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I hereby recommend that the thesis prepared under my supervision by MARSHALL R. WARREN

entitled Studies on the effect of experimental hyperthyroidism on the adult frog (Rana pipiens, Schreber).

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

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STUDIES ON THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON THE ADULT FROG, RANA PIPIENS, SCHREBER

A dissertation submitted to the

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by

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STUDIES ON THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON THE ADULT FROG, RANA PIPIENS, SCHREBER

Many investigations have been carried on in recent years in an effort to determine the relationship of the thyroid gland to development and growth in Amphibia. Most of these experiments have been concerned with the results of feeding normal and thyroidectomized tadpoles with fresh and desiccated thyroid gland tissue as well as with pure iodine. The changes involved in the metamorphosis of the tadpole to the frog afford accurate criteria for determining the effects of the thyroid gland upon this process. Although the changes during the metamorphosis of Caudata larvae are not so striking, some of them are significant enough to be used as criteria in thyroid gland experiments. The results of these varied investigations show that in practically all cases the feeding of thyroid substance or pure iodine to tadpoles hastens metamorphosis (Gudernatsch, '12, '14, '17; West, '14; Morse, '14; Lenhart, '15; and others).

The effects of the ablation of the thyroid rudiments in Rana pipiens tadpoles were first shown by Allen (1918), who found that differentiation of the soma ceased just after the hind legs had begun to grow and that metamorphosis did not occur. Feeding thyroid substance to these experimental animals caused a resumption of development even so long as four months after it had ceased. Hoskins and Hoskins ('19) showed that the larvae of Rana sylvatica and Amblystoma punctatum also failed to metamorphose after the removal of the thyroid glands. This failure was attributed to faulty metabolism, especially as regards calcium since thyroid removal causes a deficiency in calcification and ossification of the skeleton (Terry '18). Adams and Richards ('29) reported the failure of adult Triturus viridescens to molt following thyroidectomy. Injections of thyroxin into animals which had been without thyroids from 20-24 weeks caused molting after the fifth injection and again after the seventh injection. Taylor ('36) made a rather intensive study of the effects of athyroidism in Triturus torosus, Rathke. His work showed that the tissues in general exhibited a condition of nutritional deficiency and emaciation. Osborn ('36) reported an inhibition of molting in twelve species of urodeles as a result of hypophysectomy and thyroidectomy. He states that "the molting process is associated directly with the thyroid and only indirectly with the pituitary."

Although the thyroid gland has been shown to exert a significant influence upon the metamorphosis of amphibians, little is known concerning its effect upon adult forms. The effect of administration of thyroid substance to homoiothermous animals has been intensely studied. Experimental hyperthyroidism in these forms leads to an increased metabolic rate, body temperature and pulse rate, nervousness, emaciation and many other well-known symptoms. Swingle ('22) administered large quantities of physiologically active thyroid substance to adult Necturus by feeding, injecting and grafting. His results were negative. Gayda ('24) failed to note any effect of thyroid feeding on adult frogs even though the hormone must have been circulating in the tissues of the animals as was later shown by Sembrat ('25).

Since thyroidectomy is followed by such striking changes in Salientia larvae and adult Caudata, it scarcely seems plausible that experimental hyperthyroidism should be without effect in adult Salientia. For this reason a re-investigation of the problem was undertaken.

The author wishes to express his sincere appreciation to Drs. H. L. Wieman, C. K. Weichert, W. A. Spoor and W. A. Dreyer for their helpful criticisms and suggestions, E. R. Squibb and Sons laboratory for contributing a generous amount of crystalline thyroxin and to J. A. Bledsoe for his technical assistance with the photographic material.

METHODS AND MATERIALS

The experiments were performed on adult Rana pipiens received from a Chicago supply house in October and November. All of the animals were weighed and measured to determine their stage of development. No frogs measuring less than

72 mm. in body length were used since such frogs are generally immature (Rugh, '37).

The control and experimental frogs were kept under daily observation in separate large aquaria under similar conditions of light, temperature, humidity and background color. The aquaria were cleaned twice weekly with a disinfectant to prevent any infection. The basic food material consisted of fresh beef liver which was pressed into a pulp thin enough to be administered with a 10 c.c. syringe. The animals were forcibly fed with a small rubber tube fitted on an adapter for the syringe. The tube was inserted into the stomach and a certain amount of liver pulp forced out of the syringe. This procedure made it possible to give each frog a definite quantity of food. Occasionally there was some regurgitation and when this occurred some additional food, approximately equal to the amount regurgitated, was administered. It was found that if the frogs were kept in a semi-dry container for 1-2 hours after feeding that little vomiting occurred. Control and experimental animals were weighed before each feeding.

The experiments reported here were conducted over a period of two years during the winter months. The experimental animals were subjected to three different methods of treatment. In Group B1, which consisted of 75 frogs, each frog was fed twice weekly for a period of 12 weeks with 30 mgm.

of desiccated thyroid powder mixed with 1 c.c. of beef liver pulp. Each of the 20 frogs in Group B2 was fed twice weekly for 12 weeks with 30 mgm. of desiccated thyroid powder mixed with just enough water to make a thin paste. The 15 frogs in Group B3 were not fed but were given injections of Squibb's crystalline thyroxin in aqueous solution. One tenth milligram was injected into the dorsal lymph sacs of each animal twice a week for 10 weeks. Group A1, which were controls for Group B1, received 1c.c. of beef liver pulp twice weekly, Group A2, the controls for Group B2, were not fed, while Group A3, which were controls for Group B3, received 1 c.c. of amphibian Ringer's solution twice weekly for the duration of the experiment. All of the animals were in good condition at the end of the experimental period. Some of the powder-fed animals have been kept for several months with no ill effects.

The animals were sacrificed during the last week of February or the first week of March and their tissues prepared for histological study. Bouin's fixing agent was used for all of the tissues except the livers which were fixed in a mixture of 9½ parts 95% alcohol and ½ part 100% formalin and the pituitary and thyroid glands which were fixed in 10% formalin. Sections were cut at 8 microns for all of the tissues except the livers and pituitaries which were cut at 6 microns. Delafield's or Ehrlich's hematoxylin and eosin were used for staining most of the tissues. The liver sections were stained with Best's carmine method for glycogen and with Mallory's triple stain in addition to the above stains. The pituitary gland sections were also stained with Mallory's triple stain.

OBSERVATIONS DURING THYROID ADMINISTRATION

Daily examination of the experimental animals showed a progressive color change in the skin. The onset of this change was so gradual that it was difficult to determine its first appearance. At the beginning of the experiments all of the frogs, both experimental and control, were a deep brownish-green color with the usual large black pigment spots. The areas immediately surrounding these large black spots were grayish-green while the dorsolateral dermal plicae were light brown.

After 180 mgm. of thyroid powder and 0.6 mgm. of crystalline thyroxin had been progressively administered, the area around the large pigment spots changed from a grayish-green to light green. Simultaneously, the skin between these large pigment spots changed so that it was lighter in appearance than in the controls. The dermal plicae were also noticeably lighter. Further administration of thyroid substance was accompanied by more pronounced changes. The thin light-green rings around the pigment spots became deeper green and gradually spread to the entire dorso-lateral surface of the frog. In 10 experimental animals, this intense green coloration was maintained throughout the remainder of the experimental period. After the remaining experimental animals had attained the maximum green color, there was a gradual fading from green to a paler yellow shade. With the exception of the large black pigmented areas, the entire skin of the frog became yellowish in color. This coloration was maintained during the rest of the experiment.

