

THE INFLUENCE OF THYROID FEEDING UPON
THE BODY WEIGHT OF TADPOLES.

by

Bernice Crawford

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Bennet McAllen.

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There have been many experiments upon the influence of the thyroid gland upon growth and development with which are involved many important questions regarding metabolism, but up to the present time there have been, within our knowledge, no accounts of the effect of thyroid feeding upon body weight.

Gudernatsch ('12 and '14) found that thyroid feeding (giving an excess of thyroid secretion) greatly accelerates the metamorphosis of tadpoles, producing extremely small frogs with all four limbs, with no tail, with the frog type of head and with an alimentary tract greatly reduced in length as compared with the controls of the same age. Frog larvae are good forms with which to work; they are easily handled, require little

attention and the period of larval life offers splendid opportunities for one to note the effect of accelerated metamorphosis, due to thyroid feeding. There is a close relationship between the size and structure of the thyroid gland and the state of general nutrition. The effects of variations of diet upon the thyroid gland have been studied in rats by Chalmers Watson but few studies have been made of the relation of excess thyroid to body weight.

The thyroid gland deserves attention because of the frequency with which disorders of its functions are encountered and the extent to which the extract of the gland has been utilized with such good results. In the secretion the active principle concerned most with metabolism is iodothyronin, a non-protein nitrogenous material.

Since Gudernatch first published his results concerning the effect of thyroid feeding upon frog larvae there have been many experiments.

Morse fed thyroid to larvae of *Rana pipiens* and obtained the same results as Guder-

natsch, as did also Swingle, West, Lenhart, Barthelmez, Allen and others. All these found that the larvae underwent metamorphosis more rapidly upon being fed thyroid and that the frogs produced were much smaller. The reason for repeating the former experiments of thyroid feeding is to throw more light upon the problem of body weight. The questions involved in this experiment are: is the loss in weight due to thyroid feeding caused by the loss of water in the body as a whole, that is the changing of body form squeezing out the fluids of the abdominal cavity or the loss of water in the tissue due to the metabolic process?

Herring found that small doses of thyroid fed daily to white rats had but little effect on the body growth as measured in length but it tended to diminish growth as estimated by body weight.

Burge in 1917 in his paper on "The Effect of Thyroid on Catalase Content of Tissue" states that thyroid increases catalase of the blood and decreases it in the heart and probably also in the

fat and skeletal muscles. This accounts for the increased autolysis of muscular tissues as indicated by the loss of weight and strength. Thus far no work has been found that deals directly with the cause of the loss of the body weight.

Material and Method.

The experiments were begun October 24, 1921 and continued until May 25, 1922. The larvae of *Rana pipiens* and *Rana sphenoccephala* were used in all the experiments. During the winter months the tadpoles did not live long enough to metamorphose, therefore, the results will not be recorded in this paper, although they were a great aid to later work.

Experiment I.

This experiment has been considered in the light of an exploratory experiment, therefore no concrete results will be given.

The first collection of larvae was gathered from a string of shallow pools near the University campus at Lawrence, Kansas. These were *Rana pipiens* of fair size. Two groups of 50 each were selected, one to be used as control and one as experiment. Each group of 50 weighed, in mass, practically 84 grams. The first method of weighing was to remove as much excess water as possible, from the tadpoles by rolling them on cheese cloth and filter paper and placing them on dry paper on the pan of the scales. This weight, it can readily be seen, would not be exactly accurate as water on the body would evaporate during the process of weighing, but as all specimens were weighed under the same conditions the weights may be considered accurate enough. A coin was flipped to determine which should be used for thyroid feeding purposes. Each group of animals was placed in an ordinary kitchen sink and tap water was allowed to run continuously in order to provide fresh water. The sinks were well cleaned twice a week.

