1	2.9	2.7	.42	.42	.44	.42
R1066	6.8	6.9	6.8	7.1	3.5	3.5	3.5	.45	.45	.46	.44
R1067	6.1	6.4	6.5	6.5	2.3	2.4	2.5	.46	.45	.45	.52
R1068	6.5	6.7	6.6	6.7	3.6	3.8	4.0	.55	.52	.55	.62
R1069	7.7	7.9	7.9	7.9	4.3	4.6	4.6	.65	.62	.74	.70
R1070	6.8	7.1	7.2	7.2	3.4	3.6	3.5	.55	.60	.62	.68
R1071	6.8	7.0	7.0	7.3	3.1	3.3	3.6	1.30	1.36	1.45	1.45
R1073	6.2	6.5	6.3	6.2	2.9	2.9	2.7	.38	.48	.50	.52
R1074	7.1	7.3	7.4	7.5	4.0	4.2	4.6	.58	.62	.52	.75
V1078	6.0	6.1	6.0	6.4	2.6	2.9	3.0	.32	.34	.32	.40
V1079	5.5	5.5	6.0	6.2	1.9	2.3	2.7	.30	.28	.30	.28
V1080	5.7	6.1	6.5	6.5	2.7	2.7	2.8	.36	.35	.35	.40
V1083	6.8	6.9	7.1	7.4	3.0	3.1	2.9	.45	.48	.46	.50
V1086	6.6	6.6	6.9	6.9	---	---	---	.55	.60	.55	.62
V1088	6.8	6.8	7.1	7.3	3.1	4.0	5.4	.90	.95	1.18	1.20
V1091	7.1	7.4	7.7	7.7	4.5	4.7	5.5	.60	.74	.70	.85

Table 12 and Figures 22-24 are from this raw data.

Experiment 8 (continued)

	S_0	S_4	S_{10}	S_{15}
R1061	9	9	9	9
R1062	8-9	9	9-10	9-10
R1064	11	11-12	12	12
R1065	11	11	12	11
R1066	11-12	12	12	12
R1067	12-13	12	12	12-13
R1068	13-14	12-13	12-13	13
R1069	14	14-15	14-15	14-15
R1070	14-15	13-14	13-14	14
R1071	16-17	16-17	16-17	16-17
R1073	10-11	12	12-13	12
R1074	13-14	13-14	13	15-16
V1078	9	9-10	8-9	9-10
V1079	9-10	10	9	9-10
V1080	10	10	10-11	10-11
V1083	12	12	11-12	12
V1086	13-14	14	12	13-14
V1088	15-16	16-17	16-17	16-17
V1091	14-15	14-15	14-15	14-15

Experiment 9 - December 15-21, 1967

Group B-TX; 0.7% NaCl

Group F-TX; 25 µg GH

Group E-TX; 10 µg GH

	W ₀	W ₆	L ₀	L ₆	S ₀	S ₆
B813	1.7	2.0	5.4	5.7	7	7
B814	1.7	1.9	5.7	6.2	9	10-11
B815	1.6	1.6	5.5	5.7	10	11
B816	1.9	2.1	5.7	6.0	10-11	12
B818	2.5	2.9	6.1	6.6	11-12	13
B819	2.8	2.9	6.0	6.4	10	11-12
B820	2.6	2.5	6.2	6.2	14	15-16
B821	1.5	1.4	5.3	5.5	10	10
B823	2.2	2.4	6.0	6.4	10	10
B825	3.0	3.3	6.7	7.1	12	11-12
B826	2.2	2.1	5.7	6.1	10	11
B828	2.3	2.7	6.3	6.7	10-11	12
B829	2.8	2.8	6.3	6.5	11	13
E860	1.9	2.3	5.5	6.1	8-9	9
E861	1.7	2.0	5.4	5.8	8-9	10
E862	2.0	2.6	5.7	6.2	10	10-11
E863	2.0	2.4	5.5	6.1	10	11-12
E864	2.1	2.4	5.8	6.1	9	10
E865	2.5	2.6	6.1	6.4	14	15-16
E866	2.0	2.1	6.0	6.3	10	10-11
E867	1.4	1.8	5.2	5.8	9	9-10
E868	2.0	2.2	5.7	6.1	10	12-13
E870	2.0	2.1	5.5	5.8	10	10
E871	2.1	2.1	5.7	6.0	12-13	13
E872	1.6	2.1	5.5	5.9	9	9
E873	2.2	2.3	6.0	6.2	12	12-13
E874	1.8	2.1	5.6	5.9	10-11	12
F875	1.5	1.7	5.3	5.5	8-9	10-11
F876	1.4	1.6	5.2	5.5	7	7
F877	2.0	2.6	5.5	6.4	10	12
F878	2.0	2.3	5.9	6.4	9	9-10
F879	2.4	2.7	6.3	6.7	10	10-11
F881	2.8	3.2	6.5	6.8	11-12	13-14
F882	1.5	1.8	5.1	5.7	9	10
F883	2.0	2.6	5.5	6.3	9-10	12
F884	1.7	1.9	5.5	5.8	9	9-10
F885	2.3	2.8	5.8	6.5	9	9-10
F886	2.3	2.5	6.2	6.5	12	13-14
F887	2.1	2.6	5.4	5.9	8-9	9
F888	2.0	2.4	5.9	6.4	9	9
F889	2.8	3.0	6.6	7.2	10-11	12-13

Table 13 and Figures 25 and 26 are from this raw data.

