A STUDY OF THE PHYSIOLOGICAL ACTIVITY OF ADENOMATA OF THE THYROID GLAND, IN RELATION TO THEIR IODINE CONTENT, AS EVIDENCED BY FEEDING EXPERIMENTS ON TADPOLES.

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PLATES 38 TO 40.

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In 1912 and 1914 Gudernatsch[1] published the results of his experiments on feeding thyroid and other animal tissues to tadpoles. He concluded that thyroid has the "power to excite differentiation, but it lacks the power to cause growth." He used for these experiments fresh thyroid glands, the iodine content of which was not determined.

In 1914 Lenhart[2] carried out experiments along the same line as regards the thyroid, using for this purpose desiccated human, canine, sheep, and ox thyroids with iodine determinations on each specimen. He states 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," and that thyroids with sufficiently low iodine content caused earlier differentiation but did not interfere materially with growth. "It all seems a question of dosage."

In Lenhart's work non-tumorous thyroid tissue was used. Since it has been established that the action of non-tumorous thyroid on tadpoles is dependent upon the iodine content, the following study was made to determine whether or not the so called tumors (adeno-

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

[2] Lenhart, C. H., The Influence upon Tadpoles of Feeding Desiccated Thyroid Gland in Variable Amounts and of Variable Iodine Contents, *J. Exp. Med.*, 1915, xxii, 739.

mata) of the thyroid, including carcinoma, wou'd have the same action as non-tumorous thyroid and whether this action corresponds to the iodine content of the tumors.

For this purpose tadpoles (*Rana pipiens* and *Rana clamata*) were brought to the laboratory on May 8, 1915. They were fairly uniform in size (10 to 13 mm.) and age (estimated at about 1 week). The stock was kept in the laboratory in large granite basins and fed on fresh liver every day, the water (city tap) being changed once each day.

Thyroid Preparations.—Human thyroids were used in the experiments (Table I). Twenty-one specimens of desiccated thyroid were prepared from eighteen glands removed in Dr. Crile's clinic at Lakeside Hospital. Except in the case of No. 16 the portions of the fresh gland to be used were chopped fine, placed in a drying oven at 70°C. within 1 hour after removal, and allowed to remain in the desiccator from 4 to 6 days; they were then ground to a fine powder in a mortar and kept in sterile bottles until used. Iodine determinations were done on each specimen.[3] No. 16 was prepared from the same gland as No. 15 after the latter had been fixed in formalin. This was done to determine the effect of formalin fixation on the iodine content and the action on tadpoles.

The list of thyroids includes two simple adolescent colloid goiters (Nos. 12, 15, and 16) without tumors or complications; one unclassified gland (No. 3) which probably represents a stage between simple colloid goiter and diffuse colloid adenomatous goiter; and three diffuse colloid adenomatous goiters (Nos. 1, 2, 6, and 17) in which there was a diffuse colloid adenomatous change throughout the whole gland. There were also eleven glands with well encapsulated single or multiple adenomata of the fetal series in various stages of growth and differentiation from the almost pure fetal type to the well differentiated colloid or simple adenoma. Many of these showed some or all of the secondary degenerative changes frequently occurring in these tumors; namely, edema, recent and old hemorrhage, hyaline scars, and areas of calcification and cyst formation. This group includes in the order of their increasing differentiation Nos. 21, 19, 8, 4, 13, 20, 9, 10, 18,

[3] The iodine determinations were done by Dr. Marine.

TABLE I.

Desiccated Thyroid Preparations.

Thyroid No.	Specimen No.	Age.	Duration of goiter.	Clinical diagnosis.	Weight of specimen.	Pathological diagnosis.	Iodine per gm. of dried gland.
		yrs.	*yrs.*		*gm.*		*mg.*
1	11,714	57	20	Goiter, adenomatous.	700	Diffuse colloid adenomatous goiter.	0.22
2	11,714	57	20	" "	700	Diffuse colloid adenomatous goiter.	0.12
3	11,725	26	12	" " colloid, multiple.	735	Unclassified.	0.55
4	11,765	29	16	Goiter, adenomatous.	70	Hyperplastic intermediate adenoma.	0.69
5	11,818	30	18	" "	200	Intermediate adenoma, well differentiated.	1.31
6	11,819	55	31	" colloid.	225	Diffuse colloid adenomatous goiter.	0.43
7	11,821	34	7	" adenomatous.	173	Multiple intermediate adenoma, well differentiated.	0.85
8	11,825	47	15	" "	145	Intermediate adenoma.	0.06
9	11,836	44	14	" "	90	Multiple intermediate adenoma.	0.00 or trace.
10	11,836	44	14	" "	90	Multiple intermediate adenoma.	0.15
11	11,844	61	12	" multiple.	70	Multiple intermediate adenoma, well differentiated.	0.17
12	11,878	21	6	" colloid.	192	Colloid goiter, adolescent type.	0.58
13	11,886	47	40	" adenomatous.	14	Multiple intermediate adenoma.	1.23
14	11,898	58	1½	Adenoma.	273	Carcinoma; malignant adenoma.	0.00 or trace.
15	11,904	18	2	Colloid goiter. Appendicitis.	87	Colloid goiter, adolescent type.	1.00
16	11,904	18	2	Colloid goiter. Appendicitis.	87	Colloid goiter, adolescent type. (Formalin fixation.)	0.85
17	11,932	29	20	Colloid goiter.	710	Diffuse colloid adenomatous goiter.	0.18
18	11,933	55	12	Goiter, adenomatous.	545	Degenerating cystic intermediate adenoma.	0.07
19	11,938	31	16	" "	40	Intermediate adenoma.	0.06
20	11,939	44	⅓	" exophthalmic.	75	Multiple intermediate adenoma, well differentiated.	4.31
21	11,944	27	9	" adenomatous.	250	Fetal adenoma.	0.00 or trace