Both cultures of tadpoles were kept under uniform conditions of temperature, light and water supply. The first feeding of thyroid began October 24, 1921. Thyroid to be administered was mixed with wheat and alfalfa flour, egg yolk and sufficient water to make a paste. The paste was made of powdered thyroid, obtained from Armour and Company, in the proportion of 1 gram of thyroid to 6 grams of other ingredients, namely 1 gram of alfalfa flour, 4 grams of wheat flour and 1 gram of egg yolk, boiled hard and crumbled fine, and sufficient water to make a creamy paste. The paste was then spread thinly over dinner plates and allowed to dry at room temperature. Pieces of the dried paste were weighed accurately and definite amounts were given daily. Control food was made by mixing 1 gram of alfalfa flour, 4 grams of wheat flour and 1 gram of egg yolk, boiled hard and crumbled fine, with sufficient water to make a thick paste. This paste was spread over dinner plates and allowed to dry at room temperature. The control tadpoles

were fed the same definite amount as the experiment tadpoles. In the first experiments the mortality of the thyroid fed tadpoles was great and in later experiments the percent of thyroid was reduced, the proportion being .5 gram of thyroid to 6 grams of the other ingredients. Each group of this experiment was fed 70 mg of food for several days, then the amount was raised to 100, 120, 170 and 200 mg on October 27, 31, November 7 and 10 respectively. Smaller amounts proved more satisfactory.

The thyroid-fed animals ceased to grow after feeding the preparation and three weeks from the time of the first feeding, differed considerably in appearance from their controls. The head became more elongated, the tail showed indications of involution and limb buds began to appear. The stages of development may be described as follows:

- I. Slight shrinkage of body, showing hind limb buds.
- II. The total length decreased slightly, hind limbs slightly differentiated and head elongated.

III. Hind limbs larger, toes differentiated and fore limbs formed beneath the skin.

IV. Continued decrease in size, fore limbs thru the skin, hind limbs longer and tail shorter.

V. Complete or nearly complete metamorphosis.

Before the animals had reached the third stage they began to die and November 15 the remaining thyroid-fed and control cultures were killed. All specimens were preserved. The mortality was probably due to lack of knowledge of the proper amount of food and to either sewer gas in the refuse pipe of the sinks or a fungus disease retained in the sinks as supply tadpoles, later kept in the same sinks, also died.

Experiment II.

December 5, another series of tadpoles was selected for thyroid feeding. These larvæ were of the same species but somewhat larger than those used in the work just described and their size was obtained by a different method. In

this experiment it was decided to use the displacement method for comparison of sizes. The excess water was removed from the surface of the tadpoles by rolling them on cheese cloth and filter paper. They were then placed in a graduate with a known quantity of water and the amount displaced recorded. Two groups, one displacing 120.8 cc. and the other displacing 113.3 cc. were divided into smaller groups and placed in small glass containers. The group displacing 120.8 cc. was taken for thyroid feeding. Each group was fed an equal portion, by weight, of the prepared foods.

The mortality among the thyroid-fed became very great after the eighth day of feeding, each morning for a week, two or three larvae were found dead in the containers. These were all preserved. The loss then decreased and January 24 both cultures were killed and preserved. As the larvae died the volume, by displacement, was taken and finally the total volume found. The thyroid had decreased in size altho they had not undergone complete metamorphosis while the controls had increased in size.

considerably. Some of the control animals possessed tiny limb buds, though not nearly so large as those of the thyroid-fed group. The animals; after the volume was found, were dried in an electric oven, the contents of the intestines having been removed.

The two groups, at the beginning of the experiment, were not of the same volume in centimeters, the experiment being 120.8 cc. and the controls 113.3 cc. The percent of difference of the two volumes was found by subtracting the latter volume, 113.3 cc. from the former, 120.8 cc. and dividing the difference 7.5 by 113.3. The percent of difference was 6.61%. After the specimens had been dried they were weighed, the thyroid-fed weighing 2.5 grams and the control weighing 5.3 grams. These figures must then be corrected. Altho the first figures were in centimeters and the last in grams; they would, presumably, be in proportion since the specimens were chosen at random. Therefore, if the controls

displaced 6.61 per cent less than the thyroid-fed, the weight of the controls after drying would have to be corrected. This was done by multiplying the percent of difference 6.61% by the control weight and adding the result to that same weight. This gave a percent of loss in weight, due to thyroid-feeding and drying, of 55.7%. This percent shows that the loss of water was not due entirely to the squeezing out of the fluids but also to the metabolic processes.

Experiment III.

