AN ABSTRACT OF THE THESIS OF

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Title The effects of thyroidectomy on the salamander *Triturus torosus*

Abstract Approved: Nathan Fasten

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A study was made of the physiological, histological, and cytological changes accompanying various stages of the thyroidless condition in the salamander *Triturus torosus*.

Physiological studies disclosed that the thyroidless salamander is less responsive to stimuli, utilizes less oxygen, is more edematous, has a lower erythrocyte and higher leucocyte count, and possesses a skin more permeable to water than the normal controls.

Cytological investigation revealed that cells of tissues subject to rapid replacement are most seriously affected since there is a tendency for the cells to age faster. The frequency of cell division is disturbed. The rate of cell division tends to be abnormally high after several weeks or months in the thyroidless state. Later the rate of mitosis becomes lower than normal.

Histological investigation revealed that the exocrin glands especially the granular glands of the skin, the serous glands of the tongue, the cardiac glands of the stomach, and the glands of the small intestine are structurally disturbed. In addition, liver, splenic, and gonadal tissue are affected in varying degrees. Morphological changes in the glands associated with the digestion of food are evident as early as 20 days after thyroidectomy. These structural modifications seem to be such as would interfere seriously with the proper functioning of the digestive system. In the later stages of the athyroid condition, tissues of all types manifest changes characteristic of the tissues of starving animals. Grave nutritional disturbances are evident and these may secondarily give rise to disturbances in basal metabolism.

Consideration of the data obtained together with data on thyroid function from other sources tends to disprove the assumption that the thyroid principle directly affects cell respiration and ixidations. There are four separate lines of data which seem to be incompatible with this hypothesis. These are, first the apparent ineffectiveness of thyroxine on the oxidative rate of tissues not connected with the host body, secondly the latent period which must elapse after the administration of thyroxine before the full effect

is obtained, thirdly the fact that the rate of mitosis may be higher than normal in animals well advanced into the athyroid condition, and fourthly drugs capable of raising the basal metabolism have no effect on the symptoms accompanying myxedema.

The facts seem to indicate that the subnormal basal metabolism which is characteristic of the thyroidless condition may be due to a lowered irritability of the tissues and to concomitant disturbances in the exocrin glands especially those associated with the digestion and assimilation of food.

THE EFFECTS OF THYROIDECTOMY ON THE SALAMANDER
TRITURUS TOROSUS (RATHKE)

by

ALFRED TAYLOR

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THE EFFECTS OF THYROIDECTOMY ON THE SALAMANDER TRITURUS TOROSUS

Introduction

The part played by the thyroid glands in the functioning of the vertebrate organism has been investigated for only a comparatively brief period of time. Experimental removal of these glands was first performed by Schiff in 1856 without consistent results. In 1884, he repeated his investigations with more success. Schiff was also the first to attempt to alleviate the symptoms accompanying hypothyroidism and athyroidism by the implantation of thyroid tissue.

Most of the experimental work prior to 1891, however, was of doubtful value because these earlier investigations involved the parathyroids as well as the thyroids. Since 1891 numerous types of animals have been subjected to experimental procedures involving the thyroids alone. Such work has been carried out mainly on young animals since the effects of thyroid malfunction is most strikingly revealed in immature organisms.

Sharpey-Schafer (75) has summarized the effects of thyroid removal for both immature and mature animals. His list of the abnormalities accompanying thyroidectomy though made in 1924 is still quoted with approval by Cameron (14) in his book published in 1934.

Sharpey-Schafer writes:- "In young animals the most striking effect is retardation of growth. Ossification is delayed ---. The whole skeleton is more or less involved and the animals remain far smaller than controls from the same litter. The teeth are illdeveloped. The generative organs remain relatively small, and the ova and spermatozoa may not come to maturity. The pituitary becomes enlarged ---. The aorta is often found to be atheromatous. The temperature of the body is lower than normal. The abdomen is generally swollen. The skin is thickened ---. The central nervous system, especially the brain, is involved in the general arrest of development. At all ages there is marked diminution in basal and in general metabolism.

"After complete thyroidectomy in adults creatin appears in the urine ---- . The limit of assimilation of carbohydrates is raised but there is no glycaemia or glycosuria. Adiposity may supervene but there is first usually a wasting. The muscles lose tone and are weaker than in the normal animal. Anemia is generally present with lymphocytosis. Heat production is diminished. Body temperature is low, the power of heat regulation lessened. There is diminished excretion of carbon dioxide especially when the external temperature is raised, and also a diminished consumption of oxygen, but the diminution of oxygen is less than that of carbon dioxide. The sexual functions are depressed. The nervous system is markedly affected ---. Many nerve cells, especially those of the cerebral cortex, exhibit a shrunken appearance ---. The skin is dry, the hair tends to fall out ---. There is delay in the healing of fractures. The red corpuscles of the blood are diminished in number and in amount of hemaglobin but the thrombocytes and leucocytes are increased."

In the ten years that have elapsed since Sharpey-Schafer summarized the effects of thyroidectomy in his book " The Endocrin Organs" many workers have concentrated on the thyroid in an effort to explain and describe more fully the morphological and functional changes accompanying malfunction of this gland in vertebrate organisms. In addition to the accumulation of data on the organic effects of hyper- and hypothyroidism many investigators have endeavored to solve the underlying problem of how the products of the thyroid glands are related to the vital functions of the body. The question, of the primary effect of thyroxine in the economy of the vertebrate organism has been receiving much attention.

The chemistry of the thyroid glands has been studied extensively. Kendall (45) brought to a culmination work in this field when he isolated the active principle of the thyroids known as thyroxine. Harington and associates (38) have determined the structure of thyroxine. They have demonstrated it to be beta-tetra-iodo-(3,5,3,5,)-4-(4-hydroxy-phenoxy)-phenyl-alpha-amino-propionic acid. With the chemical structure solved Harington was able to synthesize thyroxine in 1927. Numerous investigators have established the fact that thyroxine is the chemical entity responsible for the effects associated with the thyroids and it has been demonstrated to be chemically the same throughout the vertebrate series of animals.

In spite of the progress which has been made, the problem of the manner in which the thyroid principle is concerned with the welfare of the body is still unsolved. Cameron (14) after a survey of the various ideas on this subject concluded that the primary function of the thyroid secretion was to facilitate cell oxidative and respiratory processes. Practically every worker of note in this field of investigation agrees with Cameron's interpretation of the available data. Later in the course of this paper after the experimental data have been presented, the prevailing concept of the way the thyroid secretion affects the bodily functions will be considered in detail. However, it may be stated here that there is much evidence against the view that thyroxine is directly concerned with cell oxidation and respiration. In fact the whole subject of the relation between the thyroid glands and the rest of the body is still, as all workers in this field agree, for the most part unknown.

Problem

In the present paper, an effort is made to approach the problem of the role of the thyroids in the vertebrate organism by a histological and cytological study of organs and tissues in relation to the athyroid condition. Data derived in this manner are then supplemented by the results of researches on thyroid gland effects from other sources and the attempt is made to analyze the known facts in relation to the manner in which the thyroid principle is concerned with the functioning of the body.

Acknowledgements

My appreciation and gratitude are extended to Dr. Nathan Fasten for the encouragement and helpful criticism which he has given me during the course of this work. I also wish to extend my thanks to Dr. H.A. Scullen and Dr. Rosalind Wulzen for their assistance and cooperation.

Materials and Methods

For this investigation, adult salamanders (Triturus torosus) of approximately the same size and weight were utilized. Controls and experimental animals were kept under identical conditions so far as possible. A diet of ground beef varied with chopped earthworms kept the animals in splendid shape. It was noted that the laboratory animals appeared better fleshed and at least as healthy as salamanders living in the wild state. Some of the specimens have been kept for over two years and have remained in good health throughout that time. No trouble has been experienced with fungus diseases or any other pathological conditions.

In the course of this work, over one hundred and fifty animals, males and females in equal proportions, were thyroidectomized. Other groups of animals were subjected to parathyroidectomy and thyroparathyroidectomy. These latter operations were performed as a check on the main problem of thyroid study.

Salamanders used in the experimental work were anesthetized by immersion in an aqueous solution of ether. Care was taken not to use concentrations of the anesthetic which would act too violently on the animals, It was found that the best procedure was to immerse the animals in a 1.5 percent aqueous solution of ether for a few minutes, then in tap water for a short time, and finally in the ether solution again until the required depth of anesthesia was obtained.

In Triturus torosus , as is true for most amphibia, the thyroids are located alongside the geniohyoid muscles just at the anterior borders of the scapulas. They are enclosed in a capsule of connective tissue and on their dorsal surfaces in close association with them are the first parathyroids. In order to remove the thyroids a lateral slit is made with fine scissors parallel to the mandible and back into the gill-vestige region. If properly done, One small cut severs the skin and superficial muscles leaving one of the thyroids exposed. No blood flows up to this time if the incision has been made correctly. With the aid of a pait of specially prepared forceps and a binocular microscope (21 times magnification) the parathyroid which clings to the dorsal surface of the thyroid is freed from its attachment. Then the thyroid, after the blood vessels which supply it are crushed, is pulled up to the surface of the incision and cut out with the scissors.

Usually there is no loss of blood. The other thyroid is removed by the same procedure.

In some instances the incisions were closed by a single suture but this is not necessary as a rule. Also the practice at first was to keep the animals after the operation in separate containers using elaborate precautions against infection. Later it was discovered that as soon as the animal had recovered from the anesthesia it was better to place it in an aquarium with a group of its kind. Healing and recovery were facilitated by this treatment.

The animals usually ate the second day after the operation. A few animals in the earlier work died as a result of the operation but after a little practice the loss from this source ceased altogether.

The thyroidectomized animals were examined daily for external changes which would indicate the onset of the athyroid condition. Groups of animals were killed at periods of 20, 40, 60, 90, 120, 140, 150, 160, 180, and 190 days after the operation. Some of these were preserved for cytological and histological examination of various types of cells and tissues. Others from these groups were utilized for determining the more general effects of thyroid removal on such factors as permeability of the skin to water, specific gravity of the deviscerated body, water content of the tissues, and general behavior.

At the same time that these experimental animals were examined, normal controls were investigated in the same manner and used as standards for comparison. Since there is considerable morphological difference between males and females in this species of salamander, comparsons were always made between animals of the same sex.

In the course of this work, it was discovered that the individual differences among the normal controls were considerable. It was found that almost every tissue and many of the individual cell characteristics varied to some degree from one animal to another. This fact necessitated the examination of a large group of normal animals in order to obtain an average picture of each structure so that in preparing standards for comparison, there would be adequate allowance made for individual differences. To still further safeguard the data from this source of error, only such changes in the experimental animals were associated with the athyroid condition as appeared in all the thyroidectomized animals and in none of the controls.

In the preparation of the tissues for histological and cytological examination, it was essential to use a uniform technique throughout the series of both experimental and control animals.