11, 7, and 5. There was one carcinoma (No. 14) of the malignant adenoma type.

In Nos. 1 and 2, 9 and 10, and 15 and 16, two preparations were made from each of three different glands. No. 20 was diagnosed as exophthalmic goiter, and No. 13 had been treated in the clinic 1 year previously for exophthalmic goiter. The others were diagnosed as simple colloid goiter or simple adenomatous goiter before operation. The carcinoma was not diagnosed as a malignant tumor before operation.

In the preparation of the specimens of adenomata the tumorous tissue was stripped from its capsule, care being taken not to include any of the surrounding non-tumorous thyroid tissue. In the case of the diffuse colloid adenomatous goiters, individual adenomatous nodules were shelled out with their capsules and the total mass was treated as described.

EXPERIMENTAL.

Series A.—May 11 to June 10, 1915 (duration, 31 days). The tadpoles were kept on a table in the center of the room so that light and temperature conditions were the same for all. Temperatures of the room and water were recorded each day.

Five tadpoles were placed in each of twenty-one granite dishes of about 500 cc. capacity, using 300 cc. of city tap water. Four basins with five tadpoles each were used as controls. Sample tadpoles were killed in formalin at the beginning of the experiment for standards of comparison. The tadpoles for experiment were fed 50 mg. of desiccated thyroids, Nos. 1 to 21, every 2nd day, alternating with fresh liver. The controls were fed fresh liver every 2nd day. The tadpoles in this series were not so uniform in size as was desired.

For this series merely the date of death in the different dishes may be given. The action of the different specimens of thyroid was essentially the same as to emaciation, growth, and differentiation as in Series B which is to be described in detail and will serve for both.

The following tabulation in conjunction with Fig. 1 gives a fair idea as to the time of death of the tadpoles and their condition at the time of death or at the termination of the experiment when they were killed in formalin.

Thyroid No.	Iodine.	Result.	Average time of death.
	mg.		*days*
20	4.31	All dead in 14 days.	12.4
5	1.31	" " " 16 "	13.8
13	1.23	" " " 21 "	12.8
15	1.00	" " " 22 "	21
16	0.85	" " " 24 "	22
7	0.85	" " " 19 "	16
4	0.69	" " " 28 "	23
12	0.58	" " " 24 "	22.4
3	0.55	Two " " 26 "; three lived.	31
6	0.43	All " " 29 "	22.8
1	0.22	All alive at end of 31 days.	
17	0.18	One dead in 25 days; four lived.	31
11	0.17	Two " " 29 "; three "	31
10	0.15	All alive at end of 31 days.	
2	0.12		
18	0.07		
8	0.06		
19	0.06	One dead in 30 days; four lived.	31
21	0.00	All but one, No. 14, lived 31 days.	
14	0.00		
9	0.00		
Controls.		Sixteen lived 31 days; no forelegs present. Four died showing no other differentiation than slight growth of the posterior leg buds.	

Definite changes were first noticed in this series on the 6th day in Nos. 20, 5, 13, 15, and 12. There was some wasting, beginning atrophy of the tail, and increased growth of the posterior leg buds. At the time of death all these, as well as Nos. 16, 6, 4, and 7, were smaller than the samples or the controls and showed much more differentiation than the controls.

As to the appearance of forelegs it is noteworthy that in the tadpoles in No. 20, four of which died in 12 days, all had visible left foreleg buds. In No. 5 all died between the 14th and 16th days and all showed foreleg buds. In No. 13 two died in 10 days with foreleg buds scarcely visible; forelegs were present on the other three. In No. 15 all died between the 20th and 22nd days; foreleg buds were present on two and doubtful on the other three.

Others in this series developed forelegs at different times corresponding closely in this and other respects with Series B. It is interesting to point out, however, that the large percentage of tadpoles living 31

days were being fed on thyroids with iodine contents of 0.22 mg. or less. It is also interesting that all these latter grew as much or more than the controls, and most of them showed a greater degree of differentiation.

Series B.—May 11 to June 28, 1915 (duration, 49 days). The procedure was the same as that in Series A, but smaller, white porcelain dishes with 150 cc. of water were used. The tadpoles in this series were quite uniform in size.

Table II and Fig. 2 give the results in this series, using the time of appearance of the first foreleg as an index to differentiation. This we think is justifiable because with the most active thyroid preparations used, and even in those tadpoles dying as early as 8 days this evidence of differentiation was present. It also offers a convenient

TABLE II.