The control frogs did not show any deviation from the color pattern which they possessed at the beginning of the experiments (fig. 1 and 2).

Another characteristic of the experimental animals was the abundance of mucous secretion given off by the skin glands. It was possible to distinguish the treated frogs from the controls by their intensely slimy skin. At the time of weighing and feeding, the excess moisture was always removed by wiping the frogs gently with a towel. After this procedure, the control animals were easily held in the hand while the experimentals retained their slimy nature and were more difficult to hold. This difficulty was enhanced by the greater activity of the treated frogs.

10 control and 10 experimental animals were put into individual containers to determine the rate at which the skin was sloughed. The controls shed their skins every 12 to 16

days while the thyroid-treated animals sloughed their skins every 7 to 10 days.

HISTOLOGICAL CONSIDERATIONS

Sections of the following tissues were prepared for histological study: integument, stomach, small intestine, liver, pancreas, kidney, gonads, thyroid and pituitary.

THE INTEGUMENT

The skin for histological study was always taken from the same region in each frog. One strip was removed from the dorsal surface between the scapulae and another was taken from the mid-dorsal region between the ilia.

In the experimental frogs of both sexes the dermal melanophores show extreme contraction. The pigment cells appear as almost spherical bodies in contrast to the normal expanded state found in the control frogs. In some cases, the degree of contraction is so great that a space appears around the shrunken pigment cell (figs. 3, 4, 5, 6).

The thickness of the epidermis in the experimental animals is greatly reduced. The epidermal layer varies from 25.2 to 33.6 microns in thickness in the treated frogs, whereas the variation in thickness in the control frogs is from 41.6 to 84 microns. The corneum of the treated animals is thinner with fewer nuclei than in the control epidermis.

The polygonal cells of the stratum germinativum are smaller and as a result the nuclei are clumped closer together. The columnar cells have practically disappeared in all of the treated animals. Instead, spherical nuclei more like those of the polygonal cells are present. The basement membrane is vacuolated and less dense than in the control frogs.

The mucous glands of the skin are greatly disturbed by the thyroid treatment while the poison glands remain unchanged. The mucous glands of the treated frogs are shrunken and are structurally abnormal. The nuclei have moved from their basal location in the cells of the control skins toward the lumina of the glands. Some of the glands are shrunken to such a degree that the nuclei appear as an aggregate in the center of the distorted gland (figs. 4, 6).

All of the connective tissue of the dermis has become more vacuolated and less compact in the thyroid-fed animals than in the controls.

THE ALIMENTARY CANAL

The small intestine, the cardiac and the pyloric regions of the stomach were studied. Sections of the stomach of the two groups of control animals do not differ markedly. The epithelium of the various regions is lower in the starved individuals and the nuclei have moved nearer the lumen rather than maintaining their normal position in the proximal portions of the cells. No difference is detected in the submucosa and glandular elements. Similar conditions are found in the small intestine. The starved animals show the typical starvation condition of the intestine while the intestines of the liver-fed frogs have a more normal histological appearance.

Thyroid administration does not markedly affect the histological picture of the stomach and small intestine. In all of the treated frogs, the vascularity of the tissue is increased. The glandular crypts of the stomach of the thyroid-fed frogs are deeper. In the thyroxin-injected frogs, the glands are less compact and the cellular units are smaller. Injections of thyroxin produce the only marked differences in the small intestine. The epithelium is very narrow and vascular with few goblet cells. The submucosa is more vacuolated and fragile in appearance.

THE LIVER

The livers of the liver-fed frogs have unusually large cells in which the cytoplasm barely stains with hematoxylin and eosin. Some of the granular cytoplasm stains slightly with hematoxylin. The "lacy" appearance of these sections as shown by Mallory's and hematoxylin stain is caused by liver capillaries engorged with blood (fig. 7). Bile capillaries have been pushed far apart by the enlarged cells. Best's stain in this tissue shows that approximately the same amount of glycogen exists as in the other group of control animals. Quantitative determinations of the amount of glycogen present are given below.

When thyroid powder is fed in addition to the liver pulp, the enlargement of the liver cells and the engorged condition of the liver capillaries with the resulting "lacy" appearance is prevented. The liver cells are reduced in size and approach the condition of the starved animals (fig. 8). The stored glycogen has a more scattered arrangement.

Administration of thyroid substance alone produces similar results regardless of the method of treatment. The liver cells are much reduced in size so that the nuclei are closely clumped together. Sections stained for glycogen indicate a reduced amount. In some of the thyroxin-injected frogs, the glycogen that can be stained is negligible.

THE PANCREAS

The pancreas of the control frogs shows distinct acini composed of large polyhedral cells with the nuclei located in the proximal portion of the cells (fig. 9). Although no secretion granules can be seen with the staining methods employed, the cytoplasm of the cells has a granular appearance. Lumina are found in some of the acini but the cells are so enlarged in most cases that the lumina are obliterated.

The pancreatic ducts of the liver-fed frogs contains some coagulated material while those of the starved frogs are empty. The islands of Langerhans of the frogs that received liver pulp are more vascular than those of the starved frogs.

Administration of thyroid powder alone or when added to the liver pulp causes a marked change in the structure of the pancreas. The acini are indistinct and the cells are smaller than those in the control animals (fig. 10). The nuclei are nearer the center of the cells which have lost much of their granular appearance. The islands of Langerhans do not appear to be increased in size although they are very vascular. Injection of thyroxin produces a more advanced state of the condition of the pancreas described above. The acini are barely recognizable and the cells are so reduced in size that the nuclei practically fill them. The pancreatic ducts are empty and the islands of Langerhans do not show any alteration.

THE KIDNEY

The sections of the kidneys of the control frogs show the usual histological picture for this amphibian tissue and need no lengthy description. The lumina of the tubules are smaller in the liver-fed frogs than in the starved animals but the cellular units are larger. In both groups of animals, the glomeruli are confined to the ventral half of the kidney and those of the starved frogs are much smaller.

The tissue from the thyroid powder-fed animals, with or without liver pulp, is similar in appearance. One of the most striking things about these sections is the abundance of coagulated material in the lumina of the tubules (fig. 11). The lumina are larger than those in the control animals but are somewhat obscured by the coagulated material. The cells of the tubules are small. Glomeruli are conspicuous due to their enlarged nature and extend further toward the dorsal surface of the kidney than do those in the control animals. Although the glomeruli appear to be more numerous in the kidneys of the thyroid-treated frogs, it is questionable whether this is actually the condition.

Injection of crystalline thyroxin has a more pronounced effect upon the kidney of the frog. Little indication of the tubules can be found. A rather homogenous mass of nuclei is found in their place. The glomeruli are evident but they are much shrunken. Some of the capsules have no glomeruli and in many cases the capsule walls are decidedly thickened (fig. 12).