Since the cytological investigations were concerned primarily with the nucleus, most of the tissues for this study were fixed in Bouins fluid and stained with iron haemotoxylin. Da Fanos silver impregnation method was found useful for bringing out the cytoplasmic bodies.

For the histological study of the tissues several techniques were employed, depending on the type of tissue investigated. In the case of the alimentary canal, Bouin's, Zenker's, and Da Fano's mixtures were found to be the most satisfactory fixatives. Bouin's and Zenker's fluids were especially valuable. Iron haemotoxylin, with or without eosin as a counterstain and thionin, with or without acid fuchsin as a counterstain were the stains used for most of this work. The spleen and kidneys were fixed with Zenker's and Bouin's reagents and stained as described for the alimentary canal. Liver was fixed in Zenker's or Bouin's fixatives and 90 percent Ethanol. Haemotoxylin with eosin as a counterstain were used as stains. Liver fixed in the Ethanol was used for the microchemical detection of the liver glycogen in accordance with the recommendations of Halliburton, Hewitt, and Robson (37).

For the integument, fixation in Bouin's fluid served best with iron haemotoxylin dedifferentiated with picric acid as a stain. This staining procedure causes the mucus and granular glands to be sharply differentiated from each other as a result of specific color tone and because the granules in the granular glands are made clearly visible, being dark brown in color in contrast to the bluish pale hue of the rest of the cytoplasm.

After much preliminary experimentation, it was found that the salamander brain was best treated for the purposes of this investigation by the Cajal silver impregnation method and by the Bouin-haemotoxylin combination. The Cajal silver impregnation method is especially valuable for the exposition of the various nerve tracts and brain nuclei while the Bouin-haemotoxylin method is useful in the study of the macro- and microscopic form of the cerebral lobes. Thionin also proved to be a good stain after the Bouin fixative.

In connection with the cytological investigations, the rates of cell division for several tissues were obtained. The mitotic activity of the skin was based on the number of mitoses observed in the epidermis per linear centimeter. For the purpose of this work, a cell was considered to be undergoing division whenever the chromosomes were visible in the form they assume during mitosis.

The rates of cell division for such tissues as the spleen, liver, and small intestine were based on the number of mitoses per square centimeter.

Before the index of mitotic activity for the cells of a tissue from an individual animal was calculated, many counts were made and several slides examined. Where linear measurements were used such as for the skin a minimum of 50 centimeters of epidermis was utilized. This work of calculating rates of mitotic activity required several weeks of painstaking study.

In addition to cytological and histological investigation of the effects of thyroidectomy, some of the more general changes accompanying the athyroid state were studied. Blood counts of both erythrocytes and leucocytes were made using a Levy counting chamber equipped with the Neubauer double ruling. Permeability of the skin to water was calculated by recording the loss of weight of experimental and control animals when exposed to the air. Specific gravities were figured by the relation of the animals weights in air to the weights of the water their bodies displaced. Percentage of water in the tissues was estimated by weighing the body immediately after death and with all viscera removed and weighing the carcass again after drying in the oven at 62 degrees centigrade until the weight became constant. The loss of weight, of course, represented the original water content of the tissues.

Modifications After Thyroidectomy

a. External appearance

Thyroid removal in Triturus torosus induces skin changes which rapidly alter the appearance of the animal. The skin becomes blackened, tends to be thicker, and assumes a texture which is entirely different from that of normal animals. (Figures 4, 5, and 6) Females are more affected than males. The male skin after a few months becomes intensely black and smooth. The female skin blackens as much as the males but assumes a rough frayed appearance. In advanced stages, the female skin may crack in places, though this condition usually occurs only when the animal is near death. During the final stages of the thyroidless condition, the female skin is so rough and thickened as to require that the animal be kept constantly submerged in water. The slightest drying at such times seems to cause the salamander much distress.

The male, as has been mentioned already, does not manifest the same type of skin changes as does the female in so far as texture is concerned. The skin in this instance, while differing emphatically from the normal type, still seems to adapt itself much better to the thyroidless condition than does the skin of the female. In advanced stages of the athyroid condition, the skin of the male salamander is soft, smooth, and pliable.

If one or more of the parathyroids are removed with the thyroids, the skin in both males and females assumes a thinner, smoother condition.

b. Permeability of the skin

Though the skin becomes thicker after thyroidectomy, it is nevertheless more pervious to water. At least water passes out through the skin more rapidly than is the case with normal animals. Since much of the blackened condition of the skin arises from the fact that moulting has ceased and several layers of cornified skin adhere to the surface of the animal, (Adams et al (2), (3), (4), and (5)) it would seem that permeability to water would be lowered. Actually, however, the tendency for thyroidectomized salamanders to perspire at a higher rate than normal controls is an invariable phenomenon.

Table 1. records the loss of water through the skin for groups of experimental animals and controls. It will be noted

TABLE 1.

Water loss by perspiration in normal and thyroidless salamanders.

animals group 1	average wt. of each grams	temperature centigrade degrees	relative humidity percent	water loss per hr. per gm. wt. grams
Controls (6)	19.52	24	41.5	.00138
thyroid-less (6)	20.8	24	41.5	.00213
group 2				
Controls (7)	20.7	20.8	30.0	.00171
Thyroid-less (7)	20.2	20.8	30.0	.00214
group 3				
Controls (6)	19.2	23.0	49.0	.00109
Thyroid-less (6)	18.3	23.0	49.0	.00131
group 4				
Controls (8)	18.4	23.2	36.0	.00183
Thyroid-less (8)	18.7	23.2	36.0	.00234

that under varying degrees of temperature and relative humidity the water loss per hour per gram of weight is always greater in the thyroidless salamanders. With increase of time in the athyroid state this tendency is intensified. The skins of individual animals which showed the syndrome accompanying the thyroidless state in an acute form were more permeable to water than those reported in the table.

c. Changes in form

Aside from the skin, thyroidless salamanders manifest changes in general form. The limbs in advanced stages are thinner in proportion to the rest of the body, the head appears narrower, the eyes are rimmed around by a lighter toned skin, and the vestigial gill pouches become accentuated.

d. Behavior

Salamanders well advanced into the thyroidless state tend to be very sluggish and slow to respond to external stimuli. On occasions, however, they display considerable activity and restlessly seek a way out of the aquarium. During the more acute stages, the animal apparently is subject to considerable pain and discomfort.

When thyroparathyroidectomy is carried out the animal manifests heithened sensibility to an extreme degree. The removal of the thyroids and the parathyroids which are normally in close association with them considerably increases the irritability of the animal.

e. Oxygen requirements

Thyroidless animals are able to remain submerged in water that has a low oxygen content much longer than is true for normal salamanders. A group of 20 experimental and 20 normal animals timed under exactly the same circumstances showed that normals came up for air on an average of once every 72 seconds while the average for the thyroidless salamanders was once every 148 seconds.

Indications are that the longer the animal has been thyroidless and the more severe the athyroid symptoms are the less oxygen per unit of time is required. At least there was a tendency for animals which had been thyroidless for long periods to be able to withstand lack of the normal supply of oxygen for longer intervals than animals which had not been in the athyroid condition for so long a time. Hence it would appear that the ability to remain submerged in water not sufficiently oxygenated for longer periods than is possible for the normal animal is not due to greater efficiency in the way of utilization of the oxygen present but is more likely due to a lowered demand resulting from a slowing down of general metabolism.

f. Edema

The experimental animals ate well. Even when the athyroid symptoms became severe they attacked their food as eagerly as the controls. In most instances, however, the experimental animals were thinner than normal. Some individuals became edematous to the point where the torso, the limbs, and the tail assumed a puffy appearance. (Figure 5). As a rule when extreme edema did arise it was of relatively short duration, but all the experimental animals were edematous to some degree. This condition seems to be generally associated with the thyroidless state.

In line with the increased water held by the tissues in the athyroid state is the fact that the specific gravity of the body as a whole is lowered. The average specific gravity for a group of 40 controls was 1.036 and for a group of 27 thyroidless salamanders the specific gravity was .990. Determination of the water content of the deviscerated bodies of a group of 15 controls and a like number of the experimental animals gave for the normals an average water content of 71.3 percent and for the thyroidless 74 percent.

g. Blood cell counts

Blood cell counts show a decided tendency for thyroidless animals to have a lower red blood cell count and a higher leucocyte count. Individuals may differ in this respect. In some instances apparently secondary conditions such as the break down of the spleen intervene and the erythrocyte count may be much increased over the normal or the leucocytes may be present in less numbers than normal. These exceptions to the general tendency are rare in the early stages but when the athyroid condition has developed fully, more variability in this regard obtains.

Blood counts made on 45 control animals gave an average for the erythrocytes of 143,000 per cubic millimeter and for the leucocytes an average of 11,800 per cubic millimeter. In the case of 33 selected experimental animals the average counts were erythrocytes 128,500 and leucocytes 13,750.

h. The viscera

Gross dissection of thyroidless salamanders reveals certain changes in the visceral organs which appear to be characteristic accompaniments of thyroid disfunction. The stomach is usually enlarged, often to twice the normal size. The liver and pancreas viewed superficially appear normal. The spleen is almost invariably enlarged and in addition may contain varying amounts of necrotic tissue. In some individuals only a small percentage of the spleen was still alive.

The adrenals are much increased in size often being several times larger than those of the normal controls. The kidneys likewise tend to hypertrophy anatomically in thyroidless animals.

i. Cerebral lobes

The brain manifests no external changes from the normal except in instances where the animal has attained an extreme athyroid condition. In such cases the cerebral lobes have a shrunken appearance and are less firm in texture.

j. Muscle tone

In all specimens, the muscles are lacking in tone. It is quite easy to pick out the thyroidless specimens from a mixed group by using the sense of touch alone. The muscles are characteristically soft and yielding in quality. However, this condition is not noticeable in the experimental animals for two or three weeks after the removal of the thyroids. Before the muscles begin to lose their tone other symptoms of thyroid insufficiency become evident such as skin effects as well as certain histological and cytological changes.

Cytological Considerations

a. The cell as a whole

The cell as a whole, in those tissues most seriously affected by thyroid removal, displays characteristic changes in comparison with the cells of normal tissues. In the skin, for example, the cells appear to age faster. Disintegrating cells are found lower down in the rows of epidermal cells than is the case with normal integument and many of the cells of the basal layer reveal distinct perinuclear spaces, a condition rarely encountered in the skin of the controls. The nuclei become more irregular in size and the chromatin material of cells above the basal layer shows not only a peculiar distribution but also an abnormal appearance. (Figures 11, 12, 13)

Distinct reactions of the cells to the athyroid condition are especially noticeable in all epithelial tissues, the spleen, subserous coat of the liver, secretory cells of the kidneys, the gonads, and the cells bordering the ventricles of the cerebral lobes. The cells of the exocrin glands also are seriously affected notably those of the mucus and granular glands of the skin; the mucus glands of the mouth, esophhagus and stomach; the cardiac and pyloric glands of the stomach and the glandular cells of the kidneys, The cytoplasm of many of these glandular cells appears to be eaten away from the lumen outward giving them a fryed, worn appearance unlike the cytoplasm of the cells of normal glands. (Figures 9-10,24-25,29-30)

The phagocytic cells of the liver, the so-called Kupfer cells, are affected. The cytoplasm of these cells contains more debris than similar cells in normal controls. The cytoplasm of the liver cells proper in advanced cases is much less dense than normal.