Experiment 10 - January 6-21, 1963

Group R - TX; 0.7% NaCl

Group S - TX; 25 μ g GH

	W_0	W_8	W_{15}	L_0	L_8	L_{15}
R902	1.4	1.7	1.7	5.2	5.6	5.6
R904	1.6	1.8	1.8	5.4	5.8	6.0
R905	2.4	3.0	2.7	6.1	6.4	6.5
R907	2.4	2.8	2.6	6.3	6.8	7.2
R908	2.4	2.7	2.7	6.3	6.8	7.1
R909	3.1		2.4	6.9		6.9
R910	1.9	2.0	2.0	6.1	6.2	6.5
R911	2.0	3.0	3.3	6.3	7.0	7.4
R913	1.6	1.9	2.0	5.7	5.7	5.9
R915	2.3	2.4	2.3	6.2	6.3	6.3
R916	3.2	3.4	3.1	7.0	7.2	7.2

S920	1.1	2.2	2.4	4.8	6.4	6.9
S921	1.6	2.6	2.5	5.5	6.7	7.3
S922	2.7	3.4	3.4	6.3	6.8	6.9
S923	1.8	2.4	2.8	5.6	6.7	7.1
S924	2.7	3.6	3.3	6.4	7.2	7.4
S925	3.3		3.3	6.7	7.2	7.2
S926	3.0	3.4	3.4	6.6	6.8	6.8
S930	2.2	3.0	3.5	6.8	7.1	7.8
S931	2.5	2.8	3.4	6.7	7.2	7.7
S932	2.7	3.2	3.0	6.5	6.8	6.8

Table 14 and Figures 27 and 28 are from this raw data.

Experiment 11 - September 20-December 5, 1967

H - hypophysectomized frogs C- sham hypophysectomized
Force fed

	Initial L	Final L	Initial W	Final W
H 1	44.0 mm	45.0 mm	6.9 gm	7.6 gm
H 7	39.0	39.0	4.7	5.0
H 8	45.5	45.5	6.9	6.85
H10	40.0	41.0	5.7	6.5
H11	44.0	44.5	7.0	7.6
H14	44.0	43.5	5.7	6.1
H19	41.0	42.5	5.7	6.6
H22	39.0	38.5	4.9	5.2
H26	43.0	45.0	6.6	6.9
H27	41.0	42.0	5.2	6.2
H32	41.0	43.0	6.0	6.6
H39	38.0	39.5	4.7	5.5
H41	42.0	43.0	6.9	6.9
H44	44.0	45.0	7.5	8.0
H45	44.0	44.0	7.6	7.5

C50	47.0	48.5	7.8	9.1
C54	40.0	43.0	5.7	6.4
C55	46.0	47.0	6.2	8.2
C67	45.0	46.0	7.6	8.7
C68	44.5	46.0	7.3	8.4
C69	44.0	46.5	6.9	7.6
C70	42.5	44.0	6.4	7.4
C71	44.0	46.0	7.2	8.0
C73	46.5	48.0	8.7	9.0
C74	42.0	44.0	4.1	6.8
C75	45.0	46.0	7.5	9.4
C76	42.0	43.5	6.1	7.3
C78	47.0	48.5	9.3	9.4
C80	40.0	43.0	5.6	6.9
C81	43.5	45.5	6.4	8.5
C83	41.5	43.0	6.5	7.4
C85	42.0	42.0	5.1	6.4

Table 15 and Figures 29 and 30 are from this raw data.

Experiment 12 - October 25-December 11, 1967

H - hypophysectomized frogs C - sham hypophysectomized

Fed live food

	Initial L	Final L	Initial W	Final W
H107	42.0 mm	43.0 mm	6.7 gm	5.9 gm
H108	43.5	43.0	6.0	5.5
H111	43.0	42.0	6.9	6.4
H112	42.5	42.5	5.7	5.6
H117	43.5	43.0	7.4	6.9
H128	46.0	45.5	7.0	7.4
H135	46.0	46.0	6.7	7.1
H136	44.0	44.0	5.3	5.5

C145	43.0	44.0	5.0	7.2
C153	42.0	42.0	5.5	6.6
C157	42.5	43.0	5.6	6.5
C158	40.5	41.0	4.9	7.5
C161	45.0	46.0	6.7	7.6
C162	42.0	43.5	6.4	7.3
C168	46.0	46.0	6.5	7.2
C169	45.0	46.0	7.0	7.0
C170	42.0	42.0	4.3	5.9
C172	44.0	45.0	6.9	8.0
C174	39.0	42.0	4.4	6.4
C176	40.5	41.5	4.6	5.3
C178	42.0	41.5	5.3	6.4
C180	43.0	42.5	5.9	6.2
C181	45.5	46.0	7.0	8.5

Table 16 and Figures 31 and 32 are from this raw data.