Series B, Arranged According to Iodine Content.

Thyroid No.	Iodine content.	Time of appearance of first foreleg.	Average time of appearance of first foreleg.	Average time of death or killing.
	mg.	*days*	*days*	*days*
20	4.31	12	12.8	12.8
5	1.31	12	12.6	12.4
13	1.23	14	17.2	17.2
15	1.00	16	21.7	23.2
16	0.85	21	25.6	25.0
7	0.85	22	23.6	24.4
4	0.69	8	19.6	20.6
12	0.58	25	25.0	28.4
3	0.55	25	32.0	38.4
6	0.43	31	37.8	39.4
1	0.22	34	42.2	45.2
17	0.18	38	40.0	42.0
11	0.17	34	43.7	42.8
10	0.15	38	40.0	45.0
2	0.12	41	41.0	29.0
18	0.07	36	38.0	33.0
8	0.06	40	44.5	47.0
19	0.06	40	41.6	39.4
21	0.00 or trace.	40	46.4	48.2
14	0.00 " "	37	45.6	46.4
9	0.00 " "	40	43.2	45.0
Controls.....		45		

and useful method of comparison in determining the action of desiccated thyroid in causing differentiation of tadpoles. This is usually a fairly decisive indicator and may be readily recognized within a period of 24 hours of its occurrence.

In comparing Series A and B it is interesting to note that in Nos. 20, 5, 13, 15, 16, 7, 4, and 12, with iodine contents varying from 4.31 to 0.58 mg., all the tadpoles were dead in each series at the end of 31 days, the duration of Series A. In No. 3 (iodine content, 0.55

TABLE III.

Series B, Arranged According to Effects Observed on the 14th Day of the Experiment.

Thyroid No.	Iodine content.	Time of appearance of first foreleg.	Average time of appearance of first foreleg.	Average time of death or killing.
	mg.	*days*	*days*	*days*
20 5 13	4.31–1.23	12–14	12.6–17.2	12.4–17.2
15	1.00	16	21.7	23.2
16 7 4 12 3 6	0.85–0.43	21–31 (No. 4 in 8 days.)	19.6–37.8	24.4–39.4 (No. 4 in 20.6 days.)
1 17 11 10 2	0.22–0.12	34–41	40–43.7	42–45.2 (No. 2 in 29 days.)
18 8 19	0.07–0.06	36–40	38–44.5	33–47
21 14 9	Trace to 0.00	37–40	43.2–46.4	45–48.2
Controls		45		

mg.), two tadpoles in Series A and one in Series B were dead. In No. 6 (iodine content, 0.43 mg.) all were dead in Series A but only one in Series B. Below this level of iodine content (0.22 to 0.00 or trace) 91 per cent of each series were still living at the end of 31 days. It is evident then that a detailed description of the results in Series B will suffice for both within the time limits of 31 days. Series B is chosen for detailed study because of its longer duration (49 days).

The action of the desiccated thyroid was striking and consistent. On the 14th day of the experiment, before the iodine contents of the different thyroid preparations had been determined, the tadpoles were grouped according to the effect and the relative iodine contents of the different preparations predicted upon this basis. The prediction was correct with iodine contents from 4.31 down to 0.43 mg. in the order given in Table III. Below the level of 0.22 individual distinctions could not be made out with certainty, but all in this group were less affected than those receiving specimens with higher iodine contents.

Protocols.

Thyroid 20.—Changes were first observed in this dish on the 5th day; that is, after the second dose of thyroid. The change consisted of beginning kite-shaped appearance, prominence of the head, diminution in size or wasting, beginning atrophy of the tail, and increased growth of the hind leg buds. Three of these died on the 12th day and two on the 14th. All showed the characteristic changes and all at the time of death had visible left foreleg buds; all were smaller than the samples or controls. One became edematous.

Thyroid 5.—The changes, first noticed on the 5th day, were the same as in No. 20. Four died on the 12th day and one on the 14th, showing marked wasting and tail atrophy; three had a frog-shaped body and two were definitely kite-shaped. At the time of death left foreleg buds were visible on three and not observed on two; all were smaller than the samples or controls; two were somewhat edematous.

Thyroid 13.—The changes, first observed on the 6th day, were similar to those in Nos. 20 and 5. One died on the 14th day, two on the 16th, and two on the 20th. The left foreleg was present on each. One dying on the 20th day had both foreleg buds. All were smaller than the samples or controls; four were somewhat edematous.

Thyroid 15.—Changes similar to the above were observed on the 6th day. All showed marked wasting, tail atrophy, and considerable differentiation. These died on the 16th, 23rd, 24th, 25th, and 28th days, respectively. Two had left foreleg buds present at the time of death; the one dying on the 28th day had

both forelegs and the body was beginning to present the typical frog shape. One in this dish was lost.

Thyroid 16.—Changes were first observed on the 7th day. One died on the 21st day, 2 in 24 days, and 2 in 28 days. The left foreleg was present on three; not observed on two. All in this dish were quite edematous; all showed typical head changes and some were assuming frog-shaped bodies.