The germ cells of the testes are very sensitive to thyroid disfunction in the salamander. In a few months after thyroidectomy, the primary and secondary spermatocytes and spermatozoa of the testes undergo complete degeneration.

b. The nucleus

Changes in the nucleus are discernible in cells undergoing rapid proliferation. As would be expected, the nuclei of cells which do not undergo mitotic division are very little affected. But the nuclei of the cells of such tissues as the epidermis, the epithelial lining of the alimentary canal, and the exocrin glands often reveal changes in shape, size, and distribution of chromatin material. Within a space of three weeks after thyroidectomy, effects on some nuclei are evident and as the athyroid state progresses more nuclei become involved.

One of the most persistent changes which involves the nucleus is an increase in the variability of nuclear size. The average size of the nuclei of the epidermal cells becomes definitely greater. Individually large nuclei are common especially in the epidermis of salamanders thyroidless for a comparatively long space of time - five or six months. Table 2. shows the changes in nuclear size of the epidermal cells of a series of salamanders killed at varying periods after thyroidectomy. The epidermal cells of normal controls serve as standards for comparison. These changes in nuclear size are all the more significant since the nuclei of comparable cells in normal animals are remarkably constant in this respect.

The nuclei of the cells of the gastric epithelium do not reveal any consistent tendency towards increased size if the average of groups of cells is considered. (Table 2). But here

TABLE 2.

Size of nuclei in normal and experimental salamanders.

animal	time thyroidless days	tissue	nuclear sizes micra	ratio to normal percent
Control	0	epiderm.	14.1-14.18	100
MFI	20	"	15.40	108.8
MF9	60	"	16.01	113.0
M8	90	"	14.85	105.0
MF4	120	"	14.00	99.0
MI	140	"	14.80	104.0
M3	150	"	15.50	109.0
MF6	160	"	15.35	108.5
M2	160	"	16.40	116.0
MF3	180	"	16.10	114.0
M4	190	"	17.20	121.0
Control	0	small intestine	18.3-18.6	100
MFI	20	"	18.9	102.0
MF9	60	"	19.0	103.0
MF8	90	"	19.2	104.0
M2	160	"	18.6	100.5
MF6	160	"	18.7	101.0
Control	0	stomach	17.6-17.8	100
MF8	90	"	19.0	107.0
MF2	150	"	18.5	105.0
M3	150	"	17.6	100.0
MF3	180	"	18.2	103.0
Control	0	forebrain	15.3-15.5	100
MF9	60	"	15.5	100.0
MF4	120	"	15.8	102.0
MF6	160	"	15.5	100.5
M2	160	"	15.4	100.0
MF3	180	"	15.3	99.50

again, there is more variability than is true for the nuclei of the gastric epithelium of normal salamanders and individual extra large nuclei are common.. A like condition is encountered among the nuclei of the cells of the various exocrin glands and the spleen.

Another pecularity of the nuclei of the cells of epithelial tissue of thyroidless salamanders is the tendency for individual nuclei to break up into fragments. (Figure 12). While only a comparatively small number of nuclei are found in this condition they are scarcely present at all in normal tissue. The epidermis more than other tissue contains these disintegrating nuclei, and the time that has elapsed since the removal of the thyroids determines the frequency of this phenomenon. The longer the athyroid state has prevailed, the more nuclear disturbances of this type appear.

c. Mitosis

Cell division, for the most part, is not appreciably disturbed. Some dividing cells, especially in the epidermis, show a disposition toward abnormal clumping of the chromosomes in the late metaphase and early anaphase. Nevertheless new cells are formed which aside from the peculiarities already mentioned differ little from the cells of normal tissue.

The principal effect of thyroidectomy on cell division is to change the frequency of mitosis. Figures 1,2, and 3 disclose