May 5, another successful experiment was started. The tadpoles in this series were hatched in the laboratory, the eggs having been procured from New Orleans. This species was *Rana sphenoccephala*. Fourteen animals were used, seven for experiment and seven for control. The average length was four millimeters. Excess water from the surface of these specimens was removed by cheese cloth and filter paper, then they were

placed in a bottle containing a known amount of water and all weighed. The weight of the specimens was then determined and those weighing 1.84 grams were chosen for thyroid feeding and those weighing 1.79 grams for controls. The first feeding of 50 mg. was May 6. The food was the same as that used in the previous work except that the amount of thyroid was decreased one half, the proportions being .5 gram of thyroid to 6 grams other ingredients as stated above.

May 14, the peculiar body shape characteristic of thyroid-fed tadpoles became noticeable, also the limb buds of the hind legs appeared. The controls showed none of these changes. As the thyroid-fed animals died, controls were killed to balance the growth and weight of the two groups. In all cases every specimen was preserved. By May 17 all of the larvae of the thyroid-fed cultures were well along in metamorphosis. Such rapid somatic changes and consequent accelerated metabolism were accompanied by slight mortality among

the animals. May 24 the specimens were killed and preserved. The average body length of the experiment cultures was 3.5 mm. and of the control cultures 5.5 mm. as compared with the initial length of 4 mm. The weight of each of the cultures also diminished, the thyroid-fed weighing .92 grams and the controls 1.435 grams. This shows a loss of weight in both groups. The thyroid-fed animals however, showed a much greater loss than the controls, the initial weights being 1.84 grams and 1.78 respectively. This loss of weight in the controls was probably due to partial starvation and weighing. Great care was taken to keep the amount of food given each group exactly the same. The controls ate the food much more rapidly than the thyroid-fed and would have eaten a greater amount but in order to keep both under the same conditions the controls were fed only the amount the experiment cultures would eat. This caused a partial starvation of the controls and accounts for some of the loss of weight in the growing tadpoles. This starvation was intermittent,

therefore it did not cause a wasting of the body that was outwardly noticeable but caused less weight as probably they had less food in their alimentary tracts. It was also possible for a discrepancy to arise in the weighing as in some cases more water might have been present on the specimens. It is much more difficult to remove excess water from living specimens than from dead ones. The loss in all cases proved to be relatively small.

After the excess water had been removed from the tadpoles, they were weighed, the contents of the intestines removed and the specimens were placed in an electric oven to dry. The question arose as to the method of weighing the killed specimens and after considering two methods, the first, to remove the excess water from the specimens and weigh them directly and the second, to weigh in water, the former was chosen as there was slight difference in the weights procured by the two methods.

The percent of loss of weight due to thyroid-feeding was found as stated above. The thyroid-fed weighed 1.84 grams and the controls 1.79 grams. The percent of difference was 2.78%. The second weighings were corrected and the percent of loss of weight due to thyroid-feeding was 37.32%. After drying, the thyroid-fed weighed .0723 grams and the controls .16 grams. The percent of loss of weight due to thyroid-feeding and drying, as calculated above, was 55.96%. The results show that about ninety percent of the loss of weight was due to water in the tissues and fluids in the abdominal cavity. The percent of solid matter was found by dividing the second weight of the experiment 8.75 grams into the dried weight .9135. This was found to be 10.4%. This leaves 92.17% of water. When the tadpoles began to metamorphose there was a contraction of the body wall and this squeezed out the abdominal fluids. Also during the process of metabolism, water was removed from the tissues.

May 13, two other series of tadpoles were selected for thyroid-feeding. These larvae were

much larger than those used in the preceding experiment and were found in a shallow pool near the University campus. The average body length of these was 6 mm. The species was *Rana pipiens*.

Experiment IV.

One series of four was divided into two lots of two each, one to be fed thyroid and the other to serve as controls. The weight was obtained by the method used in the previous experiment. The tadpoles weighing .97 grams were selected for thyroid-feeding and those weighing 1.29 grams for controls. Feeding of 20 mg. began the same day.

May 16, the typical changes of hyperthyroidism made their appearance in all of the thyroid-fed larvae. The feeding was continued until May 25. Upon this date, the specimens were killed, no mortality having resulted from thyroid-feeding. The tail of each of the experiment cultures was much reduced and the fore and hind limbs were well developed.