No evidence of a Bidder's canal has been found in sections of the kidneys studied. This confirms the findings of Rugh ('39) in his study of the testes of hibernating and pituitary-injected Rana pipiens.

THE GONADS

The gonads with the attached corpora adiposa were removed so that the gonads could be lifted by means of the fat bodies. This procedure was employed in order to prevent the manipulation of the fresh tissue with the forceps. Rugh ('39) has recently pointed out the necessity for such precaution in fixing the testes of hibernating frogs.

Due to the fact that many amphibian testes show a caudo-cephalic wave of mitoses during spermatogenesis, longitudinal sections were made of this tissue. The activity of the testes was judged by counting the number of mitoses visible in 10 microscopic fields in each of the cephalic, middle, and caudal regions of 100 sections. The magnification used was 980 diameters. The data are expressed in terms of the number of mitoses per 100 cells.

Histological study of the testes of the control and normal starved frogs shows the typical picture of the hibernating testis as described by Rugh ('39). He has described the cell types which are found in the seminiferous tubules. The number of mitoses in these control animals has been estimated to be 10 per 100 cells.

The sperm ducts, which are located among the tubules in the so-called interstitial tissue,are in a collapsed state in practically all of the control animals (fig. 13). These ducts are made up chiefly of columnar cells with typical elongated nuclei. Only in four cases have any spermatozoa been found in the ducts of the control animals. Since the lumina of the sperm ducts are well defined, it is probable that the sperm in these ducts are being gradually expelled and that handling of the organ did not cause this discharge.

The testes of the thyroid-treated animals differ markedly from those of the control animals. The scattered nature of the so-called interstitial tissue makes it appear to be reduced in amount. Although the same cell types exist in the seminiferous tubules of the experimental animals as in the controls, the proportions of the various types are much different. The majority of the spermatozoa are free in the lumina of the tubules while they are near the periphery of the tubules in the control animals (figs. 15, 16). There are numerous tubules from which the sperm cells are being discharged into the sperm ducts (fig. 17). Numbers of tubules are devoid of spermatozoa and are filled with cells in the transitional stages of spermatogenesis as indicated by the numerous mitoses. Other tubules contain cells which are typical of all stages of spermatogenesis. The number of mitoses has been estimated to be 36 per 100 cells, an increase of 26% over the controls. Another type of cell which appears as an enlarged Sertoli cell has been found in the tubules of the treated animals. These occur either singly or in groups of 3, 4, or 5 near the periphery of the tubules from which the majority of spermatozoa have been liberated (fig. 18). Varying sizes of these cells are found in different tubules and can be traced from Sertoli cells but their true identity has not been established.

The sperm ducts of the treated frogs are characteristic of frogs with active testes. The walls of the ducts are composed of cuboidal epithelial cells with their almost spherical nuclei (fig. 14). These ducts have large lumina in contrast to the almost uniformly collapsed state found in the control frogs.

Histological study of the ovaries of the control and experimental frogs did not reveal any significant difference.

THE THYROID GLAND

Sections of the thyroid glands of the control frogs show that they are composed of follicles of varying sizes which are filled with a granular colloid. The follicles in the gland of the starved frogs are smaller than those in the liver-fed frogs. The increased size of the follicles is accompanied by a reduction in the height of the epithelium and the nuclei become oval-shaped rather than spherical.

Thyroid administration causes an enlargement of the follicles in all of the experimental animals. The colloid, which is present, stains more intensely with eosin than in the above cases. The majority of the nuclei in the epithelium of the follicles are very much flattened and elongated. In some of the follicles, the epithelium is so flattened that it appears thread-like (figs. 19, 20). There is an increased vascularity in the glands of the treated frogs.

THE PITUITARY GLAND

Administration of thyroid substance to adult Rana pipiens causes a decrease in the weight of the whole pituitary gland. The average weight of the whole gland of the control animals is 0.895 mgm. as compared to an average weight of 0.764 mgm. for the treated frogs.

Histological examination of these tissues does not reveal any difference in the posterior or intermediate lobes. The cells of the anterior lobe of the control frogs vary from a columnar to a polyhedral shape. Fewer columnar-shaped cells are found in this lobe of the treated frogs and an increased number of basophilic cells is found (figs. 21, 22). The glands of the thyroid-treated frogs are more vascular.

PHYSIOLOGICAL CONSIDERATIONS

Experiments were carried out to determine whether the thyroid substance had any effect upon the physiological processes of the adult frog. Although experimental hyperthyroidism probably exerts widespread effects upon functional activity, it has only been possible to study a few. The data obtained from these experiments are based upon a single series of tests and were not performed during the initial experiments.

DETERMINATION OF OXYGEN CONSUMPTION

The oxygen consumption of the control and experimental frogs was measured during the last two weeks of the experimental period by means of a respirometer modified from the Warburg or Krogh constant volume type. Due to the size of the animals, a large chamber had to be employed for the determinations. An 850 c.c. bell jar was fitted with a number 4 rubber stopper which had two holes bored to the proper size to convey the outlet and manometer tubes. The stopper and tubes were sealed tightly in place with a special sealing wax. A small dish with an outside diameter the same as the inside diameter of the bell jar was used to hold the potassium hydroxide. A screen grid of 3/8 inch mesh was fitted on top of this dish to prevent the animal from coming in contact with the hydroxide solution. The bell jar was sealed with vaseline to a circular ground-glass plate at the bottom.

The following technique was employed in the experiments. 0.8 c.c. of kerosene of a known density, colored with Sudan III, was run into the manometer tube to fill it halfway up the scale. The frogs were weighed to the nearest 0.1 gram after the urine had been expressed. 25 c.c. of 30% potassium hydroxide were then poured into the dish. The screen grid was fitted into position and the animal placed in the chamber. The bell jar was then sealed to the ground-glass plate and the entire chamber immersed in a constant temperature water bath at 22 degrees centigrade. After the animal became quiet, the outlet tube was closed and the temperature and barometric pressure were recorded. A blank chamber was run to record any changes due to temperature and barometric pressure. At the end of three hours, the readings were recorded on a millimeter scale and the animal was removed from the chamber. The bell jar was cleaned and aerated after each determination to remove any mucus or moisture that may have collected on the walls. Each experiment was performed on a different animal.

The calculations were made according to the method employed by Evans ('39) in her experiments on aerial respiration in salamanders.

Determinations were made on both sexes of the control and experimental animals. Individuals from Groups B1, 2, and 3 of the treated animals and members of Groups A1 and 2 of the control animals were tested. The mean oxygen consumption for the various groups is presented in Table I.

TABLE I.