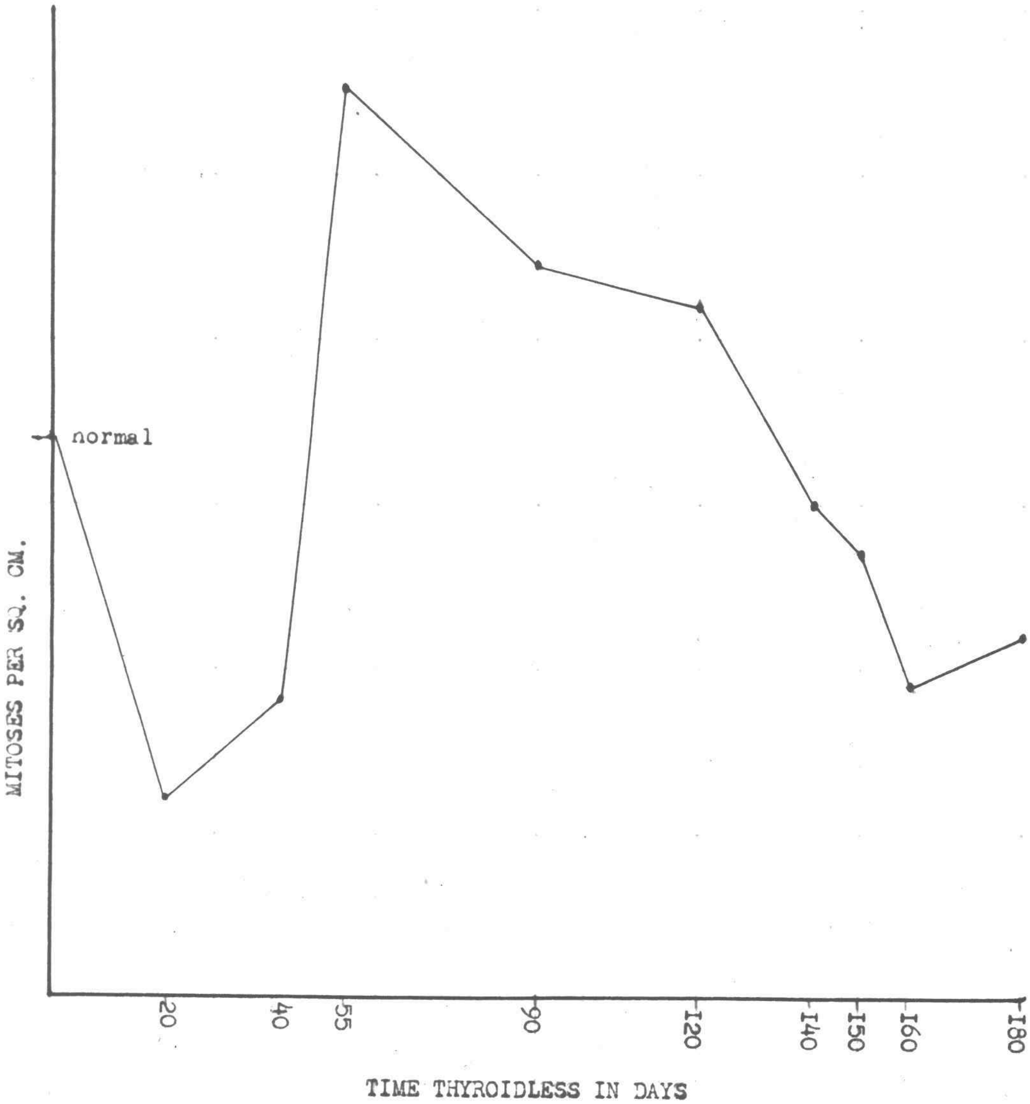

Figure I. Small Intestine

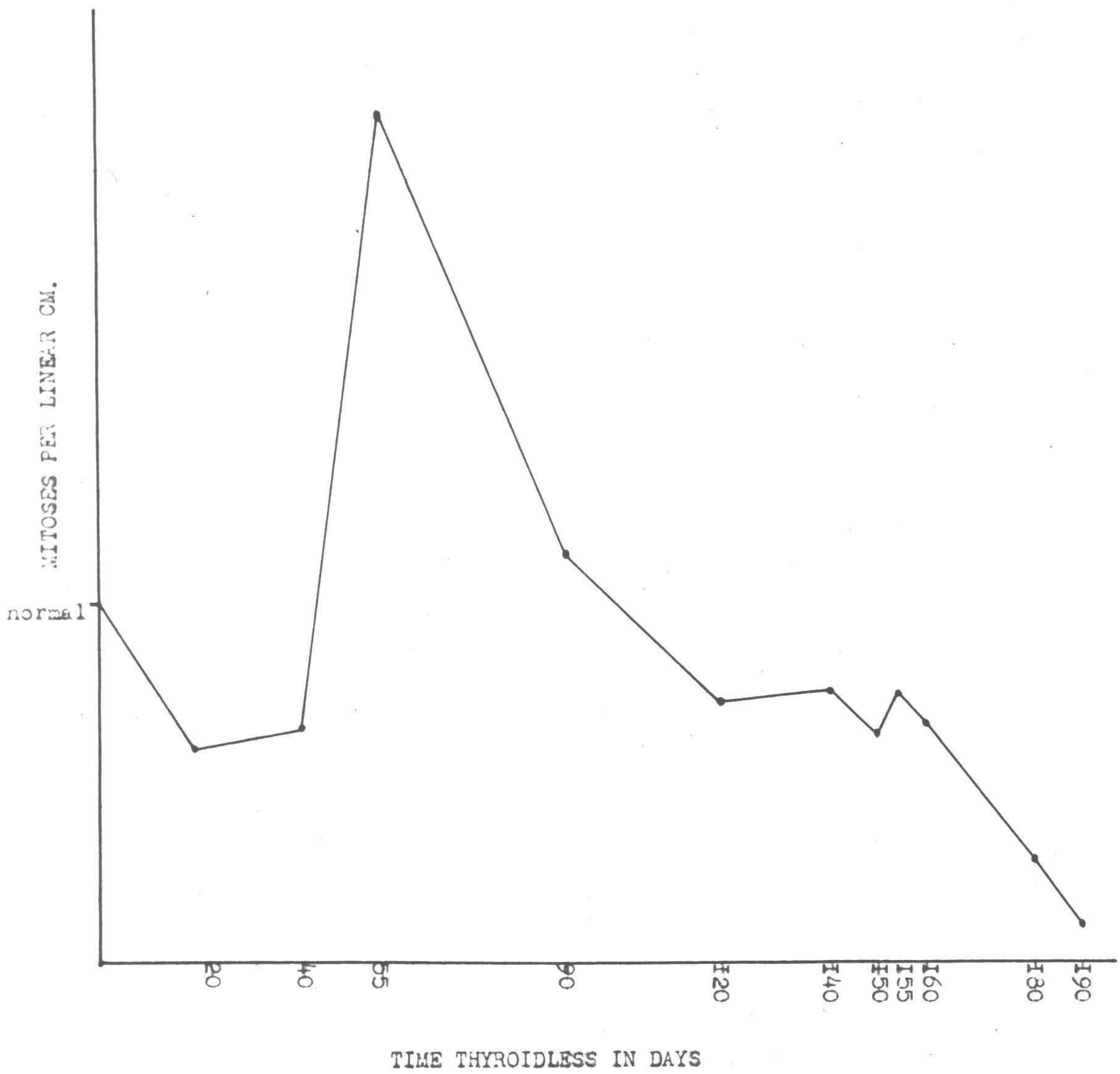

Figure 2. Epidermis

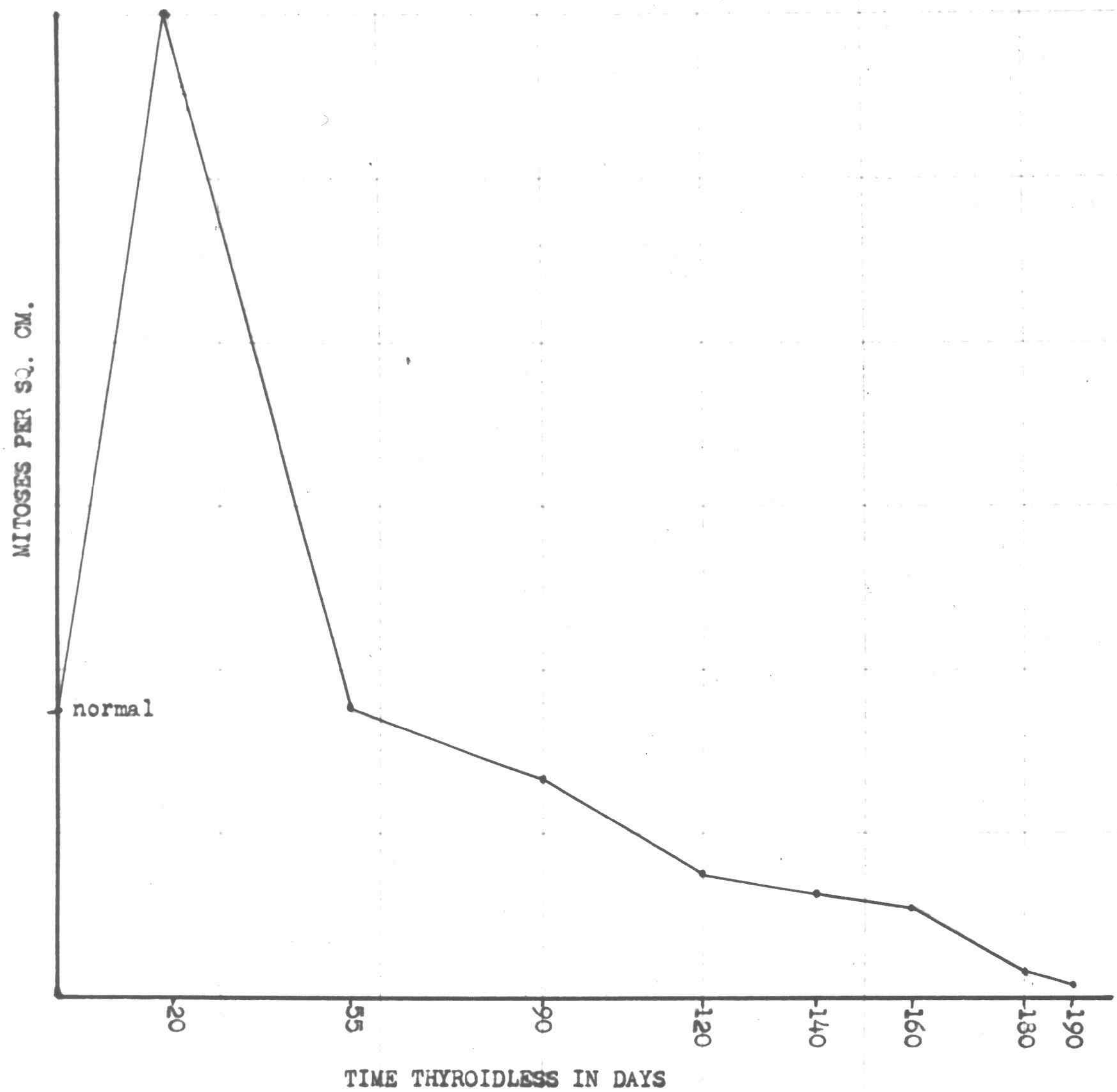

Figure 3. Spleen

a well defined tendency for the cells of the basal layer of the epidermis, of the spleen, and of the epithelial lining of the small intestine to change their rates of division. It will be noted that the tendency is for the mitotic rate to rise above normal in the first few weeks or months after thyroidectomy has been performed. Later there is a decline in the mitotic rate. After the animal has been in the athyroid state for a number of months, the rate of division in the cells of the tissues represented in the figures, tends to be decidedly subnormal.

The subserous coat of the liver, the kidneys, and the glandular tissue of various types were investigated from the standpoint of the rate of cell division but no consistent trends were evident. These tissues from one experimental animal would manifest a cell division rate much higher than normal while in the case of another thyroidless salamander operated upon at the same time the rate might be lower than normal.

d. Summary of cytological effects

The present study reveals definite cytological disturbances which are concomitant to the thyroidless state in the salamander Triturus torosus. Briefly, these are:

1. The cells of tissues subject to rapid replacement are most seriously affected.

2. The cells tend to age faster in tissues such as the epidermis, epithelial lining of the alimentary canal, and the

exocrin glands.

3. The nuclei of affected cells tend to irregularity in size and in the appearance and disposition of chromatin material.

4. The rate of cell division in tissues such as the spleen, the epidermis, and the epithelial lining of the small intestine deviates from the normal frequency.

Histological Considerations

In presenting the data on the histological effects of thyroidectomy in the adult salamander, it will be more convenient to treat each of the tissues studied from this viewpoint separately. Tissue effects were investigated under the following heads: the integument, the tongue, the esophagus, the stomach, the small intestine, the liver, the spleen, the kidneys, the gonads, and the cerebral lobes.

a. The integument

Histologically, the skin of the salamander suffers striking changes as a result of thyroid disfunction. In addition to the external changes previously described, fundamental changes in the internal integumentary structure are observable.

In both males and females the skin (epidermis) becomes thicker and assumes a leathery texture. The changes in skin thickness in salamanders thyroidless for varying lengths of time are shown in Table 3.

TABLE 3.

Thickness of the epidermis in normal and thyroidless salamanders.

animal	sex	time thyroidless days	epidermis thickness micra
Control	male	0	59.0
Control	female	0	68.5
MF1	female	20	71.5
MF5	female	50	77.0
MF9	female	60	81.5
MF8	female	90	86.5
MF4	female	120	81.5
M1	male	140	90.5
M3	male	150	65.0
MF6	female	160	74.0
M2	male	160	77.0
MF3	female	180	82.5
M4	male	190	90.5

the glands associated with the integument are apparently more disturbed by the athyroid state than any other skin structure. After a very short time in the thyroidless state, the granular glands of the salamander skin become extremely abnormal. In many instances, they appear to have broken down altogether. The spaces once occupied by them become filled with a watery liquid and debris. The mucus glands show the same tendencies as do the granular glands but in a much less acute form. (Figures 7-8, 9-10).

During the earlier stages of the thyroidless condition, the destruction of the skin glands is associated with the rapid formation of new glands from the epidermal elements of the integument. In more advanced cases this compensatory gland formation ceases almost completely. After this stage the granular glands become much rarer in the integument than normal.

Besides the epidermal and glandular elements, the connective tissue of the dermis is characteristically changed by thyroidectomy. It becomes vacuolated, less compact, and has a fragile appearance. (Figures 9-10)

b. The tongue

The effect of the thyroidless state on the tissues of the tongue are similar in general to those already described for the integument. The epithelial coat becomes thicker and more vacuolated, the connective tissue of the deeper portions of the tongue resembles that of the corresponding part of the skin, and the mucus glands are affected though not seriously.

The goblet cells of the tongue become much enlarged in size. These glands appear to be functionally disturbed. The same is true for the serous glands of the tongue. In these latter glands the space occupied by them is larger, the cytoplasm of the gland cells less dense, and the nuclei smaller than is true for corresponding cells and structures in the tongue tissue of normal controls. The granules of the serous glands of thyroidless animals are present in smaller numbers and stain more lightly. (Figures 14-15).

c. The esophagus

This portion of the alimentary canal manifests changes in the epithelial layer comparable to those mentioned for the tongue and the integument. The glandular elements especially the goblet cells reflect serious structural and functional disturbances in the instance of animals thyroidless for a few months. There seems to be a tendency for the goblet cells to be present in increased numbers. In some cases these glands become so numerous as to occupy the greater part of the epithelial surface.

The mucus glands of the esophagus show the same type of abnormalities as those characteristic for mucus glands under athyroid conditions in other tissues. In extreme cases the esophageal glands as a whole appear to be so disturbed morphologically as to render normal functioning difficult.

d. The stomach

The stomach resembles the esophagus in its reaction to the absence of the thyroid principle from the body. However, there is more glandular tissue present in the case of the stomach and accordingly thyroidectomy has a more disturbing effect on this organ.

The cardiac glands found in the anterior half of the salamander stomach are adversely affected. Within three weeks after thyroidectomy has been performed, these glands show changes in general structure which indicate impairment in the digestive processes in which the cardiac glands are involved. The main and most consistent change observable in these glands is a wearing away of the gland cells from the vicinity of the gland ducts outwards. This structural modification is so marked that the masses of cardiac glands under high magnification appear to be worn into holes. (Figures 26,27,28,29,30).

The mucus glands of the stomach are of lesser importance than the cardiac glands. They do not show as much structural change.

The pyloric glands located in the posterior half of the stomach are affected almost as seriously by the thyroidless state as are the cardiac glands. They become enlarged, the nuclear material of the gland cells becomes smaller and there is a general tendency for these glands to show structural abnormalities. No specific change in the cytoplasm of the pyloric glands is evident since the cytoplasm of these glands is normally clear and transparent and hence it is difficult to detect possible morphological variations from the normal.

The secretory epithelial border of the stomach manifests characteristic changes in structure. The mucigenous border is invariably much wider and more massive, the tall columnar cells form a wider border than normal, and both the nuclei and cells as a whole tend to be longer and narrower in shape than comparable cells from the stomachs of control salamanders.