Experiment 13 - November 27, 1967-January 24, 1968

Group A - 0.7% NaCl

Group B - 5 and 10 $\mu\text{g/day}$ prolactinGroup C - 25 and 50 $\mu\text{g/day}$ prolactinGroup D - 5 and 10 $\mu\text{g/day}$ GHGroup E - 25 and 50 $\mu\text{g/day}$ GH

	L ₀	L ₃₀	L ₅₆	W ₀	W ₅₆
A 58	46.5mm	47.0 mm	49.0 mm	9.8 gm	9.3 gm
A 65	45.0	45.5	47.5	8.3	8.9
A160	43.0	44.0	44.5	7.4	8.5
A177	41.0	41.5	42.5	5.2	6.4
A201	47.0	47.0	48.0	7.7	9.0
A207	44.0	44.0	46.0	5.9	7.1
A218	44.0	44.5	45.0	6.6	7.4
A219	42.5	42.5	43.0	6.0	7.1
A220	46.0	45.5	46.5	7.9	9.5
A223	40.0	41.0	42.5	5.9	7.6
A261	42.0	42.0	43.0	5.9	7.6

B 66	42.0	42.5	44.5	6.1	7.6
B 62	47.0	48.0	50.0	9.9	10.3
B 49	44.0	44.0	45.0	8.7	7.6
B173	46.0	46.5	47.5	6.7	8.1
B221	43.0	43.0	44.0	5.9	7.2
B225	46.5	47.5	48.0	7.6	8.6
B227	44.5	45.5	47.0	6.4	8.1
B240	43.0	42.5	44.0	6.7	7.8
B242	42.0	42.5	43.5	5.5	7.0
B253	44.5	45.0	45.5	4.8	7.3
B258	42.0	42.5	45.0	4.8	7.3

C200	46.0	46.0	47.5	7.6	8.3
C203	45.0	45.0	47.0	6.7	8.2
C209	44.0	44.0	44.5	6.1	7.0
C213	47.0	47.0	47.0	6.9	7.6
C265	43.5	44.0	46.0	5.5	7.3
C229	44.0	45.0	45.0	7.4	7.3
C232	46.0	47.0	48.0	8.1	10.1
C236	39.5	40.5	42.0	4.8	6.7
C241	40.0	40.0	42.0	5.5	6.9
C251	43.0	43.5	45.0	5.8	7.7
C259	42.5	43.0	44.0	6.0	7.1
C202	42.5	45.0	47.0	6.9	8.6
C204	42.0	43.0	43.5	6.9	7.7
C233	41.0	41.5	43.0	5.7	6.8

Experiment 13 (continued)

	L ₀	L ₃₀	L ₅₆	W ₀	W ₅₆
D205	46.0 mm	47.5 mm	49.0 mm	7.4 gm	9.0 gm
D206	42.5	43.0	45.0	6.0	7.6
D212	44.0	44.0	45.0	6.5	7.8
D230	45.0	45.0	47.5	6.4	8.1
D231	43.0	42.5	44.0	6.1	7.2
D237	44.5	45.5	46.5	6.3	7.8
D244	42.5	42.5	45.0	5.4	8.0
D245	47.0	47.5	48.5	7.1	8.7
D252	40.0	40.0	42.5	4.9	7.0
D254	41.0	41.0	44.0	5.1	7.7
D255	39.0	39.0	41.0	4.3	6.4
D256	43.5	43.5	44.5	6.1	7.1
D243	42.0	42.0	45.0	5.1	7.3

E210	43.5	45.0	46.5	6.6	8.1
E214	40.0	41.0	44.5	3.8	6.9
E215	44.0	45.5	46.5	6.1	7.6
E216	46.0	47.0	47.5	7.9	8.3
E217	42.0	44.0	45.5	5.7	8.1
E234	43.0	44.0	46.5	6.8	9.1
E238	46.0	46.5	47.5	7.6	8.8
E246	42.0	44.0	46.0	5.8	8.0
E248	45.5	46.0	46.5	6.6	8.2
E249	44.0	45.0	47.0	6.7	8.5
E257	41.5	42.5	45.0	5.5	7.9
E260	43.0	44.0	46.5	6.1	7.9
E247	47.0	47.5	48.5	8.3	9.4

Table 17 and Figures 33-37 are from this raw data.

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