The control animals for this series showed n one of these changes but had seemingly increased in length. The weights of both cultures were then taken. The thyroid-fed animals weighed .34 grams and the controls weighed .985 grams as compared with .97 grams and 1.29grams, respectively, initial weights. This showed a loss of weight of .63 grams for the experiments and .305 grams for the controls. The contents of the intestines were removed and the specimens were dried. The final weight of the thyroid-fed animals was .042 and of the controls .084. The difference of the two initial weights .97 experiment and 1.29 control was .32 and the percent of difference was 32.9%. To correct the second weighings, this percent of difference 32.9 was multiplied by the control weight of the second weighing .985 and the result .3240 was subtracted from that weight making the corrected control weight .661 grams. From this weight was subtracted the second experiment culture weight .34 and the result .321 was divided by .661, giving

48.21% the percent of loss of weight due to thyroid feeding. The percent of loss of weight due to drying and thyroid feeding was also computed.

The correction for the thyroid-fed weight after drying was made by multiplying the percent of difference .329 by the experiment .042 and adding .042 to the result 0.0138 obtaining .0558. This result was then subtracted from the control weight, .084 and that result, .0882, divided by .0558 giving 50.52% the percent of loss of weight due to thyroid-feeding and drying. This result was well balanced with that of the former experiments.

Experiment V.

The second series numbered about 75. These were divided into two lots of approximately equal weights, 21.36 grams for thyroid-feeding and 20.11 grams for controls. Feeding began the same day. Food, prepared as for the previous experiment and weighing 50 mg. was given every other day. The larvae of both groups were divided into smaller groups and kept in small glass containers

under exactly the same conditions of heat and light.

May 16, all the body changes characteristic of thyroid-fed larvae were discernable and by May 20, seven days after the first feeding, the posterior limb buds were well developed. The animals also showed shortening of the tail, lengthening of the head and bulging of the eyes. The controls did not change except to seemingly increase in length. By May 23 the hind limbs were well differentiated and the fore limbs had developed, altho they did not show any great differentiation. In order to complete the work the animals were not allowed to undergo complete metamorphosis but were killed May 25. The thyroid-fed and controls weighed 8.75 grams and 16.48 grams respectively, showing a decrease in weight in both but to a much greater degree in the thyroid-fed. The percent of loss of weight due to thyroid-feeding was determined, as in the previous experiments, and was found to be 50%. The specimens were then dried,

after the contents of the intestines had been removed. The thyroid-fed weighed .9135 grams and the controls weighed 1.330. As in previous work the percent of loss of weight due to thyroid feeding and drying was found. This result was 35.32%.

Conclusions.

At the beginning of this paper, it was intimated that the loss of body weight might be due to one of ~~two~~ things, in the metabolic process, the rapid burning up of the tissue or the loss of fluid in the abdominal cavity. By feeding thyroid preparation to frog larvae, a change is brought about in the body within a few days; the animals become reduced in size and begin to metamorphose. Almost complete metamorphosis can, with careful feeding, be produced within a short time of two or three weeks or even a shorter time in the spring. The animals have the characteristics of the frog yet they are greatly reduced in size. The thyroid-fed show a contraction of the body wall due to the change of form in development. This causes a squeezing out of the fluids of the abdominal cavity. There is also a reduction in size due to the loss of water in the tissues. In order to determine the difference in weight resulting