If one studies the gastric tissue from animals well advanced in the thyroidless state, all of the abnormalities mentioned above are greatly magnified. It appears that lack of the thyroid principle leads to serious malformation in the glandular elements of the stomach.

e. The small intestine

The small intestine, in agreement with the rest of the alimentary canal, is disturbed chiefly in its glandular elements by thyroid disfunction. In addition to changes in the goblet cells comparable to those found in the same glands in the esophagus, the epithelial layer as a whole is affected. The nuclei of the outermost layer of cells are decidedly nearer the lumen of the intestine than is true for normal tissue. This condition becomes accentuated as the athyroid state is prolonged. Table4, and figures 31-32 illustrate this tendency. It will be noted that in normal tissue the nuclei of the surface layer of columnar epithelial cells are two to five times as far back from the free edge of the cells as with thyroidless animals.

f. The liver

The subserous coat of the liver is affected by thyroidectomy. This capsular portion of the liver becomes thicker and more differentiated from the remaining tissues of the organ. In salamanders, this portion of the liver serves as the site for the formation of lymphocytes and eosinophil myelocytes. In the thyroidless animals there is a marked increase in the numbers of eosinophils in the subserous layer.

Throughout the liver tissue proper there is a very striking enlargement of the sinusoids and in extreme cases vacuole-like spaces permeate the liver substance. One of the very earliest responses of the liver tissue to the athyroid state is observed in the hepatic cells. After a very short period in the thyroidless condition these cells become smaller than the liver cells of the controls and after a few months have elapsed they shrink to less than one half the normal size. At the same time they become more homogeneous in appearance resembling in some respects the hepatic cells from the livers of starving animals. (Maximow and Bloom (56)).

Microglycogen determinations show the livers of athyroid salamanders to possess a richer store of glycogen than is the case with the livers of normal animals.

g. The spleen

During the first few weeks of the athyroid state, the spleen appears to undergo little specific change histologically. It enlarges and there is an active proliferation of the splenic cells but otherwise the tissue at this period appears normal.

Later distinct changes are observable. The outermost fibrous layer becomes thicker and lighter in color. There is a tendency for necrosis to set in which in extreme instances may result in the death of the major portion of the gland. Even before this stage develops, there is a noticeable diminution of the red pulp and the spleen tissue is paler than normal.

h. The kidneys

Like the other organs considered, the kidneys (mesonephros) appear to be progressively disturbed as the athyroid state is continued. Within three weeks, the glandular elements manifest morphological disturbances. The lumen of the secretory tubules becomes enlarged and the cellular elements become smaller. The collecting tubules likewise become enlarged and in some cases the enclosing cells are reduced to a thin ring. (Figures 24-25).

The glomeruli show morphological changes in association with thyroidectomy. The arterioles become much enlarged and in some instances appear to be empty. The capsule enclosing the glomerulus becomes larger, so much so that in cross section the glomerulus fills only a small part of the capsular space, a condition in contrast to the normal appearance where the glomerulus practically fills the capsule. (Figure 24-25).

The outer fibrous layer of the amphibian kidney has a frayed, less dense appearance in thyroidless animals as compared with the controls and it also tends to be thicker than normal.

i. The gonads

In the salamander the athyroid condition is accompanied by complete disorganization of the gonadal tissue. Both testes and ovaries are affected though the testes are more sensitive to thyroid disturbance and are more quickly reduced to a characterless mass. The changes which occur in gonadal tissue are of such a devestating nature that to all intents and purposes, it ceases to have a part in the structure of the organism. (Figures 16-17).

j. The cerebral lobes

Careful examination of the fiber tracts, nuclei, and cell sizes failed to reveal any definite change which could be ascribed to the lack of thyroid secretion. Before passing judgement on the morphological reactivity of brain tissue to thyroid disfunction, it will be necessary to make a more exhaustive investigation of these parts than was within the scope of the present study. In the foregoing consideration of the histological changes accompanying thyroidectomy in the salamander, tissues such as glandular and epithelial tissues which are subject to rapid replacement are most seriously affected. Brain tissue is not of this type and for that reason may be less disturbed structurally by the athyroid condition.

k. Summary of histological effects

Histological changes affecting many tissues have been shown to characterize the athyroid condition in the salamander Triturus torosus. These may be summarized as follows:-

1. The glands of the integument and of the digestive tract manifest varying degrees of morphological disorganization.

2. The subserous layer of the liver and the liver tissue proper show structural abnormalities. The quantity of stored glycogen is greater in the livers of thyroidless salamanders.

3. Structural changes in the spleenic tissue are evident together with a tendency for necrosis to develop.

4. Kidney tissue suffers marked changes in the malpighian capsules and in the collecting and secretory tubules.

5. Gonadal tissue tends toward morphological disorganization.

6. Brain tissue of the cerebral lobes appears to undergo no structural changes as a result of thyroidectomy.

Discussion

Microscopic examination of the tissues of thyroidectomized animals has been limited, most workers investigating the physiological and gross anatomical changes accompanying this operation. However, before attempting to evaluate the implications of the data obtained in the present investigation, it is necessary to consider briefly the pertinent results of other workers in this field. This is necessary even though the greater part of the work to be reviewed is concerned with thyroid research carried out from a different standpoint than the present work. Results of a physiological or functional nature added to data more morphological in character should give a much clearer view of the problems under consideration.

a. The integument and thyroid malfunction

Adams and associates (2,3,4,5) studied the integument of thyroidectomized Triturus viridescens. They were concerned mainly with the connection between the thyroid and hypophysis and moulting. They showed that the athyroid condition inhibits moulting in the salamander thereby producing an increase in the cornified layers of the epidermis and a blackening of the animal generally. They also reported a distinct increase in the thickness of the epidermis as a whole.

Alderman (6) working also with Triturus viridescens reported similar skin changes after thyroidectomy. Noble and Bradley (61) reported comparable integumentary changes in the thyroidless lizard. In this instance moulting was not entirely inhibited as it is among the salamanders investigated but thyroid removal lengthens the interval between successive moults.

Contrary to these results is the report of Schaefer (74) who stated that in the case of the garter snake, thyroidectomy increases the frequency of moulting while feeding thyroid substance tends to inhibit it. It is difficult to understand why the garter snake should prove an exception in this respect.

The influence of the thyroid glands on the plumage of birds has been studied by many workers - Horning and Torrey (39), Parhon and Parhon (66), Torrey and Horning (81), Zavadowsky (90), Brambell (12), Giacomini (32), Giacomini and Taibell (33), Krizenecky and Nevalonnyi (47) (48), Occhipinti (62), Landauer (51), and Sainton and Simmonnet (72). The results of these workers and many others are in agreement in demonstrating that feeding of thyroid substance greatly increases the moulting and growth of the feathers in fowls. As Giacomini and Taibell have shown feather growth is so accelerated by thyroid feeding that there is insufficient time for the feather structure to be completed hence the change from the cock type of feathering to a more hen-like form/

The influence of the thyroid gland on hair and wool growth has also received much attention. Chang (17) after considering both hypo- and hyperthyroidism and its effects on hair growth suggested that there may be a functional optimum of the glands secretion necessary for normal growth. Insufficient thyroid hormone is accompanied by hair loss, while increased thyroid secretion above a certain degree increases hair growth.

Huestis and Yocom (42) working with Peromyscus reported increased hair growth as a result of thyroxine injection.

Simpson (77,78) reported that lack of thyroid secretion in sheep was accompanied by a lighter fleece and incidentally less growth in the horns.

Youmans (89) investigated the effects of thyrotoxicosis on skin tone. He concluded that the skin reflects the metabolic rate of the subject. The higher rate of metabolism is associated with better skin tone and the lower rate tends to produce a coarser more leathery type of skin.

Hoskins (41) reported that increased thyroid secretion is accompanied by hyperactivity of the skin glands and this condition is reflected in increased perspiration in the case of man. Wiggers (87) stated that there was no sweat secretion from human skin when there was thyroid insufficiency.

The various investigators on the relation between the skin and the thyroid glands agree generally on the following essentials:-

1. Absence of the thyroids is associated with a leathery, dry thickened epidermis and where moulting is usual this is either

entirely inhibited or the intervals between moults is lengthened.

2. Feeding thyroid substance to, or the injection of thyroxine into an otherwise normal animal is associated with increased moulting, increased hair growth or feather growth as the case may be and a general improvement in the tone of the skin. The dermal glands become hyperactive.

In addition to the above, the present investigation has shown that there are histological and cytological changes of importance in the integument associated with thyroid disfunction. It has been shown that the dermal glands, especially the granular glands are seriously affected leading to complete atrophy of the glands in extreme cases.

b. Alimentary canal

In the present paper data have been presented demonstrating structural changes in the epithelium of the mouth and esophagus in association with the thyroidless condition, comparable to the effects observed in the integument. Little work has been done by others on this portion of the digestive tract. Zavadowsky and Slotow (92) reported that in dogs the secretion of saliva is diminished in the athyroid state.

The stomach and small intestine in their relation to the thyroid secretion have been investigated extensively. Rogers, Rahe, and Ablahadian (70), Chang (18), Coelho and Rocheta (20), and Brown (13) have shown that the acidity and quantity of the secretion of gastric juice is delicately responsive to thyroid disturbances. Hyperthyroidism increases the acidity and quantity of the gastric secretion while hypothyroidism has the opposite effect. Wiggers (87). Such functional variations in the gastric glands are what would be expected from the data presented in this paper on the morphological changes in these glands which invariably accompany the thyroidless condition.

If the glands of the stomach are disturbed by thyroid dysfunction, one would expect insufficient or oversupply of the thyroid principle to disturb protein metabolism. Many workers have shown this to be the case. Miyazaki (59) reported that the injection of thyroxine markedly increases the specific dynamic action of proteins. Loeper, Lemaire, and Tonnet (54) stated that the thyroid is concerned with maintaining protein equilibrium in the blood. Dann, Chambers, and Lusk (23) reported that thyroidectomy prevents the increase in protein metabolism which is usual after the administration of phlorizin. Kommerell (46) found that thyroidectomized dogs are more reactive to thyroxine on a meat diet. Under these circumstances the basal metabolism is raised higher and lasts longer per unit quantity of injected thyroxine. Weymuller, Wyatt, and Levene (86) reported comparable results in the case of children. Wiggers (87) stated that nitrogen metabolism was reduced in individuals suffering from hypothyroidism.

The above reports indicate a close relation between protein metabolism and the thyroid gland. Other workers have shown the metabolism of carbohydrates and fats to be in some way linked with the thyroid principle. Thus Silberstein (76) reported a close connection between fat metabolism and the thyroid glands and Baumann (9), Miyazaki (59), Weymuller et al (86), and Hoskins (41) have reported likewise for carbohydrates.

It is evident that work done from a functional standpoint confirms the results given in the present paper. On the one hand the fact is emphasized that thyroid disfunction produces maladjustments in the digestion and assimilation of food and on the other, the glandular elements so necessary for proper digestion and assimilation are shown to be disturbed structurally.

c. Kidneys, liver, spleen, and gonads

Davidson (24) reported that hypothyroidism in man is sometimes associated with nephrosis and in such cases thyroid treatment brings relief. Goldberg (34) reported that thyroidectomized sheep and goats suffer renal abnormalities. Hoskins (41) stated that thyroid deficiency has a depressive effect on the kidney glands. In the present paper, it has been shown that striking morphological changes are evident in the kidneys of thyroidectomized salamanders.