Thyroid 7.—Changes were first observed on the 7th day. Three died in 22 days, and two in 28 days. The left foreleg bud was present on all; all showed typical changes and were assuming frog bodies; slight edema in all.

Thyroid 4.—Slight change of shape was first observed on the 7th day. These tadpoles died in 8, 22, 23, 24, and 26 days. All were smaller than the controls; four were edematous. They showed various degrees of tail atrophy and were slightly kite-shaped. The three dying in 8, 24, and 26 days, respectively, had left foreleg buds. None were observed on the other two.

Thyroid 12.—Slight change of shape was first observed on the 7th day. One died in 26 days, two in 28, and two in 30. The first was highly edematous and showed no differentiation beyond the controls; no foreleg bud. The others were small and had frog-like bodies; the left foreleg bud was present on each; tail atrophy one-half to two-thirds.

Thyroid 3.—A slight change of shape was first observed on the 8th day. One died in 28 days, one in 36, two in 40, and one in 48. The first had a tadpole body, no tail atrophy, posterior leg buds about the same as the controls and no forelegs, and was highly edematous. The second had slight tail atrophy and the left foreleg bud through the skin; it was smaller than the control. The other three had frog-like bodies; both forelegs were present on each; tail atrophy one-third to two-thirds.

Thyroid 6.—In this dish it was doubtful whether definite changes could be made out on the 8th day. The tadpoles died in 31, 36, 39, 42, and 49 days. At the time of death all had left forelegs; the third and fourth had both forelegs, and on the fifth the right foreleg was present under the skin. Three had typical frog-shaped bodies and two were slightly frog-shaped.

Thyroid 1.—No change was observed up to the 8th day. Four died in 40, 43, 45, and 49 days, respectively. All were well differentiated frogs with almost complete atrophy of the tail. One which lived 49 days had a tadpole body and no forelegs. This is the first tadpole of the series fed on thyroid with iodine of 0.22 mg. or above which lived the 49 days of the experiment. Above this level of iodine content the tadpoles showed progressive degrees of wasting, and early differentiation in proportion to the iodine content. In none of these did the thyroid-fed tadpoles keep up with the controls as to growth (size), but of course they showed greater differentiation. Below this level of iodine content the thyroid-fed tadpoles grew as well as, and in many cases better than the controls (growth inversely proportional to the iodine content). At the same time there was a greater degree of differentiation among the low iodine-fed tadpoles as compared with the controls.

Thyroid 17.—In the frogs in this dish forelegs appeared in 38, 39, 40, 41, and 42 days. The first four developed into normal frogs with well developed and functioning fore- and hind legs and were killed in formalin on the 41st day. The last one developed into a normal frog and was killed on the 46th day.

Thyroid 11.—One died in 34 days, with beginning atrophy of the tail, slight changes about the head, and no forelegs. This one was smaller than the controls. The other four developed forelegs in 34, 39, 46, and 46 days, respectively. Two of these were killed in 42 days, and the last two died on the 48th day. All four were well developed frogs.

Thyroid 10.—Forelegs appeared in 38, 39, and 43 days on three. These developed into normal frogs; one died on the 40th day and two were killed on the 43rd and 46th days. The other two became highly edematous, showed no greater differentiation than the controls, and were killed on the 48th day.

Thyroid 2.—In this dish the tadpoles died in 11, 22, 34, 37, and 41 days. The first three maintained the tadpole body, had no atrophy of the tail, and developed no forelegs. Some of these were counted accidental deaths and not attributed to thyroid action. The fourth, dead on the 37th day, was becoming frog-shaped; no tail atrophy; no forelegs. The fifth was the only one that developed a foreleg (41st day). This one had a well developed frog-shaped body; tail atrophy about one-half.

Thyroid 18.—One of these was lost; two died in 15 and 35 days; and two lived 41 days and were killed in formalin. The first (15 days) showed little or no differentiation beyond the controls; no forelegs. The second (35 days) had well developed hind legs and was assuming a frog-shaped body; no forelegs or tail atrophy. The third and fourth developed forelegs in 36 and 40 days, respectively, and were killed on the 40th day. One of these was a well developed frog with almost complete atrophy of the tail; the other developed good fore- and hind legs, with little atrophy of the tail, and was highly edematous.

Thyroid 8.—Four developed forelegs in 40, 42, 48, and 48 days, respectively. One, which lived 49 days, had a tadpole body, no tail atrophy, and no forelegs. The first three developed into normal frogs with functioning fore- and hind legs. The fourth had both forelegs but a tadpole body and little atrophy of the tail. The fourth and fifth, both having a tadpole body, were larger than the controls.

Thyroid 19.—Two died in 28 and 35 days, respectively, without forelegs. Tail atrophy had begun, one having a tadpole body, the other becoming frog-shaped. Two developed forelegs in 40 days, became normal frogs, and were killed on the 43rd day. The last one developed forelegs on the 45th day, and when killed on the 48th day was fairly well differentiated.

Thyroid 21.—Forelegs appeared in 40, 46, 48, 49, and 49 days, respectively. The first developed into a normal frog and was killed on the 45th day. The other four lived 49 days; two of these had well developed frog-shaped bodies, tail atrophy one-third, and functioning fore- and hind legs; one had the left foreleg; and the last one had the left foreleg under the skin.

