THE INFLUENCE UPON TADPOLES OF FEEDING DESICCATED THYROID GLAND IN VARIABLE AMOUNTS AND OF VARIABLE IODINE CONTENTS.

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PLATE 78.

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In 1912 and 1914 Gudernatsch[1] reported his studies of the variable effects upon the growth and differentiation of tadpoles produced by feeding different kinds of animal tissues, such as thyroid, thymus, muscle, pancreas, liver, testicle, etc. His most striking findings were that thyroid feeding hastened the differentiation of the tadpoles, at the same time inhibiting their growth, so that he was able to obtain pigmy frogs; and that thymus feeding prevented or delayed their differentiation but favored their growth, so that giant tadpoles resulted. He used fresh tissues, and, in the case of the thyroid, without determining the amount of iodine present.

In view of the known relations of iodine to thyroid activity, it seemed probable that the iodine content of the thyroid fed might also modify its effect on tadpoles. With this in view a supply of tadpoles was brought to the Laboratory on May 9, 1914. These tadpoles were of uniform size, and their age was estimated at about one week.

The stock tadpoles were kept in large granite baking dishes. Those used for experimental observations were kept in small granite basins of about 200 cc. capacity, in which were placed a few small stones. The water in all the basins was completely changed twice

[1] Gudernatsch, J. F., Feeding Experiments on Tadpoles. I. The Influence of Specific Organs Given as Food on Growth and Differentiation, *Arch. f. Entwcklngsmechn. d. Organ.*, 1912–13, xxxv, 457; Feeding Experiments on Tadpoles. II. A Further Contribution to the Knowledge of Organs with Internal Secretion, *Am. Jour. Anat.*, 1913–14, xv, 431.

daily. The experimental basins were kept on tables in the middle of a large room, so that all would be exposed to similar light and temperature conditions. The room temperature was recorded every afternoon. After a few changes by way of trial, it was decided to feed the stock with fresh hog's liver every day, while the experimental animals were fed liver and thyroid on alternate days. The liver was cut up into small pieces, but not crushed. In the earlier experiments the liver was put into the basins in the forenoon and left till late in the afternoon, but this was abandoned because on hot days there was evidence of fermentation or putrefaction which led to the death of some of the tadpoles. The plan of allowing the liver to remain in the basins for one hour and then changing the water eliminated this danger even in the hottest weather. The thyroid was fed in the form of dried powder, in each case the iodine content having been previously determined by Dr. Marine. In the earlier experiments ten tadpoles were placed in each dish, with about 200 cc. of city tap water. Later but five tadpoles were placed in this quantity of water. Beyond taking photographs of the several series, no objective measurements were made of the changes produced.

The questions particularly studied may be summarized as follows: First, the effect upon both growth and differentiation of non-thyroid iodine; *e. g.*, potassium iodide and iodalbin (Parke, Davis & Co.). Second, the effect of thyroid feeding; (a) feeding constant amounts of a series of desiccated thyroids containing progressively increasing percentages of iodine; (b) feeding different quantities of one particular thyroid; (c) feeding thyroid obtained from different species of animals. Third, an attempt to counteract the effect of the thyroid feeding by keeping the tadpoles exposed to cold, by the use of cracker dust, quinine, egg white, egg yolk (both cooked and uncooked), and egg yolk extracted with acetone.

It may at once be stated that the effect of the potassium iodide solutions was negative. As to iodalbin, the results were indefinite. The animals showed early tail absorption, and most of them showed some emaciation at the time of their death; but they all developed some disease resembling a general body edema. Iodalbin contains about 21 per cent of iodine so loosely bound that the toxic effects from free iodine had to be considered, and hence these results cannot be accepted as suggesting a thyroid-like action for iodalbin. Since these observations were made Morse[2] has published positive results from artificially iodized proteins, and states that the effect is comparable to that produced by thyroid iodine. Further observations must be made before this can be accepted, since there is no conclusive evidence that artificially iodized proteins exhibit an iodothyreoglobulin-like action.