However, the kidneys do not appear to react so quickly to the athyroid condition as do the integument and the lining of the alimentary canal. Hence the structural changes observed may have been due to the general disturbances suffered by the organism as a whole. Confirming this is the fact that the kidneys are not markedly affected until the thyroidectomized animal begins to exhibit the more acute symptoms of the athyroid condition. The glandular elements of the kidneys in common with the exocrin glands generally do appear to be directly affected by the thyroidless state. It may be therefore that both primary and secondary effects are involved in the case of these organs.

The liver appears to have received very little attention from the standpoint of its relation to the thyroid principle. Many investigators have reported the fact, confirmed again in the present paper, that the livers of hypothyroid animals tend to contain a richer store of glycogen than normal. Parhon (64) reported that glycogen storage in the liver bears an inverse ratio to thyroid activity in rabbits, dogs, sheep, and guinea pigs. Coggeshall and Greene (21) working with the albino rat showed that the liver glycogen was reduced by thyroid feeding.

The spleen has received little attention except that it has been reported to be larger than normal in thyroidless animals. Weil (85) .In the present paper it has been shown that not only does the spleen become enlarged in athyroid animals but there is a tendency for necrosis to develop. It is not known whether the spleen reflects the general bodily disturbances accompanying the athyroid state or whether there is a more direct connection with the thyroid principle.

Investigators who have observed the effects of the thyroidless state on the gonads invariably agree that these organs are adversely affected though here again it is unknown whether the effect is a primary or secondary one in so far as the thyroid secretion is concerned.

Goldberg (34) working with sheep and goats stated the gonads of thyroidectomized animals were smaller but still functional. Doderlein (25) reported that the testes were more seriously affected by the athyroid condition than were the ovaries. In the present paper, it has been shown that in the salamander the testes in advanced cases of athyroidism are completely degenerated with no functional sperm present. the Ovaries are almost as seriously affected.

d. Edema in thyroidless animals

Parhon (65) reported that the tissues of thyroidless sheep contained more water than the tissues of normal controls. Bernardbeig and Sendrail (11) stated that water injected subcutaneously in the ears of thyroidectomized rabbits was absorbed faster than water injected into the ears of normal rabbits. Ferrari (30) working also with rabbits concluded that lack of thyroid secretion was accompanied by an edematous condition in the tissues.

In the present paper, it has been shown that the tissues of thyroidless salamanders contain a higher percentage of water than the tissues of the normal controls.

e. The central nervous system

Everyone who has had any contact with thyroidless animals of any kind has noted the apparent sluggishness of response, the lack of spontaneous activity, and the generally apathetic demeanor which characterizes animals in this condition. In the present investigation such behavior has been recorded but very little in the way of specific morphological change has been observed in the nervous system itself. Some investigators have reported anatomical changes in the central nervous system in association with the thyroidless condition. Thus Kunde (49) reported lesions in the spinal cords of thyroidectomized rabbits. He reported also that there was progressive paralysis among his experimental animals. Sharpey-Schafer (75) reported neurons in the cerebral lobes of thyroidless animals to be shrunken and abnormal in appearance.

Most workers, however, have inferred disturbances in the nervous system from the observed functional disturbances. Chaney (19) noted that persons suffering from hypothyroidism exhibited tendon reflexes so slow as to be quite characteristic of the condition. He showed that ordinarily changes in metabolism do not have this effect but even a slight inadequacy of the thyroid secretion was followed by slower tendon reflexes. Thyroid feeding ot thyroxine injection always brought the reflex time back to normal

Oswald (63) reported that thyroglobulin feeding increased the sensitivity of the nervous system but seemed to affect some nerves more than others. Liddell (52) found that thyroidless sheep and goats made more errors in maze learning than the normal controls. Zavadowsky (91) reported that lack of thyroid secretion was accompanied by a gradual lessening of nerve sensitivity. He stated that conditioned reflexes in dogs under such conditions tend to slower response. Zavadowsky (92) continuing the same type of investigation thought the slower response of the conditioned reflexes in athyroid dogs was due to lower sensitivity of the cerebral lobes.

f. Sympathetic nervous system

Many workers have considered the problem of the relationship between the sympathetic nervous system and the thyroid glands. Cannon and Smith (15) reported that massage of the thyroids (cat) increased the heart rate 33 percent and that stimulation of the cervical sympathetic trunk had a similar effect. Stimulation of the cervical sympathetic trunk of thyroidless cats however, they found to be without effect on the rate of the heart beat. They concluded therefore that stimulation of the sympathetic nerves in the region of the cervical trunk caused an increase secretion of the thyroid principle. Cannon and Smith's data can be explained just as well on the basis of the thyroid secretion increasing the irritability of the sympathetic nerves or of the tissues activated by them.

Pottenger (68) from clinical data concluded that both the thyroid glands and sympathetic nervous system are concrened in the skin changes accompanying hypothyroidism. Kalnins (44) found that thyroxine in small doses increases the excitability of the para-sympathetic nerves of the intestine while large doses paralyze them. Meyer (57) considered that thyroxine affects both metabolism and the sensitivity of the autonomic nervous system. Pick (67) stated that the thyroid acts directly on all the tissues and that this condition in turn affects the sensitivity of the various parts of the nervous system. Bergwall and Kuschinsky (10) and Cutler and Schnitker (22) reported that the excitability of the sympathetic nervous system is increased as a result of the injection of thyroxine into the body. Kuntz (50) stated that many of the symptoms associated with hyperthyroidism arise as a result of the generally lowered threshholds in the sympathetic nerves due to excess thyroid hormone.

The work of these investigators seems to have established a relationship between the thyroid secretion and the sensitivity of at least some parts of the autonomic nervous system or of the tissues served by nerves of this type.

From the foregoing brief survey of data pertinent to the role of the thyroids in the economy of the vertebrate animal and from the data presented in the present paper, some general principles may be formulated which appear to be true for those aspects of the problem considered here.

I. The glands of the skin, the digestive tract, and perhaps of the kidneys and liver are disturbed both morphologically and functionally by thyroid disfunction. It appears likely that exocrin glands whereever found are sensitive to the condition of the thyroids.

2. The sympathetic and parasympathetic nerves and the central nervous system appear to be in some way dependent upon the thyroid secretion for their proper functioning.

3. The rhythm of mitosis in tissues which are normally subject to relatively rapid replacement is changed by thyroid malfunction. The athyroid condition first effects an abnormally high cell division rate to be followed later by a rate that is abnormally low.

4. The liver cells of animals in the athyroid condition resemble those present in the case of starving animals and the tissues in general of thyroidless animals tend to be more vacuolated, more transparent, and more fragile in appearance indicating serious nutritional disturbances.

g. Thyroxine and cell oxidations

The generally accepted theory which seeks to account for the interrelationship between the thyroid glands and the functioning of the vertebrate animal is that the thyroid principle governs the rate of metabolism of the body and that it does so through its effects on the oxidative processes of the individual cell (Cameron (14)). All other effects, it is thought, may be considered to be secondary to the primary one of interference with cell respiration. The assumption is that changes in metabolism are directly connected with changes in the quantity of thyroid principle present in the body.

Before commenting on this hypothesis, it is necessary to review data from various workers who have investigated the problem of cell respiration and the problem of cell respiration in its relation to the active principle of the thyroid secretion.

If thyroxine stimulates the respiration and oxidation of the individual cell then one would expect the metabolic rate of developing embryos and of tissue apart from the body to be increased by this substance. Woodruff and Swingle (88) using cultures of paramecia reported that no acceleration in division arose from adding thyroxine to the culture but rather the tendency was for division to be inhibited if the concentration of thyroxine was relatively high.

Meyer (58) reported that thyroid extract does not increase the oxygen utilization of yeast cells. Torrey (82) reported that thyroxine retards the cleavage rate of paramecia. Sturm (80) showed that thyroid tissue itself does not differ in oxygen utilization and fermentative power from other tissues of the body.

It might be objected and rightly that yeast cells or protozoa would not necessarily be expected to respond to thyroxine. The evidence, however, appears conclusive against the idea that thyroxine has any stimulating effect on tissue strips taken from vertebrate animals. Myhrman (60) reported that thyroxine had no stimulating effect on tissue oxidation and Dodds (27) after an exhaustive investigation stated that the thyroid principle had no stimulating effect either on the respiration or glycolysis of any tissue that had been freed from its normal connection with the host body. Cameron (14) wrote, " The thyroid principle has not been shown to influence any specific chemical reactions in vitro."

In contrast to the above many workers have shown that tissues in vivo are markedly affected in their respiratory activity by the hyper- or hypoactivity of the thyroids. Among the many workers who have confirmed this may be mentioned - Arnoldi (7), Verebely (83), Dye (29), Gerard and McIntyre (31), Rutsch (71), and Stare and Elvehrem (79).

Apparently there is no direct evidence for believing that the thyroid glands have an immediate influence on cell oxidations or cell respirations. The evidence indicates that only cells which are a part of a living vertebrate organism are affected in this regard by the thyroid principle. If the effects of the thyr_oid secretion were directly on the individual cells as is generally postulated - Aub, Bright, and Uridil (8), Weil (85), Dodds and Dickens (26), Hogben (40), and Cameron (14) - it should affect the oxidation of vertebrate cells in vitro.

Recently Dodds (27) has shown that chemical compounds such as 4:6-dinitro-o-cresol which can be used to raise the basal metabolism of a vertebrate animal also increases the metabolic activity of tissues in vitro. He found, however, that while this compound was capable of raising the basal metabolism of myxedematous patients above the normal level, it had no effect on the symptoms which characterize this condition. In a specific instance the drug was used on a man exhibiting typical myxedematous symptoms. His basal metabolism was raised to plus 20 without in any way ameliorating the effects of the disease. Thyroxine was used beginning at the same basal rate of metabolism at which the drug was commenced and the basal metabolism rose to plus 10 at which time the patient was perfectly normal and free from all signs of the hypothyroid condition.

Such findings as these throw still more doubt on the postulate that the thyroid glands act directly on tissue oxidation or tissue respiration. In fact the implication is that changes in basal metabolism so long considered to be directly related to the thyroid principle are really secondary effects. Otherwise drugs which restore the metabolic rate in myxedematous patients would at the same time alleviate the symptoms associated with that condition. The justification for this hypothesis in the past has been the fact that the hypothyroid condition is always accompanied by a lowered basal metabolic rate and the hyperthyroid state by an increased basal metabolic rate. Since the basal metabolic disturbances follow the thyroid malfunctions, it has been assumed that the latter was the cause and the former the effect in a very direct sense.

Another fact which makes it seem improbable that the thyroid glands can directly affect cell oxidation is the latent time which must elapse after the feeding of thyroid substance before increased metabolism ensues. This delay is such that no perceptible reaction is discernible for two or three days and the peak of the effect is not attained for ten to fourteen days. Facts such as these point to the possibility that disturbances in cell oxidations in the case of thyroid malfunction are an effect rather than a cause of the symptoms present.