MEAN OXYGEN CONSUMPTION OF ADULT FROGS

Group of Animals	Sex	Av. Weight in Grams	No. of Determinations	mm^3/Gm./Hr. consumed and Standard Error
		A. CONTROL ANIMALS		
1. Liver-fed	Male	52.6	9	64.7 ± 1.01
	Female	53.8	6	63.2 ± 0.76
2. Normal-starved	Male	47.8	9	45.3 ± 0.99
	Female	50.2	6	43.0 ± 0.83
		B. EXPERIMENTAL ANIMALS		
1. Thyroid Powder plus Liver-fed	Male	45.4	9	93.6 ± 2.15
	Female	48.6	6	87.7 ± 1.06
2. Thyroid Powder-fed	Male	40.8	4	60.4 ± 0.89
	Female	49.6	4	60.1 ± 0.59
3. Thyroxin-injected	Male	38.8	4	59.8 ± 1.12
	Female	42.7	4	58.7 ± 0.83

The mean difference between the amount of oxygen consumed in the control males of Group A1 and A2 is 19.4 mm^3

and between the females of the two groups is 20.2 mm^3. This difference can be accounted for by the fact that those in Group A1 received beef liver pulp twice weekly while those in Group A2 were not fed. The mean difference in oxygen consumption between the control males of Group A1 and the experimental males of Group B1 is 28.9 mm^3 and between the females of the two groups is 24.5 mm^3. Since both of these groups were fed beef liver twice weekly, it is apparent that the increased oxygen consumption of the experimental animals is associated with the administration of thyroid powder.

When the males of Group B2 experimental animals are compared with the control males of Group A2, a difference of 15.1 mm^3 of oxygen consumption is noted. Similar comparisons of the females of the two groups shows a difference of 17.1 mm^3. Comparing the experimental animals of Group B3 with the control animals of Group A2, a difference between the amounts of oxygen consumed by the males is 14.5 mm^3 and between the females is 15.7 mm^3. The increase in the amount of oxygen consumption of the experimental animals in Groups B2 and B3 over oxygen consumption of Group A2 is evidently due to the administration of thyroid substance since these frogs were not fed.

DETERMINATION OF BLOOD SUGAR

Before the blood was removed for the sugar determinations, kymograph records of the rate of heart beat were made to correlate with similar records on other frogs. The blood was taken from the ventricle of the heart and allowed to flow down the walls of a previously oxalated vial. The samples of blood were immediately corked and stored in a refrigerator until the tests were made. Analyses were performed within 24 hours after the removal of the blood.

15 samples of blood were obtained from each series of animals, including the thyroid-treated, control and normal-starved frogs. The sugar analyses were made according to the method of Hagedorn and Jensen. Kymograph records of the rate of heart beat were prepared for 15 frogs in Group A1, 10 in Group A2, 15 in Group B1 and 10 in Groups B2 and B3.

The results indicate that there is no significant difference in the percentage of blood sugar of the experimental and control animals. Blood sugar values of the control frogs vary from 0.036 to 0.128 mgm. while those of the treated animals vary from 0.053 to 0.165 mgm. Since the amount of blood sugar in the frog is so small and the individual variation so great, a larger number of determinations will be required before definite conclusions can be made. Olmsted ('24) states that "the blood sugar of frogs at room temperature is so low that it is hardly titratable by the Schaffer-Hartman or the Hagedorn-Jensen method." This fact has been confirmed in the present study and further work is being planned for a more detailed study of the blood sugar level in Rana pipiens.

The study on the rate of heart beat of the various animals shows that the administration of thyroid substance has no effect. Counts made from the kymograph records ranged from 59-62 contractions per minute at room temperature in both types of animals.

LIVER GLYCOGEN ANALYSES

Liver glycogen analyses were made according to the method of Good, Kramer and Somogyi ('33) in Groups A1 and 2 and in Groups B1, 2, and 3. No attempt has been made to study the sexual differences and the data includes results from both males and females.

Benedict's method was employed for the quantitative determination of sugar. The neutralized hydrolysate was poured into a graduated burette and titrated against the boiling Benedict's solution. The quantity of liver solution necessary to discharge the blue color of the reagent contains 0.05 gm. sugar.

The results of these tests are given in Table 2.

TABLE 2.

PERCENTAGE OF GLYCOGEN IN THE LIVER

Group of Animals	Av. Weight of Livers in Gms.	No. of Determinations	% of Sugar
A. CONTROL ANIMALS			
1. Liver-fed	3.5	15	7.03
2. Normal-starved	1.5	15	6.85
B. EXPERIMENTAL ANIMALS			
1. Thyroid Powder plus Liver-fed	2.2	15	4.25
2. Thyroid Powder-fed	0.8	10	1.84
3. Thyroxin-injected	0.6	10	1.05

The data given in Table 2 show a marked dimunition in the glycogen content of the liver as a result of the administration of thyroid substance. It is evident that the peculiar histological picture of the livers of Group A1 frogs is not caused by a storage of glycogen. The increased weight of these livers is apparently due to the storage of some other material, probably protein, and to their increased vascularity. The livers of some of the thyroxin-injected frogs are so depleted of their glycogen that it is impossible to obtain any precipitate during the procedure.

BODY WEIGHT

The data obtained from the bi-weekly body weights are recorded in Table 3. Both males and females are included in the group average.

It will be noted that the 25 starved frogs of Group A2 show a gradual decrease in body weight throughout the experimental period. The total loss in weight over the 12 weeks is 2.9 grams. The slight fluctuations in the curve are of no significance.

The 50 control liver-fed frogs in Group A1 show an increase of approximately 1.7 grams for the experimental period. The sudden decrease in weight during the second week is difficult to interpret. This may be due to some inability of the frogs to become accustomed to the type of diet or method of feeding.

The effect of the administration of thyroid substance on the body weight of the frogs differs according to the method of treatment. The 75 thyroid powder plus liver-fed animals of Group B1, which lost 3.7 grams over the 12-week period, show a slight increase during the first two weeks of the period and then a sharp decline to the 5th week. The 0.8 gram increase in weight between the 5th and 6th weeks and the 1.4 grams increase between the 8th and 9th weeks are probably due to a decrease in temperature. Although the temperature remained almost consistently between

17°C. and 18°C., at the end of 4½ weeks it fell to 13°C. and at the end of 7½ weeks to 12°C. It may be that below 12° or 13°C. the activity of thyroxin is inhibited so that an increase in weight due to the administration of liver is affected. In this experiment, the males are more seriously affected than the females.