The effect of thyroid feeding was very marked and closely associated with both the iodine content and the amount fed. The details will be exhibited later.

As to factors protecting against the effect of thyroid feeding, only two were found which were certain in their action; namely, exposure to cold and feeding carbohydrate in the form of cracker dust.

We may now examine more in detail the positive results.

The Effect of Thyroid Feeding.

Preparations of desiccated thyroid from human, canine, sheep, and ox glands were used. The human thyroids were obtained from Dr. Crile's clinic and include simple and exophthalmic goitres. All acted alike qualitatively. The sheep and ox glands available were too few to furnish an extended series of experiments, and may be dismissed from present consideration with the statement that there is no reason to believe that, with the material available, one could not get as gradated a series of results as we shall show can be gotten by the use of desiccated dog thyroid. With human glands a gradated series of effects was obtained, but it was not so sharp as with dog's thyroid. This is to be expected because of so many unknown factors in the life history, treatment, etc. Then, too, some of the thyroids had been in 10 per cent formalin for a day or two before desiccation. As regards the effect of formalin one can only state that it does not destroy the thyroid effect.

As examples of the experimental findings, the following protocols are exhibited.

Series I, Dog Thyroid.—Dishes 1, 2, and 3. The tadpoles in this experiment received 50 mg. respectively of three thyroids whose iodine contents were 0.05,

[2] Morse, M., The Effective Principle in Thyroid Accelerating Involutions in Frog Larvae, *Jour. Biol. Chem.*, 1914, xix, 421.

1.40, and 2.92 mg. of iodine per gm. of dried thyroid. This dosage was given every other day, as in all other experiments, unless otherwise indicated. The feeding was started May 16. No liver was fed in this case after the thyroid was started. The tadpoles in Dish 3 were all dead as early as 15 days, and in Dish 2 in 11 days. These were instances of high iodine contents. Four of the tadpoles in Dish 1, receiving thyroid of low iodine content, were living and active as late as Aug. 3—79 days—when the experiment was terminated. These tadpoles were about the size of the controls, but more differentiated, presenting formed (jointed) hind legs. Those in Dishes 2 and 3 died early, were much emaciated, and only slightly differentiated as compared with the controls.

Series I, Dog Thyroid.—Dishes 4, 5, and 6. This experiment is the exact duplicate of the previous one, except that liver was fed on alternate days. The tadpoles in Dish 6 were all dead in 10 days, and in Dish 5 in 2 days,—instances again of early death after feeding with thyroid of high iodine content. Those in Dish 4, getting low iodine thyroid, showed one tadpole still alive and active after 79 days. The tadpoles in this dish developed functional hind legs, were of large size, and had long, well preserved tails. Compared with the controls they showed no emaciation, but marked differentiation. They were larger than those in Dish 1, perhaps due to the liver feeding. In contrast, the tadpoles in Dishes 5 and 6, getting high iodine thyroid, died early, with much emaciation and before there was time for much differentiation. The emaciation was extreme. They literally melted down, the tails rapidly disappearing.

Series I, Dog Thyroid.—Dishes 7, 8, and 9. Here conditions were the same as in the first and second experiments above, except that the thyroid was given only twice. The tadpoles in Dishes 8 and 9, receiving high iodine thyroid, were all dead in 16 and 10 days, respectively, while those in Dish 7, getting low iodine thyroid, were not all dead till 57 days had passed. Those in Dishes 8 and 9 were the more emaciated. Differentiation was not especially affected in any. This experiment shows that only two doses of thyroid of a certain iodine strength will initiate emaciation and lead to early death, the effect being more marked in the case of thyroids with higher iodine contents. Gudernatsch also observed that one feeding with thyroid was sufficient to induce the emaciation and death.