In the present paper it has been shown that the rate of cell division in such tissues as the epihelial lining of the small intestine, the epidermis, and the spleen is stimulated by thyroidectomy and that the rate rises sharply, only falling below normal as the animal becomes seriously impaired. Such mitotic activity would seem to be incompatible with the theory that the thyroid glands are concerned with tissue oxidation and respiration. This is so since Loeb (53) and Warburg (84) have shown cell division to be inhibited if the oxygen supply is depressed. Mathews (55) demonstrated that agents such as cold, quinine, and anesthetics which are known to reduce oxidation also prevent cell division. It appears that though the act of mitosis can not be shown to utilize extra oxygen over and above the usual requirements of the cell as indicated by Gray (35), it will not take place at all if the oxygen supply is in anyway deficient.

The stimulus toward the increased mitoses observable in thyroidless animals probably results from the accumulation of autolyzed tissue, which has been pointed out as being characteristic of those tissues most affected by the athyroid condition. The hypothesis that cell division is stimulated by the presence of autolyzing cells seems feasible from the work of Chambers and Scott (16), Dustin (28), Gutherz (36), and Isawaki (43).

Summarizing the case against the thyroids being directly concerned with cell oxidations and cell respiration, the follow-points are evident:-

1. Thyroxine does not increase the metabolic rate of vertebrate tissue if it is separated from its normal connection with the body. This fact is incompatible with the theory.

2. Chemical compounds which raise the oxidative rate of the individual cells and the basal metabolism of the organism as a whole, do not in any way amelliorate the pathological conditions associated with myxedema. This fact is also irreconcilable with the prevailing hypothesis.

3. The latent time which must intervene after the injection of thyroxine before the full effects are evident seems to be against any conclusion that there is a direct connection between the thyroid principle and the oxidative processes of the cells.

4. The increased rate of cell division which follows thyroidectomy is a fact difficult to reconcile with the idea that the oxidative processes of the cells of thyroidless animals are depressed.

h. The probable function of the thyroids

It has already been shown from the data furnished by the present investigation and by the results of other workers that the thyroidless state in an animal is associated with grave disturbances in the digestion and assimilation of the various types of foods, especially of the proteins. Histological and cytological examination of the digestive glands of thyroidless animals reveals characteristic changes which indicate a lowered rate of activity. It appears that the nutritional requirements of the body are not fulfilled in athyroid animals and this condition alone would account for many of the symptoms associated with thyroid disfunction. If, as seems probable, protein metabolism is especially disturbed then the organism would be under the necessity of using a certain amount of its own muscle protein for the repair and upkeep of more vital organs and this condition would be revealed by the appearance of creatine in the urine. Sharpey-Schafer (75) reported creatine as typically present in the urine of individuals deficient in thyroid secretion.

Assuming, however, that lack of thyroid secretion disturbs the digestion and assimilation of essential foods mainly because the glandular elements of the digestive tract are not functioning properly, the question then arises, why are these glands adversely affected. Obviously if the disturbances in the glands initiates the nutritional deficiences, the glands themselves must react to thyroid deficiency before the general bodily metabolism has been affected. Later, after the utilization of necessary foods had become disorganized, then the glands themselves would suffer from this cause along with the other tissues of the body.

The data, as yet, are insufficient for solving this problem. It is possible that the apparent influence of the thyroid secretion on the sensitivity of nerve and other tissue may be an important factor. Absence of thyroids might in this instance initiate the disturbances so evident in the exocrin glands of the integument and digestive tract. Abderhalden and Wertheimer (1) stated that the results of their researches on the thyroids tended to the conclusion that thyroxine affects the basal metabolism by way of the sympathetic nervous system, but Ring, Dworkin, and Bacq (69) reported that the effect of thyroxine on the basal metabolism was unaffected by sympathectomy. More recently, Sawyer and Brown (73) reported that the denervated heart in cats was more sensitive to adrenine when thyroxine was present in the system. The hearts of thyroidless cats do not respond to the injection into the blood of adrenine. It has been pointed out already that the skeletal muscles of the thyroidless salamander are characteristically lacking in tone and Sharpey Schafer (75) stated this condition to be an accompaniment of the athyroid condition among all animals that have been investigated. These researches and the results of numerous others cited previously indicate that insufficient thyroid secretion may primarily affect the excitability of organs and tissues in general

Whatever the mediating factors may be, it appears from the present investigation that there are morphological disturbances in the dermal and digestive glands which arise before the animal has been seriously affected otherwise. At the stage when the mitotic activity has not yet risen above normal in such tissues as the spleen, small intestine, and epidermis the destructive changes in these glands are well advanced. The cardiac glands shown in figures (28-29) were taken from a salamander which had been thyroidless for only twenty days. This particular specimen was still vigorous and, aside from a blackened skin, appeared normal in its behavior. The emaciation which later develops in thyroidless salamanders had not had time to make its appearance. There would not have been any nutritional deficiency evident in such a space of time even if no food had been given to the animal. Salamanders are slow to show the effects of starvation.

On the other hand, microscopic examination of the tissues of animals well advanced into the athyroid state reveals characteristics comparable in many ways to those common to the tissues of starving animals. Liver cells, as has been pointed out, shrink to less than their normal size, connective tissue is looser, more fragile in appearance, and cells subject to spesial wear and tear such as those on free surfaces age faster. In other words, the appearance of the tissues themselves points emphatically to the presence of nutritional disturbances within the organism.

It would seem from the foregoing that lack of thyroxine in the body is accompanied by such destructive effects on the glands essential for normal nutrition as to seriously impair bodily functions. It is possible that this in turn is responsible for the lowered metabolism so characteristic of thyroidless animals. If, as is here suggested, the changes in basal metabolism are secondary to such effects as impaired nutrition and lowered sensitivity of the tissues, it becomes understandable why such drugs as dinitro-o-cresol, though capable of raising the basal metabolism of the body, are without effect on the symptoms which accompany the thyroidless condition and why the rate of cell division may be temporarily higher in a thyroidectomized animal than in one whose thyroids are functioning normally.

Summary

1. A group of 150 salamanders (*Triturus torosus*) were thyroidectomized for the purpose of studying the relation between the thyroids and the remaining structures of the body.

2. Observations coupled with laboratory studies were made on the effects of the athyroid state as revealed through cytological, histological, and physiological manifestations of the organism.

3. The studies reveal that the thyroidless salamander as compared with the normal salamander possesses the following characteristics:-

a. It is less responsive to stimuli.

b. It utilizes less oxygen.

c. The body has a lower specific gravity.

d. The tissues are more edematous.

e. There is a lower erythrocyte count.

f. There is a higher leucocyte count.

g. The skin is more permeable to water.

4. Cytological investigation of the effects of thyroidectomy revealed the following:-

a. The cells of tissues subject to rapid replacement are most seriously affected.

b. The cells tend to age faster.

c. The nuclei manifest abnormal irregularity in size and in the disposition of chromatin material.

d. The frequency of cell division is disturbed.

5. Histological investigation disclosed the following data:-

a. Exocrin gland tissue, especially the granular glands of the skin, the serous glands of the tongue, the cardiac and pyloric glands of the stomach, and the glands of the small intestine are structurally disturbed.

b. Epithelial tissue is adversely affected.

c. The sinusoids of the liver are enlarged, the hepatic cells are smaller, and the subserous layer thicker than normal.

d. Necrotic tissue is found in the spleen and there is a diminution of the red pulp.

e. The arterioles of the nephritic glomeruli are enlarged and disturbances in the secreting and collecting tubules are evident.

f. Gonadal tissue is completely disorganized.

g. The cerebral lobes were not found to respond by any specific morphological change as a result of the athyroid condition.

6. Consideration of the data obtained together with data on thyroid function from other sources tends to disprove the assumption that the thyroid principle directly affects the oxidation and respiration of the individual cells. The following facts appear to be opposed to such an assumption:-

a. Thyroxine has been shown to be without effect on the oxidative rate of tissues whose connection with the host body has been broken or on the oxidative rate of tissues in vitro.

b. There is a latent period of ten to fourteen days which must elapse after the administration of thyroid secretion to a normal animal before the full effect is obtained.

c. The rate of cell division is at first higher than normal in thyroidless animals even though cell division cannot take place if the oxygen supply is depressed.

d. Drugs which are capable of raising the basal metabolism of myxedematous patients to the normal level or higher have no effect on the symptoms associated with this condition.

7. The facts indicate that the lowered basal metabolism which accompanies the thyroidless condition may be due to a lower-

ing of the irritability of the tissues and to disturbances in the exocrin glands particularly those associated with the digestion and assimilation of food.

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PLATES

Plate 1

Explanation of figures

Figure 4. The salamander on the right is the normal control (male). The animal on the left (male) has been thyroidless for 50 days. Note blackened leathery appearance of the skin.

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Plate 2

Explanation of figures

Figure 5. The animal on the right (male) is the normal control. Animal on the left (male) has been thyroidless for 70 days. Pronounced edema is evident.