Thyroid 14.—Forelegs appeared in 37, 45, 48, 49, and 49 days, respectively. The first died in 37 days, with a tadpole body, slight changes about the head, and the left foreleg under the skin; it was about the size of the controls. The second developed into a normal frog with functioning fore- and hind legs. The third had a well developed frog body, tail atrophy one-third, and the left foreleg present. The fourth and fifth were becoming frog-shaped, had slight tail atrophy, and left forelegs under the skin.

Thyroid 9.—Four developed forelegs in 40, 40, 45, and 48 days, respectively. These were well differentiated, had functioning fore- and hind legs, and various degrees of tail atrophy. The fifth was killed on the 43rd day; highly edematous.

Controls.—There were four dishes with five tadpoles in each. These received fresh liver every 2nd day. The first one developed the left foreleg on the 45th day and died on the 48th day, a well differentiated frog. On the 49th day there were four dead, one with well developed hind legs and a left foreleg, also beginning tail atrophy; one with well developed hind legs and no forelegs; the other two were well preserved tadpoles with short posterior leg buds, no forelegs, and no tail atrophy. Fifteen lived for 49 days and were well preserved tadpoles.

Fig. 2 shows the condition of the tadpoles in this series at the time of death or termination of the experiment when they were killed in formalin. The different groups are arranged according to the decreasing iodine content with the controls and samples last. The individuals of each dish are arranged from left to right according to the time of death; *e.g.*, in No. 20 the first three from left to right died in 12 days and the fourth and fifth died in 14 days.

We have no satisfactory explanation for the peculiar edematous appearance of some of the tadpoles. This was observed in individuals in different dishes without regard to the iodine content and appeared in some of the controls.

Series C and D were for the purpose of determining the action of desiccated thyroid on a different variety of tadpoles and at varying ages of this species (*Rana catesbiana*).

Series C.—May 25 to June 10, 1915 (duration, 17 days). (Fig. 3.)

Tadpoles averaging 4 cm. in length were used in this series. Three were killed in formalin at the beginning of the experiment for comparison; three were used as controls and fed fresh liver every 2nd day; the experimental tadpoles were fed 50 mg. of Thyroids 20, 15, 12, 17, and 14 with iodine contents of 4.31, 1.0, 0.58, 0.18, and 0.0, or trace, every 2nd day, alternating with fresh liver. At the beginning of the experiment the tadpoles had an average length of 4 cm., just visible posterior leg buds, tadpole bodies, and no sign of forelegs.

The controls all lived 17 days and were killed in formalin. They had an aver-

age length of 4.3 cm., maintained the tadpole body, and showed slight increase in the posterior leg buds as compared with the samples.

Thyroid 14.—All lived 17 days and were killed in formalin, had an average length of 4.2 cm., tadpole bodies, slightly better growth of the posterior leg buds than the controls, and no forelegs.

Thyroid 17.—All these were lost.

Thyroid 12.—One was lost. One died in 12 days, showing marked wasting and tail atrophy; considerable growth of the hind legs; left foreleg bud present; beginning frog-shaped body; length 2.5 cm. The third lived 17 days and was killed in formalin. This one was 3 cm. in length; the body was becoming frog-shaped; left foreleg bud present; tail atrophy about one-third.

Thyroid 15.—Two died in 11 days. Each measured 2.3 cm.; there was marked atrophy of the tail; posterior leg buds 5 mm. long; left foreleg bud present on one and not on the other; heads frog-shaped. The third died in 12 days; length 2.2 cm.; left foreleg present.

Thyroid 20.—All died in 10 days, measuring 2, 2.2, and 2.2 cm., respectively. All showed marked tail atrophy; left foreleg present on each; bodies becoming fairly frog-like.

This series shows that there is no qualitative difference in the action of desiccated thyroid on the different varieties of tadpoles used.

Series D.—May 27 to June 7, 1915 (duration, 12 days). (Fig. 4.)

For this experiment two sets of tadpoles of different ages with three in each set were used. The individuals of each age were alike as to size and condition of development. The older ones averaged 8.5 cm. in length, had posterior leg buds slightly over 2 cm. long, and had no forelegs. The younger set averaged 6.5 cm. in length, had just visible posterior leg buds and no foreleg buds. One of each age was killed in formalin at the beginning of the experiment for comparison. Another of each age was used for control and fed fresh liver every 2nd day. A third one of each age was fed 50 mg. of Thyroid 20 every 2nd day, alternating with fresh liver.

Both forelegs were present on the older thyroid-fed tadpole on the 7th day; the left foreleg appeared on the corresponding control on the 9th day. There was marked atrophy of the tail of the thyroid-fed tadpoles. The younger control had no forelegs; and the posterior leg buds were 8 mm. long; it maintained a tadpole body. The younger thyroid-fed animal had a frog-shaped head and body; left foreleg present; posterior legs 15 mm. long. The older tadpoles at the end of the experiment showed about the same amount of development of fore- and hind legs on both the controls and the thyroid-fed animal. There was marked tail atrophy in the latter and practically none in the former. The older thyroid-fed tadpole had become a well developed frog, while the corresponding control had the same characteristics to a slighter degree.