Series II, Dog Thyroid.—In this experiment a series of thyroids containing respectively 0.05, 0.08, 0.18, 0.54, 0.71, and 1.40 mg. (Figs. 1 to 6) of iodine were fed in 50 mg. doses every other day, beginning May 23. Liver was given on alternate days. As early as four days the series as a whole showed a progressive decrease in size and activity in proportion as the iodine percentage increased. Within five days a most remarkable difference was seen, from large active tadpoles in Dish 1, getting the thyroid of lowest iodine content, to markedly emaciated, inert, and highly metamorphosed tadpoles in the dish getting the highest iodine thyroid. At the end of 72 days there was one tadpole living in each of the first three dishes. All were dead in Dishes 4 and 5 within 19 days, and in Dish 6 all were dead within 11 days. The number of days that intervened before the first tadpole died in each dish of the series ran as follows: 8 (accidental), 52, 33, 17, 9, and 5 days. For the second dead in each dish the figures ran: 54, 54, 35, 19, 11, and 7 days. For the third dead: 59, 68, 41, 15, and 11 days. This clearly shows that the death rate parallels the iodine contents. As to differentiation the notes cannot be given in detail, but by way of summary it may be stated that the tadpoles getting high iodine thyroid (Dishes 4, 5, and 6) emaciated so rapidly and died so soon that little differentiation took place. In 41 days the tadpoles in Dish 1 had formed hind legs and were larger than the controls; *i. e.*, they showed marked differentiation together with growth instead of emaciation. On the same date the tadpoles in Dish 2 showed formed hind legs, but were smaller than those in Dish 1, while those in Dish 3 compared in every way with the controls. The final result then seems to be a balance between the tendency to emaciation and to hastened differentiation, and all degrees of differentiation may be associated with all degrees of size. Gudernatsch's more uniform results were undoubtedly due to using thyroid of more constant or high iodine content.

As previously stated, experiments were also made in which the quantity of a particular thyroid fed was varied; *e. g.*, feeding in 10, 20, 30, 40, and 50 mg. doses of some one particular thyroid. The first experiment of this kind to be reported is Series IV, where a dog thyroid containing 1.40 mg. of iodine per gm. of dried gland was fed beginning June 1. The number of days when all were dead ran, respective to the increasing amounts given, 20, 10, 8, 7, 8. Emaciation was very rapid and marked in all, so much so that in the larger doses there was little time for any differentiation. The tadpoles in Dish 1, on the other hand, getting only 10 mg. of thyroid, proceeded within 14 days to the formation of front and hind legs, large frog mouth, and prominent eyes.

Series VII, Dog Thyroid.—Here as in the previous experiment increasing doses were given of a thyroid containing only 0.54 mg. of iodine. Here the number of days within which all were dead were 32, 37, 37, 39, and 42, not very strikingly different. Emaciation was of little consequence in this series, and death was probably largely due to advanced differentiation, which was hastened in all as compared with the controls.

On the whole, the experiments of varying the quantity of thyroid fed are not nearly as clear cut as those where the iodine percentage was varied. I feel, however, that it is only a matter of obtaining a thyroid of suitable iodine content and arranging the quantities fed in a suitably gradated series, in order to get a well gradated series of effects.

The Protective Effect of Feeding Cracker Dust.

Series VIII, Human Thyroid.—In conjunction with some experiments on the possible inhibiting effect of quinine on metabolism, the following experiment was made. One group was fed cracker dust in addition to the regular liver feeding, and the other liver alone. Both groups received 50 mg. of human thyroid with an iodine content of 2.58 mg. per gm. of dried gland, every second day, beginning June 16. Dates of death were of little importance in this case, as they were mostly due to drowning on account of the high degree of differentiation reached. The tadpoles receiving cracker dust became large and acquired functioning hind legs, front legs, and frog-shaped bodies. Those receiving no cracker dust were smaller, had formed hind legs, but no front legs; on the whole they were more nearly like tadpoles, the first more like frogs. Both sets were larger than the controls.