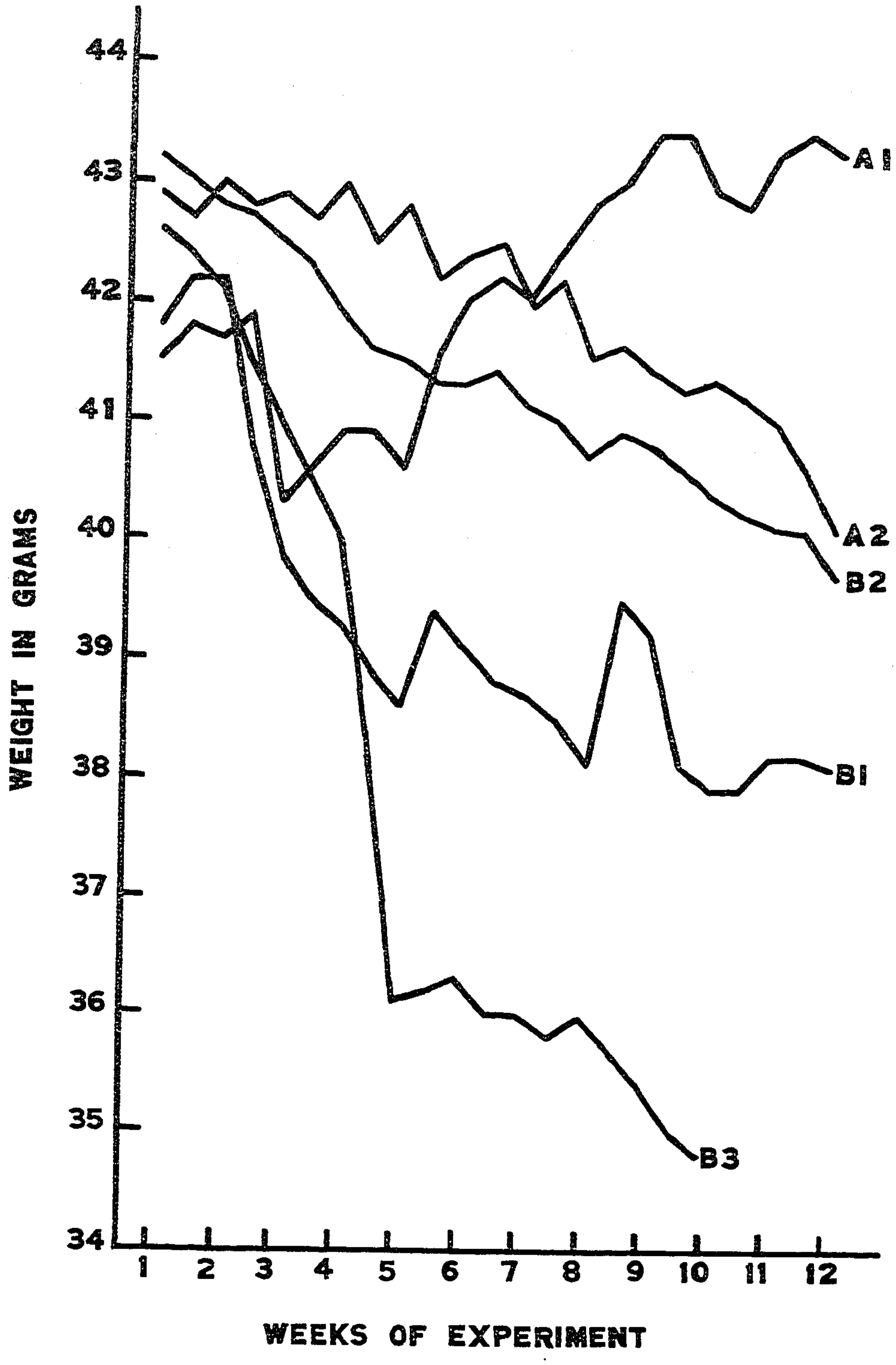
The 20 thyroid powder-fed frogs in Group B2 show a gradual decrease in body weight with a total loss of 3.7 grams. The general trend of the curve is downward but the temperature effects described above seem evident. A break in the decline is found between the 5th and 6th weeks and again between the 8th and 9th weeks. Female frogs are less severely affected. A curve for the males alone would show a total loss of 4.2 grams during the 12 weeks. This is perhaps due to the almost negligible effect of thyroid substance upon the ovaries of the females which constitute a considerable portion of the female body weight.

The body weight of the 15 thyroxin-injected frogs of Group B3 is most severely affected with a total loss of 7.8 grams for 10 weeks. The thyroxin appears to exert its maximum effect during the first five weeks. The interruption in the decline of the curve is again noted between the 5th and 6th weeks and between the 7th and 8th weeks. It would seem that the thyroxin which is injected responds more rapidly to temperature effects than that which is administered orally. The inhibition in the loss of body weight due to the

TABLE 3

BODY WEIGHT CURVES

CONTROL & EXPERIMENTAL FROGS



temperature appears about 4 days earlier in the thyroxin-injected animals.

DISCUSSION

The influence of the thyroid gland upon the integument of amphibians has only recently received much attention. Athyroidism in adult Caudata is accompanied by striking changes in the integument as shown by Adams and co-workers ('29, '30, '32), Alderman ('33), Taylor ('36) and Osborn ('36). Thyroidectomy causes a distinct increase in the thickness of the epidermis due to the inhibition of molting. Noble and Bradley ('33) have reported similar changes in the integument of the thyroidless lizard. The fact that molting occurs when thyroid substance is given to these athyroid animals indicates that the thyroid gland alone is the functional factor.

Experimental hyperthyroidism has a pronounced effect upon the integument of the adult frog. The skin is sloughed off at a faster rate causing the epidermis to be reduced in thickness and is accompanied by definite cellular changes. Although the mucous glands are severely affected, the poison glands show no change.

Numerous investigators have studied the effect of thyroid substance upon the cutaneous system of many animals. Simpson ('24) and Chang ('26) agree that the thyroid gland is important in the growth of wool, hair and horns.

Administration of thyroid substance to fowls produces noticeable changes on molting and growth of feathers (Cole and Reid, '24; Torrey and Horning, '25; Zavadovsky and coworkers, '26, '27; Krizenecky and Nevalonnyj, '27 and others). The two latter groups of investigators have reported molting and depigmentation in fowls following thyroid treatment. Hoskins ('33) pointed out that the thyroid gland is linked with the activity of the skin glands in humans.

Some of the important factors which influence color change in amphibians are temperature, humidity, color of background, light, ozygen supply and pituitary extirpation (Hogben, '24). Corona and Moroni ('98) and Bigney ('19) showed that injections of adrenalin caused extreme contraction of the melanophores of the frog. McCord and Allen ('17) produced the same effect by feeding pineal glands to frogs. Injections or implantation of pituitary glands cause expansion of the melanophores (Hogben and Winton, '23; Huxley and Hogben, '21). Most workers agree that the hormone which stimulates chromatophore expansion is formed in the intermediate lobe of the pituitary gland. However, Steggerda and Soderwall ('39) believe that some substance is "emitted by the pars tuberalis which counteracts the melanophore dilating substance, Intermedin, which is known to be formed in the pars intermedia."

The contraction of the melanophores under conditions of experimental hyperthyroidism may be due to one or more of several conditions: 1) After a prolonged period of time hyperthyroidism may suppress the secretory activity of the intermediate lobe of the pituitary and prevent the release of intermedin, 2) thyroid substance may stimulate the adrenal gland to eliminate unusually large amounts of adrenalin, 3) or the response may be due to the increased metabolic rate. Further experimentation will be necessary to determine the exact conditions responsible for this chromatophore change.

The surprising number of persons with thyroid disfunction who suffer from gastro-intestinal disturbances has initiated much study of this interrelationship. Hypothyroidism is accompanied by lowered gastric secretion and subacidity while hyperthyroidism has the opposite effect (Rogers and coworkers, '19; Chang, '30; Coelho and Rocheta, '30; Verbryche, '31; and Brown, '31). Protein, carbohydate and fat metabolism are altered in relation to the functional activity of the thyroid.

The histological picture of the gastro-intestinal tract in the present work does not offer much evidence concerning the functional activity of the tissue. Whether increased gastric secretion accompanies the increased depth of the glandular crypts in the stomach has not been determined.

The liver is known to show varying degrees of response to thyroid disfunction. One of the more constant responses of this organ to thyroid feeding is reduction in glycogen content.