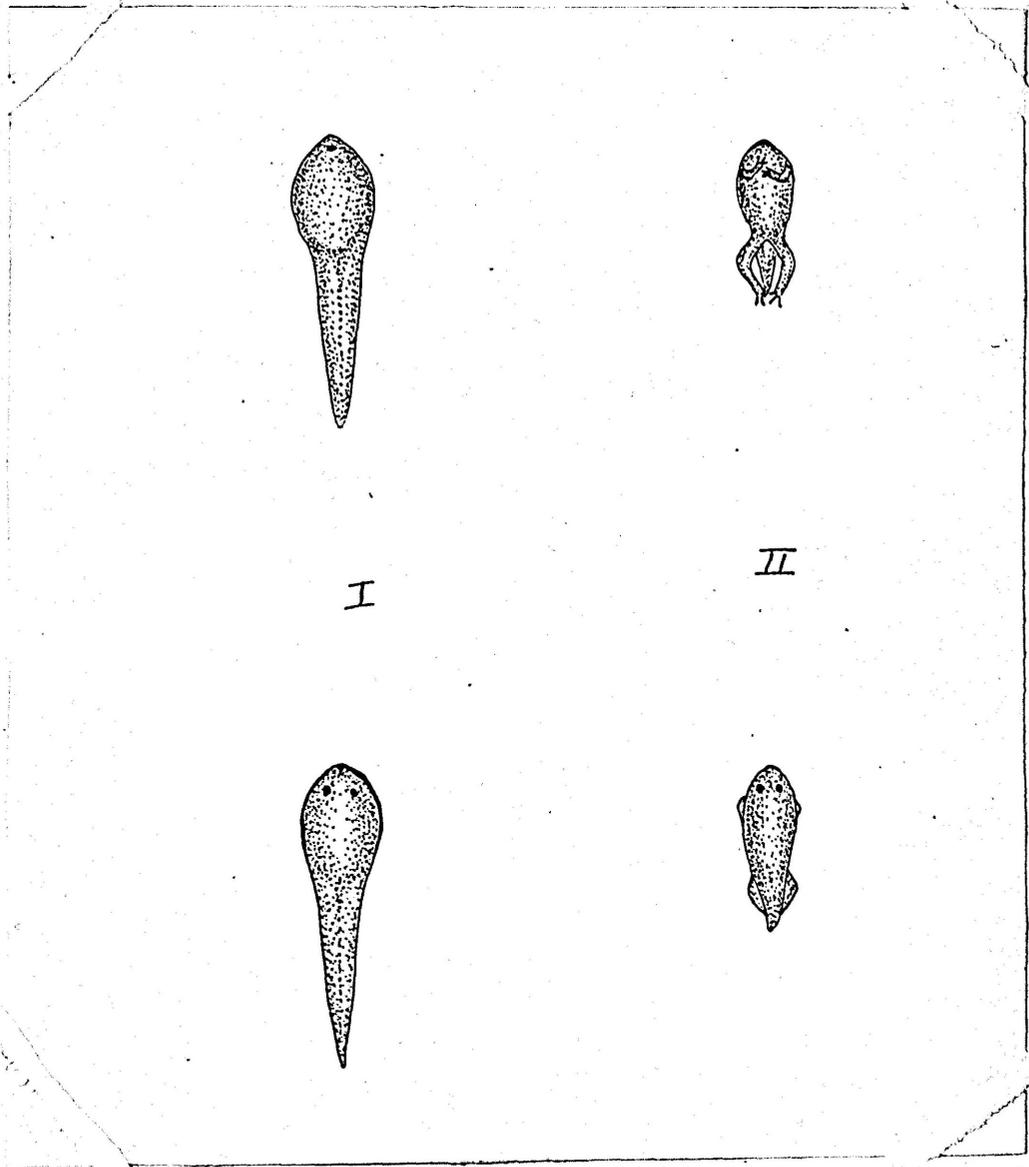
from thyroid feeding the above comparisons have been made. The results obtained in this experiment appear to indicate that hyperthyroidism causes a reduction in weight due to the squeezing out of water and to a greater degree the burning up of the tissue during metabolism.

I wish to take this opportunity of acknowledging my indebtedness to Dr. B. M. Allen of the University of Kansas for suggesting this problem and for the interest he has shown in the work.

Table of Weights and Per cents of Loss.

No. of Exp.	Initial wt.		Second wt.		% loss due to thyroid feeding	Dried wt.		% loss due to thyroid feeding
	Exp. Vol. in cc.	Cont. Vol. in cc.	Exp.	Cont.		Exp. grams	Cont. grams	
II	120.8	113.3				2.5	5.3	55.7
	grams	grams	grams	grams				
III	1.84	1.79	.92	1.43	37.32	.0723	.16	55.96
IV	.97	1.29	.34	.985	48.21	.042	.084	50.22
V	21.36	20.11	8.75	16.48	50.00	.9135	1.330	35.32

No. of Exp.	Date Started	No. Days	Amount fed Each Group	Number specimen	
			milligrams	Exp.	Cont.
II	Dec. 5	50	70	50	50
III	May 5	20	50	7	7
IV	May 13	12	20	2	2
V	May 13	12	50	38	45
Total				97	104



Control and Experiment Tadpoles, Exact Size,
showing decrease in size during metamorphosis.

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