Series XI and XII received every other day dog thyroid with an iodine content of 1.40 mg. Each series was divided into 5 dishes getting 10, 20, 30, 40, and 50 mg. of thyroid, respectively, beginning June 30. Series XI got cracker dust every second day alternately to the thyroid feeding. Within 8 days the cracker series showed a very distinct progressively increasing emaciation, proportional to the increasing amounts of thyroid fed. This bears out the previous experiments on the effect of variable quantity of thyroid. As early as the sixth day the other group, not getting cracker, showed a marked absorption of tails with decreased activity, while on the seventh day the disparity between the two groups was exceedingly well marked. All the non-cracker group were like small round balls with short conical tails. Owing to the severity of the reaction there was not much difference between the different members of this group. The death dates show a marked shortening of life in the group not receiving cracker. We may conclude then that the feeding of cracker dust delays the tendency of thyroid, when sufficiently active, to hasten death and also tends to prevent emaciation.

The Protective Effect of Exposure to Cold.

Believing that the effects thus far observed were largely due to the well known pharmacological action of thyroid of increasing metabolism, it was thought this action might be lessened by exposing the tadpoles to a lower temperature. Being cold-blooded animals, this would tend to lower their metabolism. With this in mind the following experiments were made.

Series V, Dog Thyroid.—To ten tadpoles kept in a refrigerator were given every second day 50 mg. of a thyroid (dog) containing 1.40 mg. of iodine, which had by previous experiments been shown to have a marked effect. The first dose was given on June 1. All the tadpoles were dead in 27 days, while of the controls kept at room temperature all were dead in 9 days. The controls became markedly emaciated. Those on ice became emaciated toward the end; but earlier, while the controls were still living, they were distinctly larger and less emaciated than the controls. Gradually their tails became absorbed, their bodies smaller, hind and front legs developed along with a frog facies, so that shortly before death they were small but well differentiated; *i. e.*, really pigmy frogs. The controls emaciated so rapidly that there was little differentiation.

Series VIII, Human Thyroid.—These tadpoles were fed cracker dust every second day in addition to the regular liver feeding, and were given every second day 50 mg. of a thyroid containing 2.58 mg. of iodine. Thyroid was started on June 16. One dish was kept on ice, one at room temperature. Of those on ice four were still living at the end of 49 days, when the experiment was terminated, while all of those kept at room temperature were dead in 28 days. Those on ice were of good size, with well preserved tails and slight hind leg buds at the last date of observation, Aug. 3. Those kept at room temperature, compared on the same dates with those on ice, were always larger. Also their differentiation went much further, in that they developed functioning hind legs, front legs, and frog-shaped body and head. And while they died earlier, death was not due to emaciation but to drowning, owing to their complete differentiation.

We may interpret this experiment as follows: At room temperature the stimulation of metabolism by this particular thyroid was not sufficient, in the presence of a more sufficient food supply (cracker), to lead to emaciation, but on the contrary the animals grew large and practically completely differentiated, meeting death by drowning. In the tadpoles kept on ice, metabolism was lowered by the cold so that the tadpoles grew only a little and differentiated only slightly; that is, the stimulating effect of thyroid on metabolism was to some extent counteracted. In the first experiment (Series V) cold protected against the extreme emaciation produced by a certain thyroid at room temperature. In the second experiment (Series VIII) cold tended to counteract the mild stimulation of a certain thyroid which at room temperature led to a high degree of differentiation.

DISCUSSION AND CONCLUSIONS.

We may conclude that the feeding of dried thyroid gland to tadpoles causes an early differentiation in proportion to the quantity fed or the percentage of iodine content of the gland used. With the larger doses and the higher iodine percentages, metabolism is stimulated to such an extent that the animals emaciate rapidly and die early, before there is time for much differentiation. With smaller amounts and lower iodine percentages the size of the animals is roughly inversely proportional to the amount or percentage, so that a close association of differentiation with pigmy size is not characteristic of thyroid feeding as such, as Gudernatsch seems to conclude. One may see early and marked differentiation along with large size. It all seems a question of dosage. The larger sizes are associated with slower differentiation, the smaller sizes with more rapid differentiation, and the smallest sizes may show no differentiation at all, due to the extremely rapid and marked emaciation, and early death. Non-thyroid iodine does not have this effect. The thyroid effect is inhibited by exposure to cold and by cracker feeding. Exposure to cold probably acts by lowering metabolism; cracker feeding, by substituting food other than the animal's own tissues to meet the increased demands caused by the stimulating effect of the thyroid feeding.