The enlarged condition of the hepatic cells and the increased vascularity of the liver in control frogs fed liver pulp are of significance. Walter and Addis ('39) report a 37% increase in the liver weight of rats fed large amounts of dried liver. They point out that this is not due to any substance peculiar to liver tissue since feeding dried whole kidneys produces similar results. When liver tissue is fed, this increase in weight is accompanied by a 42% increase in liver protein. They have shown further that thyroxin causes a 16% increase in liver protein in fasting rats. The increased size of the hepatic cells in the liver-fed frogs may be due to a storage of protein. No protein analyses were made, however, to confirm this assumption.

It is doubtful whether a direct relationship exists between the pancreas and the thyroid gland, but that an indirect relationship exists seems to be generally accepted. Numerous investigations suggest an antagonistic action between these two glands. The results obtained in this work are probably due to a general effect upon the entire frog rather than to a specific action on the pancreas.

Whether the effects of administration of thyroid substance on the kidney are due merely to the increased metabolic rate or to some specific action on the kidney is difficult to determine. Thyroxin is known to affect the distribution of water in the body by its effects upon the body tissues. Under such conditions the kidney may respond to the general altered effect upon the various body tissues rather than to a direct action of the thyroid substance. Some investigators indicate that thyroxin does not affect the kidney directly but causes a nervous reaction which increases the permeability of the capillaries or causes a mobilization of the salts in the blood.

Taylor ('36) described progressive changes in the kidney of the athyroid salamander.

The nature of the interrelationship between the thyroid gland and the gonads in mammals is uncertain although it has been recognized for many years. It is thought that the pituitary is the necessary intermediary in this relationship. The results of investigations dealing with the effect of thyroid administration on animals are conflicting (Schockaert, '31; Da Costa and Carlson, '33; Cohen, '34; Smelser, '39; and others). Döderlein ('28) reported that the testes of rats are more severely affected by thyroidectomy than the ovaries. Cohen ('34) did not observe any histological changes in the testes of thyroid-fed rats although they increased in weight. Smelser ('39) obtained a slight loss in testis weight and a severe depression of spermatogenesis by feeding thyroid substance to rats.

Although the presence of the thyroid gland is essential for growth and metamorphosis of tadpoles, neither the removal nor the administration of thyroid substance affect the gonads or germ cells (Allen, '18; Swingle, '18; and others). Taylor ('36), however, has reported that both the ovaries and testes of the adult salamander are severely affected by athyroidism.

Experimental hyperthyroidism in the adult Rana pipiens under the conditions reported here is accompanied by marked changes in the testes while the ovaries show no change. In view of previous work on hyperthyroidism, it seems more likely that the testis changes are due to the increased metabolic rate rather than to a stimulation of the pituitary gland.

The response of the thyroid glands of mammals to the administration of thyroid substance and iodine compounds seems to depend on the dosage and the age of the animals (Herring, '17; Cameron and Carmichael, '20, '22, '24; Gray and Rabinovitch, '29; Cohen, '34; and others). Cohen ('34) states in her paper on hyperthyroidism in the male rat that the thyroid glands of treated animals show follicles with low epithelium and abundance of colloid.

The cyclic changes in the thyroid glands of the frog indicating a storage of colloid in winter and its release in summer have been described by Sklower ('25). Swingle ('19) found that the thyroid glands of iodin-fed frog larvae are larger than those of the control frogs and the follicles contain more colloid. Judging from the height of the follicular epithelium, thyroid administration in the adult frog brings about a resting condition of the thyroid glands accompanied by a collection of colloid within the follicles.

The interrelationship of the thyroid and pituitary glands has been well established. Removal of the hypophysis causes a marked involution of the thyroid gland while injections of pituitary extracts cause an activation of the gland.

Increased gonadotropic potency of the hypophysis of the male and female rat as a result of thyroid feeding has been reported (Evans and Simpson,'30; Van Horn, '31; and Cohen, '34). The anterior lobe of the pituitary gland of thyroid-treated rats has more and larger basophilic cells than the control animals (Severinghaus, Smelser, and Clark, '34; Campbell, Wolfe, and Phelps, '34; and Cohen, '34). Campbell and coworkers found a subnormal weight for the pituitaries of female thyroid-fed rats while Cohen noted an increased weight in similarly treated males.

The increased number of basophilic cells in the anterior lobe of the pituitary gland of the adult frog is not accompanied by an increase in their size. Further studies are being planned to test whether the cellular changes observed have any relation to the gonadotropic potency of the gland.

Various factors are known to affect the respiratory rate of frogs. The results of the earlier investigations on the oxygen consumption of the frog show great variations (Hill, '11; Krogh, '16; Joel, '19; Iizuka, '26; and others). More recent work on oxygen consumption of amphibians indicates that much of the lack of correlation may have been due to the methods employed (Helff, '27; Wilder, '37; Evans, '39).

Since the work of Magnus-Levy ('95), it has been known that the concentration of thyroxin in tissue affects the output of carbon dioxide. Although many factors have been shown to affect the action of thyroxin, it is generally agreed that the thyroid substance markedly increases the amount of oxygen consumed in tadpoles and frogs (Joel, '19; Groebbels, '22; Abelin and Scheinfinkel, '23; Helff, '27; and others).

The present work on the adult Rana pipiens confirms the above results. It will be noted that the amount of oxygen consumed by the experimental animals in Groups B2 and 3 is lower than in Group B1 when both are compared with their controls. This may be due to the decreased body weight, as Helff ('27) has suggested, rather than to a decrease in metabolic rate against excessive oxidation, as Abelin and Scheinfinkel ('23) have indicated.

Although numerous investigators have studied the effect of thyroid activity on the blood sugar content, no consistent results have been obtained under conditions of hyperthyroidism. No increase in blood sugar was found in rabbits and dogs (Kuriyama, '17; Hancher and associates, '25; and Shpiner, '27). A doubtful increase has been reported in sheep (Bodansky, '24) as a result of thyroid treatment. Riddle and his associates ('24) believe that the thyroid gland is concerned in the regulation of the blood sugar level in pigeons.

Administration of thyroid substance for a prolonged period does not alter the blood sugar level in Rana pipiens to an significant extent. It is, of course, possible that an even more prolonged treatment will ultimately bring about changes in the blood sugar level.

It has been pointed out that the administration of thyroid substance greatly decreases the glycogen content of the liver (Kuriyama, '17, '18; Romeis, '23; Coggeshall and Greene, '33; Frazier and Friedman, '35; Drill, '37; and others). A decrease in liver glycogen after treatment with thyroid substance has been found in the present study on a poikilothermous animal. Less pronounced effects are observed in the experimental animals fed thyroid powder plus liver pulp. It may be that this is due to the utilization of the thyroxin in the oxidation of food material before it can exert its full influence upon the glycogen stored in the liver.

Administration of thyroid substance is not always accompanied by a decrease in body weight if carefully controlled amounts are administered. If excessive amounts are given, the metabolic rate will exceed the level of food intake and a loss of weight occurs (Kendall, '29).

The experiments dealing with the effects of thyroxin on metamorphosis of tadpoles show that a decrease in body weight occurs. Despite the fact that the frogs in Group B1 were fed liver in addition to the thyroid powder, their loss in body weight approximates that of the animals in Group B2. It is possible that the food material is oxidized before it can be assimilated by the tissues. The more direct method of treatment of the frogs in Group B3 may account for their severe loss of weight.