Comparison of samples, controls, and Thyroid 20 in Series C and D shows that desiccated thyroid of high iodine content administered to tadpoles of the same variety at different ages and stages of development produces the same effect; namely, immediate cessation of growth, rapid metamorphosis as evidenced by atrophy of the tail, increased growth of the posterior legs, and the development of forelegs. The younger the tadpoles at the beginning of thyroid feeding, the smaller the metamorphosed frog, and *vice versa*.

DISCUSSION AND CONCLUSIONS.

It seems evident from the foregoing experiments that the so called tumors (adenomata) of the thyroid possess the property of taking up iodine and metabolizing it into the active combination in the same way that the non-tumorous thyroid tissue does, although not so readily nor to the same degree, and the action on tadpoles of feeding desiccated tumorous thyroid tissue does not differ qualitatively from feeding desiccated non-tumorous thyroid tissue. The action in either case depends upon the iodine (active iodine) content, and in the case of the adenomata bears no constant relation to the state of their growth or differentiation.

Examination of Tables II and III shows that in the main this is true. There are, however, certain discrepancies as to time of death, appearance of first forelegs, degree of emaciation, and rate of growth in certain dishes of the series, the action being not quite parallel to the iodine content. Some of these discrepancies may be explained in part by accidents of feeding, slight differences in size, age, and susceptibility of the different tadpoles receiving the same thyroid, and also by the variations in the amount of thyroid consumed by the different individuals in the same dish. Lenhart has shown that the action of the same thyroid varies with the quantity fed. Another important factor which has to be considered is the condition of the iodine itself. It was suspected at the time of these experiments that the iodine might be present in an active and an inactive form, but no satisfactory proof of this assumption, at the beginning of these experiments, was at hand. Support of this point has been afforded by the work of Kendall on the isolation of the active principle of thyroid and the separation of the iodine into two fractions. Since the com-

pletion of our experiments Marine[4] has demonstrated by means of perfusion experiments *in vivo* and *in vitro* that iodine is rapidly taken up by the thyroid cells, and though the iodine increase in the perfused lobe may be 1,000 per cent in 2 hours as compared with the control lobe, yet the action on tadpoles is no greater. It then becomes an important question to determine the time required by the thyroid to take up inorganic iodine and manufacture it into the active thyroid principle.

It is known that iodine is rapidly taken up by the thyroid, and in man the iodine content of the thyroid is subject to greater variations than in animals on account of the prevalent therapeutic use of iodine and the iodides in goiter and other conditions; even the iodine used in preparing patients for operations would increase the iodine content of the thyroid in a short time, so that one might expect such variations in the action of a given thyroid preparation fed to tadpoles as appear in these experiments.

In this connection it is interesting to note (Table II) that Thyroid 20 with 4.31 mg. of iodine was only slightly more active than No. 5 with 1.31 mg. of iodine. Two possibilities have to be considered here. First, No. 20 may have active iodine slightly greater than 1.31 mg. and the balance present as inactive iodine. Second, No. 5 with 1.31 mg. of iodine might represent the maximum possible effect under the conditions of the experiment and a larger quantity of active thyroid iodine could produce no greater effect.

Of course with the lower iodine contents the variations in effects might well come within the limits of errors of observation. Also the percentage error would be greater in the iodine determinations, accidents of feeding, etc.

Our conclusions as to the effect of feeding desiccated thyroid to tadpoles agree in general with those of Lenhart. The action of the thyroid depends not upon a specific stimulus to differentiation but upon a stimulation of metabolism in general in proportion to the active iodine and the quantity consumed. High iodine contents produce

[4] Marine, D., Demonstration *in Vitro* of the Specific Affinity of Thyroid Cells for Iodin, *Proc. Soc. Exp. Biol. and Med.*, 1915, xii, 132. Marine, D., and Feiss, H. O., The Absorption of Potassium Iodid by Perfused Thyroid Glands and Some of the Factors Modifying It, *J. Pharm. and Exp. Therap.*, 1915, vii, 557.

rapid emaciation, at the same time resulting in differentiation even in tadpoles dying in 8 to 12 days. Low iodine contents result in differentiation at an earlier period than the controls. Tadpoles fed on thyroid with practically no iodine grow better than the controls, in this instance the thyroid acting simply as a food.

Finally, the interest that the results of these experiments may have in connection with the question of function in tumor tissue should be pointed out. To those who hold that tumor lacks the capacity for physiological function, the adenomata of the thyroid could not be consistently regarded as tumors. To those who hold physiological function as a possible property of tumor tissue, the adenomata might be regarded as tumors. Future studies might warrant a recognition of different grades or degrees of tumor. On this basis the fetal adenoma (very little differentiation) might represent a higher degree of tumor than the diffuse colloid or simple adenomatous thyroid in which the adenomatous nodules are present to a great extent throughout the whole gland and are well differentiated. It is certain that there are all grades and degrees of growth and differentiation in the life history of fetal adenomata of the thyroid, from the pure fetal, undifferentiated adenoma with little or no iodine to the simple or colloid adenoma, well differentiated and with varying amounts of iodine approaching that of normal thyroid.