Gudernatsch in his earlier paper speaks of the thyroid as stimulating metabolism, which leads to early differentiation and suppresses growth. Later he seems to lean to the view that the thyroid possesses some specific influence on differentiation. It may all be a matter of words, but our present conception is that we are simply dealing with the well known action of thyroid on metabolism. As the iodine content increases, the thyroid increasingly stimulates the metabolism of the tadpole, which undergoes changes in size, increased growth or rapid emaciation, according to the strength of the action. The tadpole being a larval form, the tissues first to be stimulated to increased metabolism, and later the first to be consumed, are naturally those tissues whose normal function is approaching a normal end, and which, in the normal course of events, are about to undergo metamorphosis. Hastening of differentiation seems then to ensue not as a specific stimulation of differentiation, but only to be the normal result of the stimulation of general metabolism. The seeming specificity of the result lies not in a new action of thyroid, but in its application to a living organism at a specific time in its development.

Most important, of course, is the confirmation of what we may be justified in regarding as an established fact; namely, that the activity and potency of the physiologically active substance of the thyroid is measurable in terms of its percentage iodine content.

Finally, it may be pointed out that the reaction of tadpoles to thyroid feeding is so sensitive that the procedure might well serve as a biological test for the activity of thyroid tissue, superior even to chemical methods.

EXPLANATION OF PLATE 78.

FIGS. 1 TO 6. Photographs of Series II, dog thyroid experiments. Experiment begun May 23 and photographed 7 days later. All were fed 50 mg. of thyroid every other day.

No. 1	received	thyroid	containing	0.05	mg.	of	iodine	per	gm.	dried.
" 2	"	"	"	0.08	"	"	"	"	"	"
" 3	"	"	"	0.18	"	"	"	"	"	"
" 4	"	"	"	0.54	"	"	"	"	"	"
" 5	"	"	"	0.71	"	"	"	"	"	"
" 6	"	"	"	1.40	"	"	"	"	"	"

No change is seen in Nos. 1, 2, and 3 because of the short time interval and the low iodine content of thyroid used, while Nos. 4, 5, and 6 show the characteristic increasing effect of thyroid paralleling the iodine content.


Fig. 1.


Fig. 2.


Fig. 3.

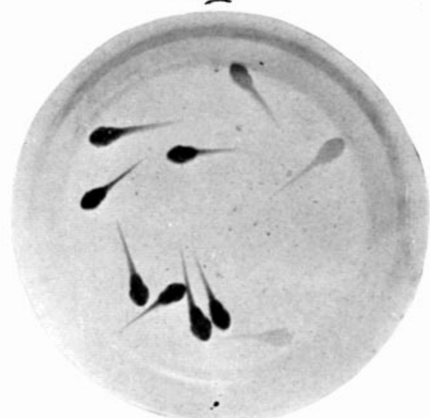
Fig. 4.

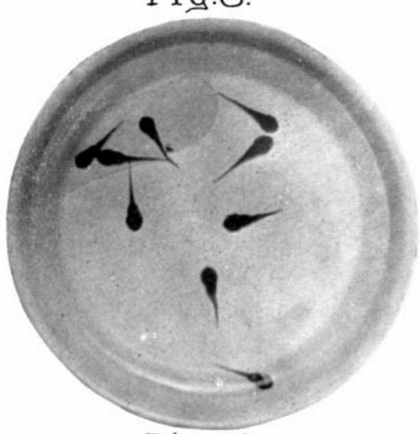
Fig. 5.


Fig. 6.

(Lenhart: Influence of Desiccated Thyroid Gland upon Tadpoles.)