The influence of temperature upon the activity of the thyroid gland is well known. Adler ('20) found that during hibernation there is a regression and atrophy of the thyroid gland of the hedgehog. The thyroid gland is less active at high temperatures and its iodine content is increased. The results of the work reported here suggest that below 12°C. the oxidative action of thyroxin is inhibited. This may be due to a direct effect of temperature on the chemical reactions involved or perhaps an inhibition due to the inability of the tissues of the body to respond to the oxidative processes. The fact that the thyroid powder plus liver-fed frogs gain weight at lower temperatures indicates that there is no inhibition of the digestive processes in the hyperthyroid state.

SUMMARY

1. Some of the effects of prolonged administration of thyroid substance on the adult frog, Rana pipiens, have been studied.
2. Histological examination of the various tissues revealed the following effects:
    a) An extreme contraction of the melanophores of the skin.
    b) A reduction in the thickness of the epidermis and definite cellular changes as a result of the corneal layers being sloughed off at a rapid rate.
    c) Mucous glands of the skin are severely affected with no effect upon the poison glands.
    d) The cells of the liver are reduced in size and the storage of some material, probably protein, is inhibited.
    e) Acini of the pancreas are less distinct and are composed of smaller cells than in the controls.

f) Lumina of the kidney tubules are larger and filled with an abundance of coagulated material. Cellular units are smaller but the glomeruli are larger than in the controls. Injections of thyroxin cause a degeneration of renal tubules and glomeruli.

g) The testes show increased spermatogenesis with a liberation of spermatozoa into the sperm ducts while the ovaries are apparently unaffected.

h) Follicles of the thyroid gland are increased in size due to a storage of colloid causing the epithelium to be flattened.

i) The pituitary gland is decreased in size with smaller cellular units and an increased number of basophilic cells.

3. The physiological effects of thyroid feeding are:

a) Increased rate of oxygen consumption.

b) No significant effects on the rate of heart beat or blood sugar level.

c) A pronounced reduction in liver glycogen.

d) A gradual decrease in body weight so long as the temperature remains above 12° C.

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PLATE 1.

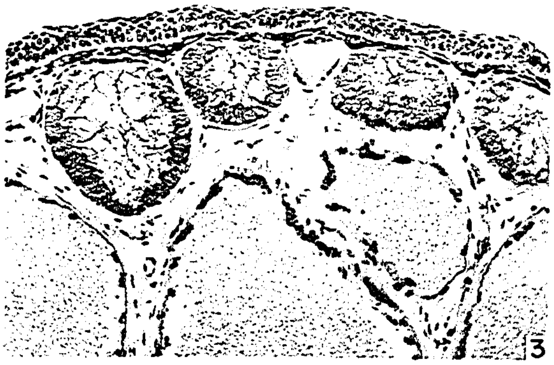
Explanation of Figures

1. Control frog above. Lower animal received 270 mgm. of desiccated thyroid powder.
2. Control frog above. Lower animal received 510 mgm. of desiccated thyroid powder.
3. Photomicrograph of section (x320) of skin of a control frog from the dermal plicae region. Note the thickness of the epidermis, the expanded melanophores, and mucous glands.
4. Photomicrograph of section (x320) of skin of a thyroid-treated frog from the dermal plicae region. Compare with figure 3.
5. Photomicrograph of section (x320) of skin of a control frog from region between the ilia. Note the thickness of the epidermis, the expanded melanophores, and mucous glands.
6. Photomicrograph of section (x320) of skin of a thyroid-treated frog from region between the ilia. Compare with figure 5.

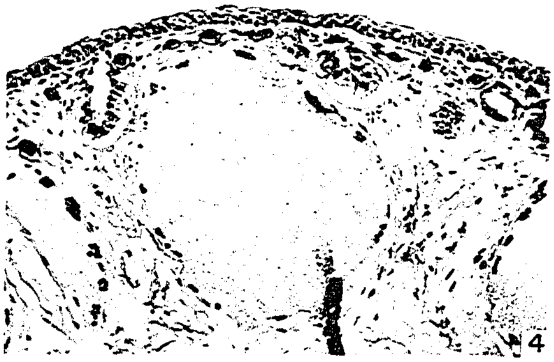
PLATE I

1

2



3



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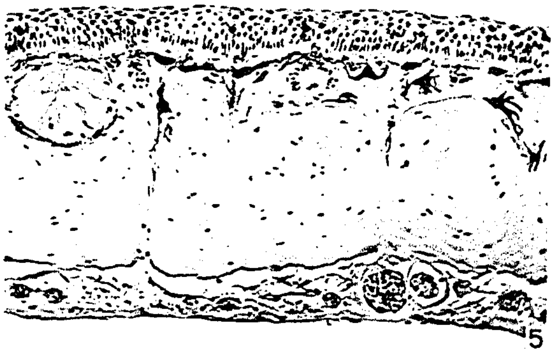


5

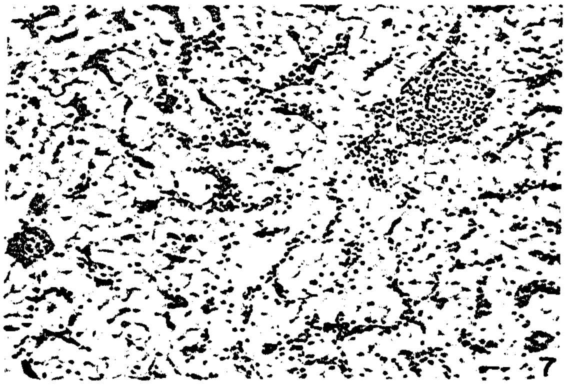
6

PLATE 2.

Explanation of Figures

7. Photomicrograph of section (x320) of liver of a control liver-fed frog. Note the enlarged hepatic cells and the engorged liver capillaries.
8. Photomicrograph of section (x320) of liver of a thyroid-fed frog. Compare with figure 7.
9. Photomicrograph of section (x320) of pancreas of a control frog. Note the distinct acini and position of the nuclei.
10. Photomicrograph of section (x320) of pancreas of a thyroid-fed frog. Compare with figure 9.
11. Photomicrograph of section (x160) of kidney of a thyroid-fed frog. Note the coagulated material in the lumina of the tubules.
12. Photomicrograph of section (x160) of kidney of a thyroxin-injected frog. Note the absence of tubules and the condition of the glomeruli.
13. Photomicrograph of section (x700) of a sperm duct in the testis of a control frog. Note collapsed state of the duct with absence of a lumen.
14. Photomicrograph of section (x700) of a sperm duct in the testis of a thyroid-fed frog. Note size of the lumen with spermatozoa.