EXPLANATION OF PLATES.

PLATE 38.

FIG. 1. Series A. The condition of the tadpoles at the time of death or after they had been killed in formalin, at the end of 31 days.

PLATE 39.

FIG. 2. Series B. The condition of tadpoles at the time of death or after they had been killed in formalin, at the end of 49 days.

PLATE 40.

FIG. 3. Series C. The effect of desiccated thyroid on *R. catesbiana* tadpoles as compared with the larvæ of *R. pipiens* and *R. clamata.*

FIG. 4. Series D. The effect of desiccated thyroid on tadpoles of different ages.

Series A.

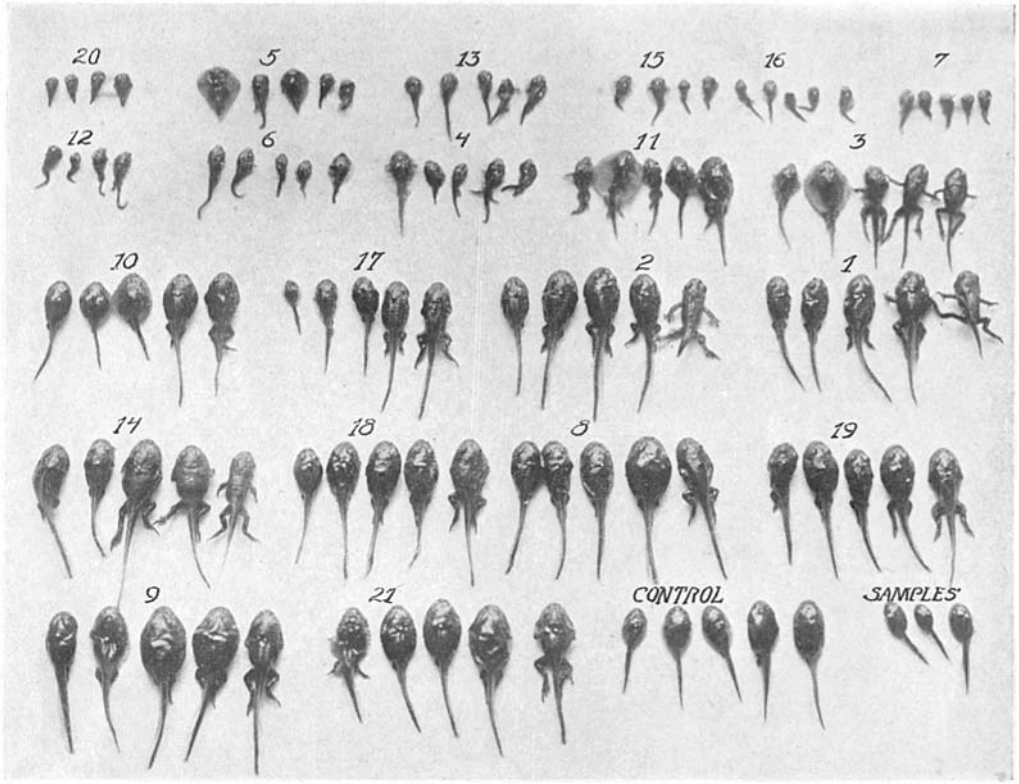

FIG. 1.