PLATE 2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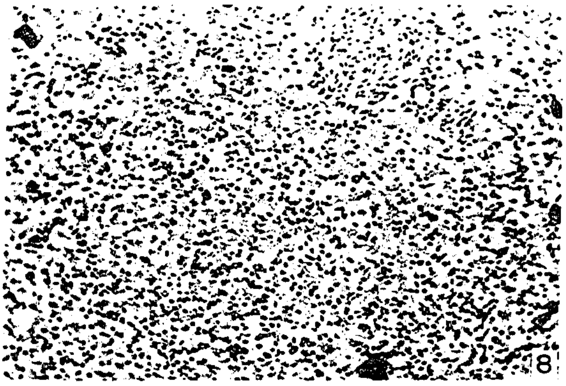
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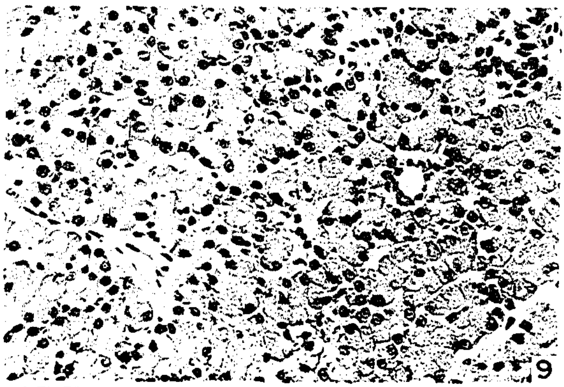
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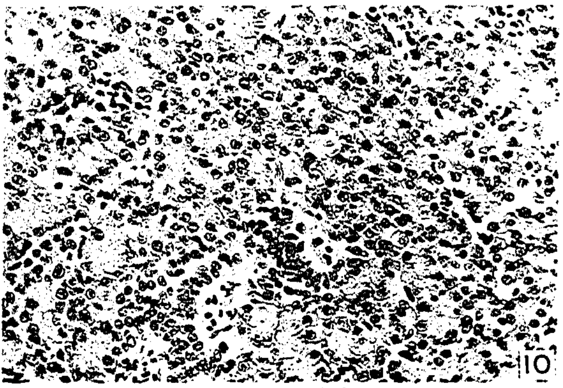
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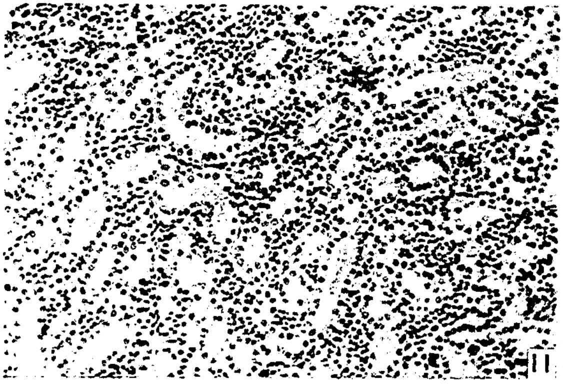
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PLATE 3.

Explanation of Figures

15. Photomicrograph of section (x160) of testis of a control frog. Note the position of the spermatozoa and the amount of so-called interstitial tissue.

16. Photomicrograph of section (x160) of testis of a thyroid-fed frog. Compare with figure 15.

17. Photomicrograph of section (x700) of a sperm duct of a thyroid-fed frog. Note the spermatozoa being emptied from the seminiferous tubules into the duct.

18. Photomicrograph of section (x700) of a seminiferous tubule of a thyroid-fed frog. Note the large cells in the tubule.

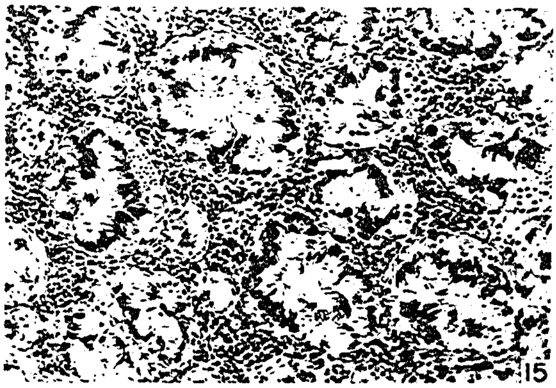
19. Photomicrograph of section (x700) of a thyroid gland of a control frog. Note the height of the epithelium of the follicle and the granular colloid.

20. Photomicrograph of section (x700) of a thyroid gland of a thyroid-fed frog. Compare with figure 19.

21. Photomicrograph of section (x700) of the anterior lobe of the pituitary gland of a control frog. Note the size of the cells and the number of basophilic cells.

22. Photomicrograph of section (x700) of the anterior lobe of the pituitary gland of a thyroid-fed frog. Note the increased number of basophilic cells and the size of the cells.

PLATE 3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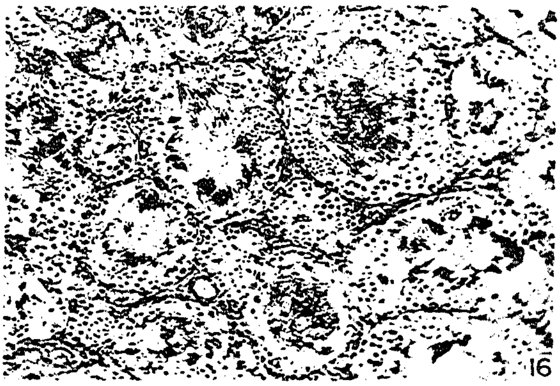
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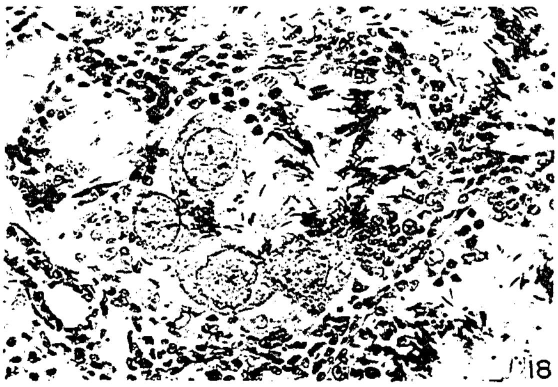
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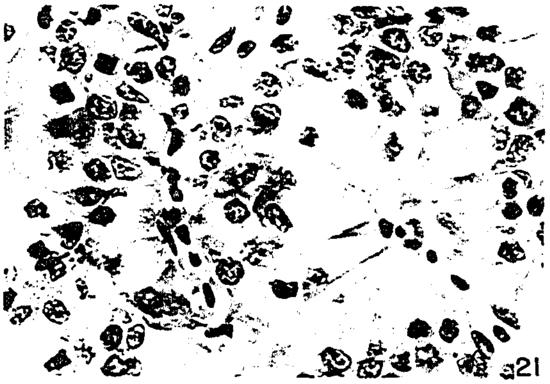
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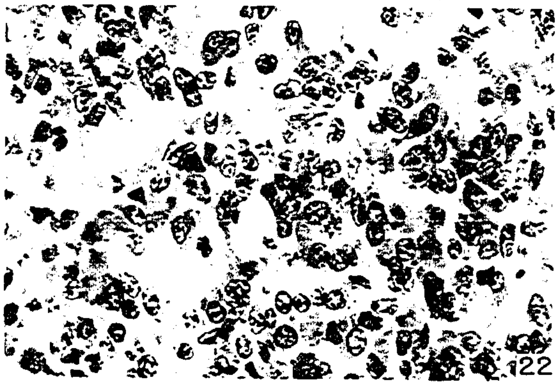
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