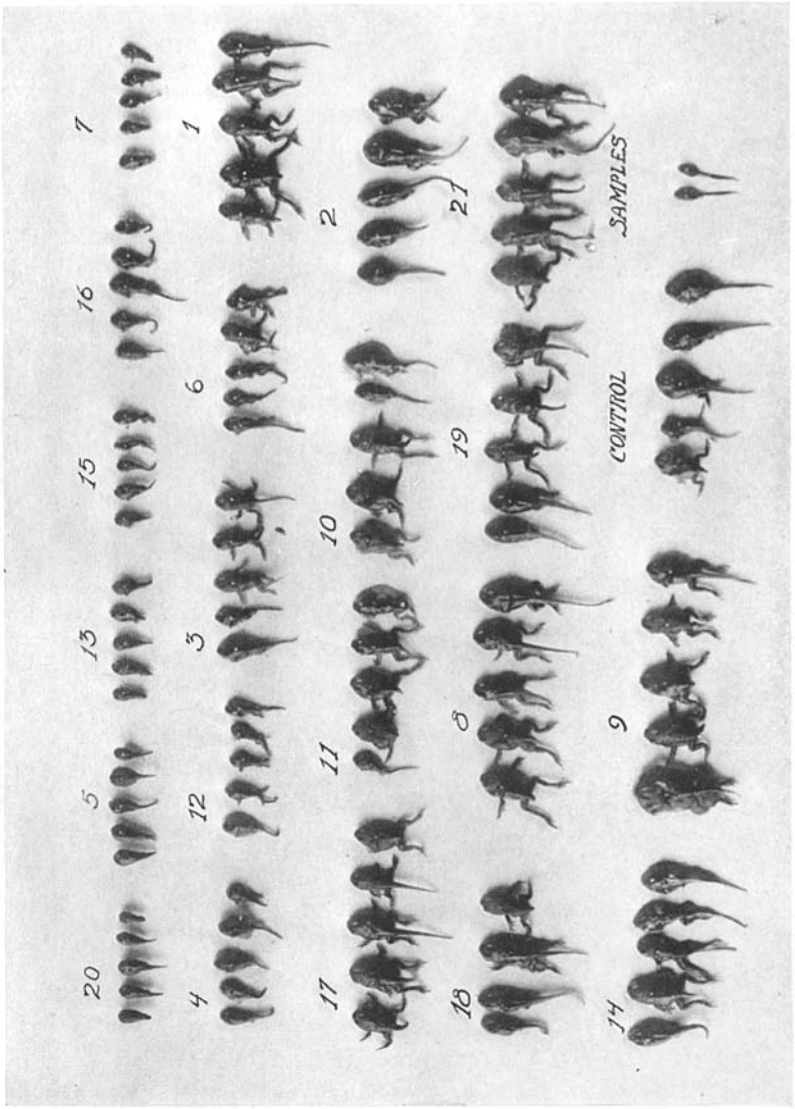

FIG. 2.

(Graham: Adenomata of the Thyroid Gland.)

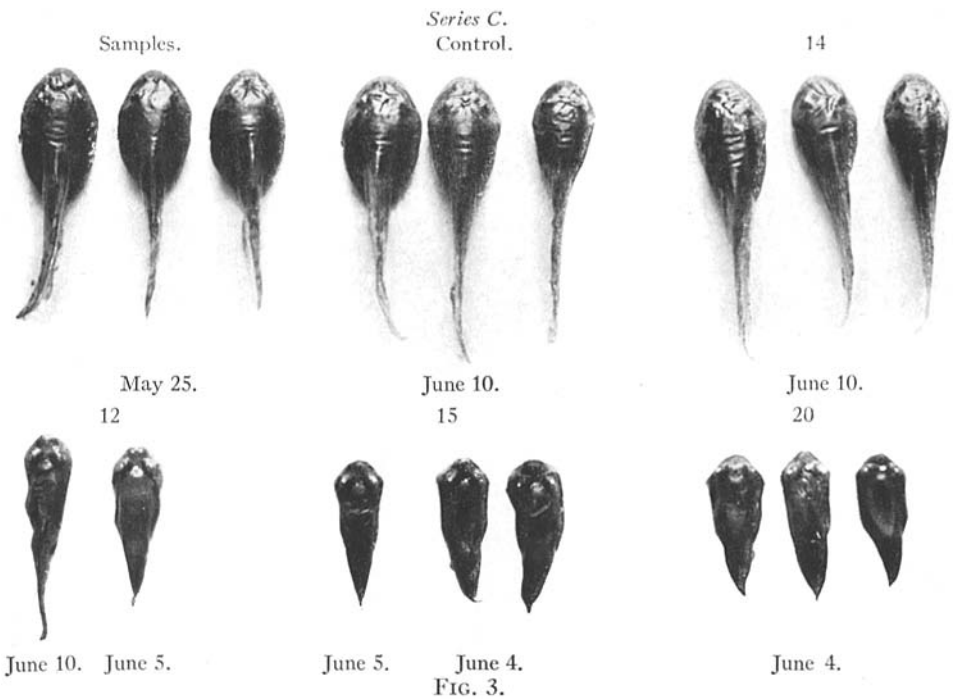

FIG. 3.

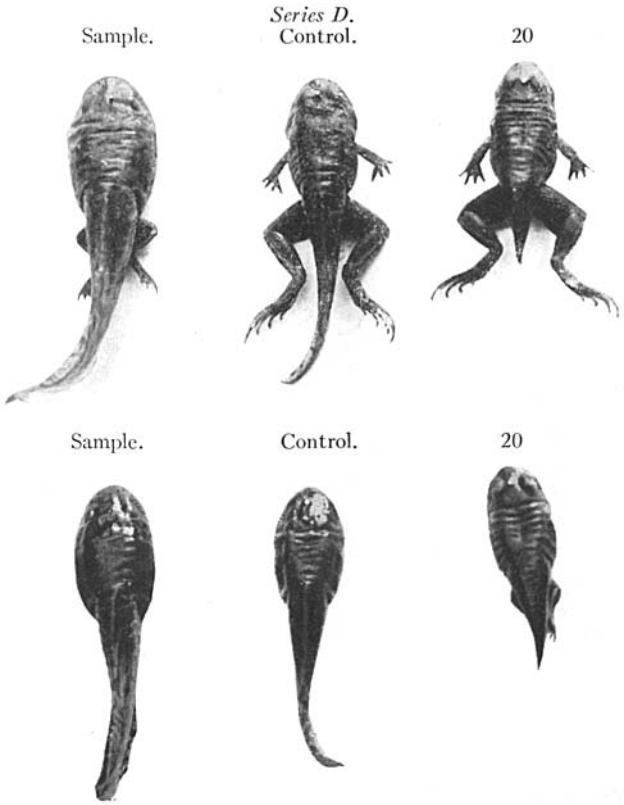

FIG. 4.

(Graham: Adenomata of the Thyroid Gland.)