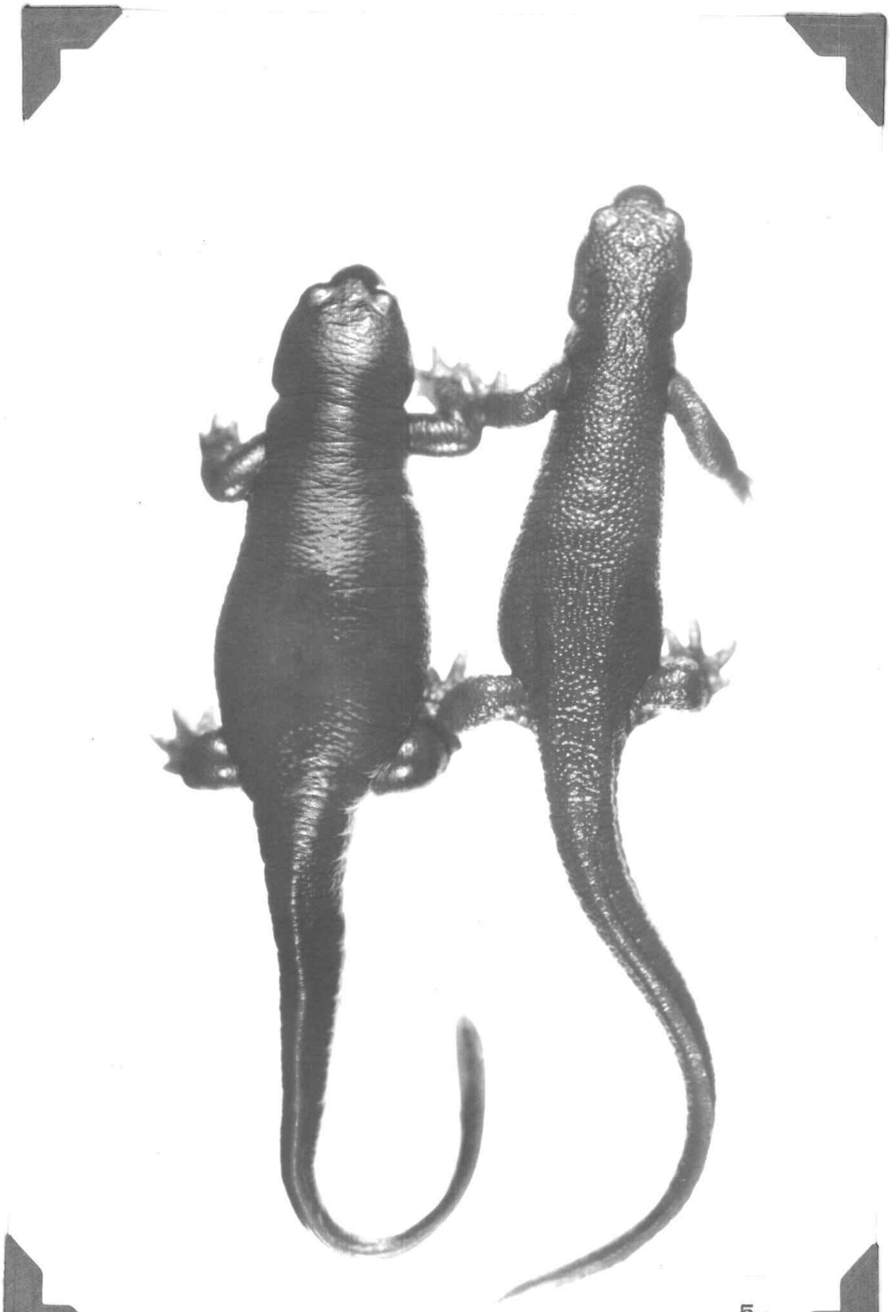

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Plate 3

Explanation of figures

Figure 6. The animal on the right is the normal control (male). The animal on the left is a female thyroidless for 120 days. Note the rougher skin of the thyroidectomized female. as compared with thyroidless males - figures 4 and 5.

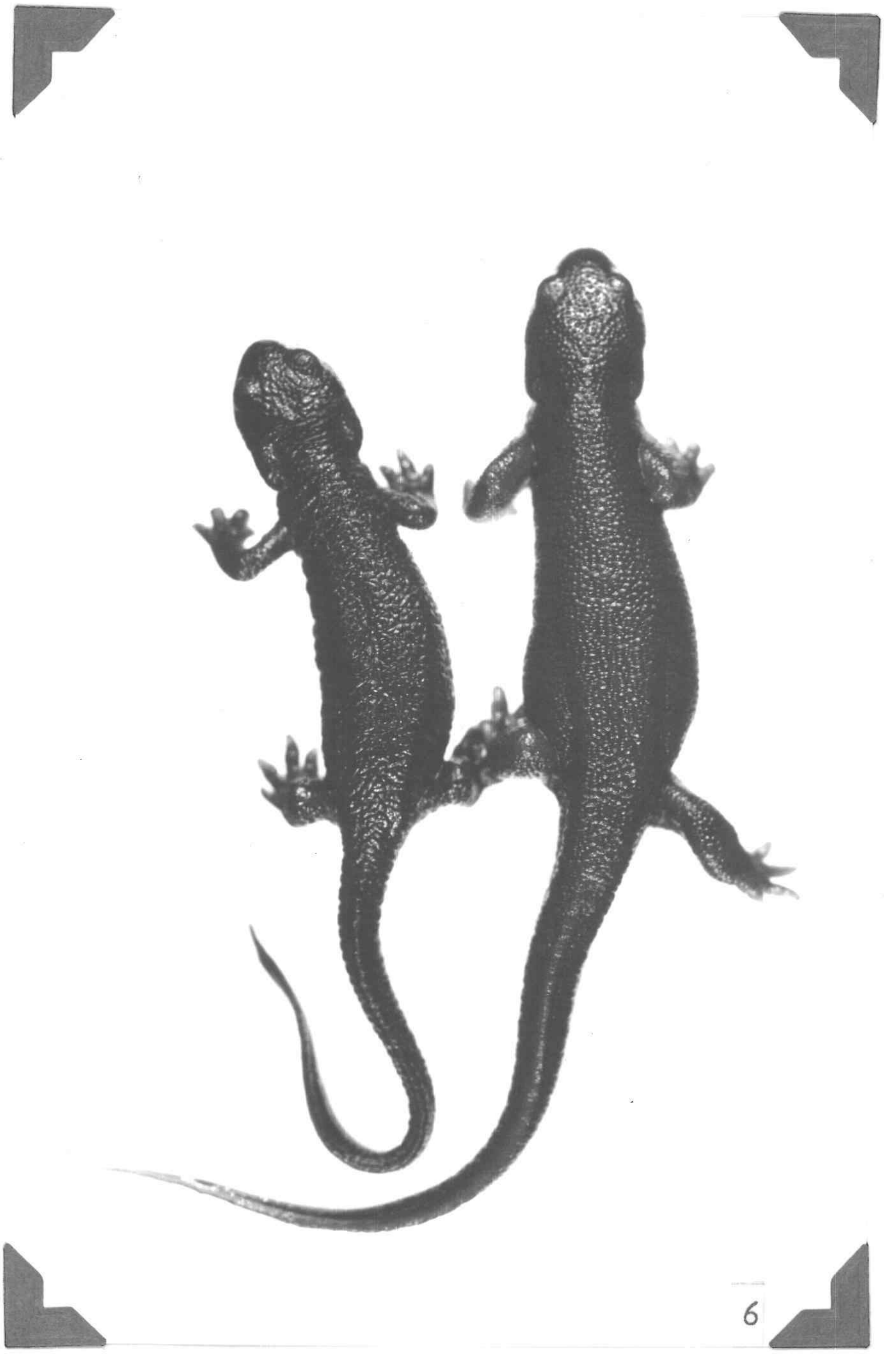

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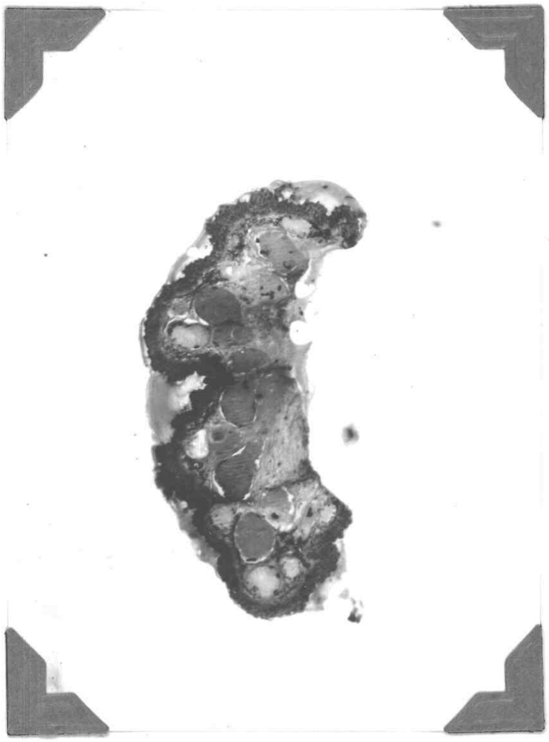
Plate 4

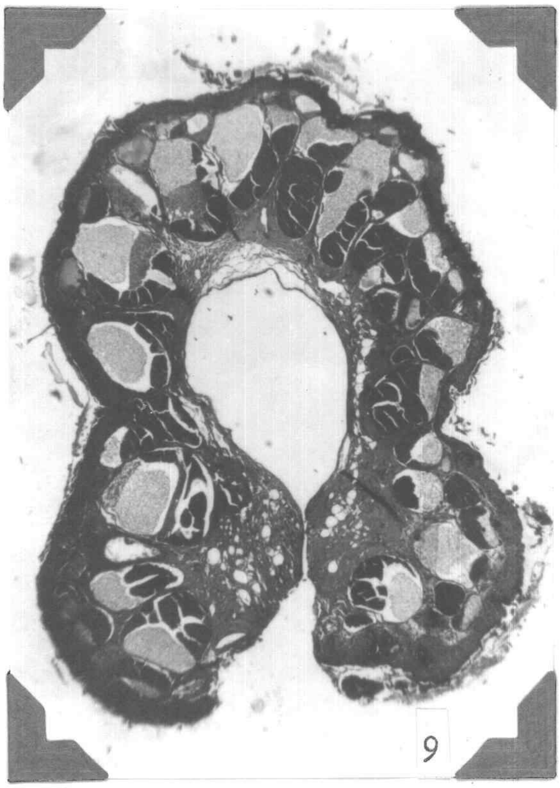
Explanation of figures

Figures 7 and 8 represent sections through the skin. Magnification 100. Figure 8 was prepared from skin provided by normal control. Figure 7 shows skin cross-section from experimental animal MF1 thyroidless 20 days. The breakdown of the large granular glands is evident.

Figures 9 and 10 are photomicrographs of sections through the gill vestige region. Magnification 100. Figure 10 represents the normal control. Figure 9 was taken from tissue furnished by experimental animal MF6 thyroidless 156 days. Granular glands are disturbed. The fragile worn appearance of connective tissue is also well shown.

7





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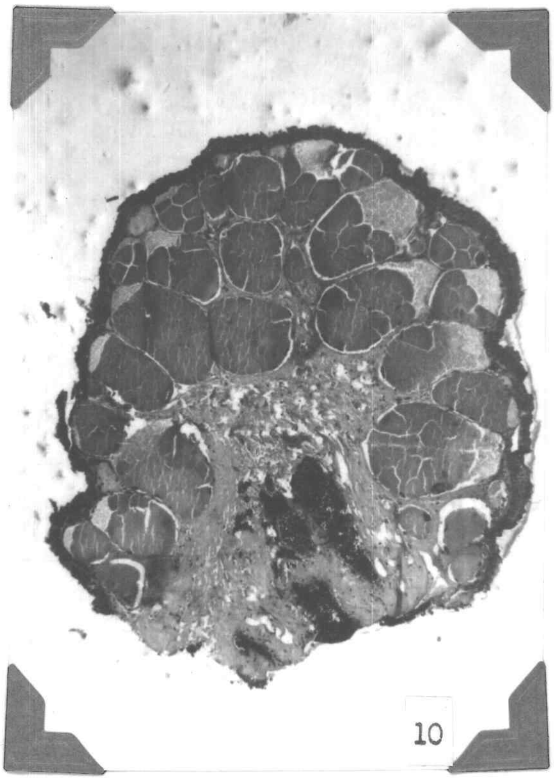

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Plate 5

Explanation of figures

Figures 11, 12, and 13. Photomicrographs of sections through the epidermis. Magnification 1500. Figure 11 represents the control. Figure 12 was prepared from tissue furnished by experimental animal MF4 thyroidless 120 days. A typical case of nuclear fragmentation as well as peculiar chromatin distribution are shown. Figure 13 prepared from tissue furnished by experimental animal MF6 thyroidless 155 days. Distinct perinuclear spaces and generally abnormal appearance are shown.

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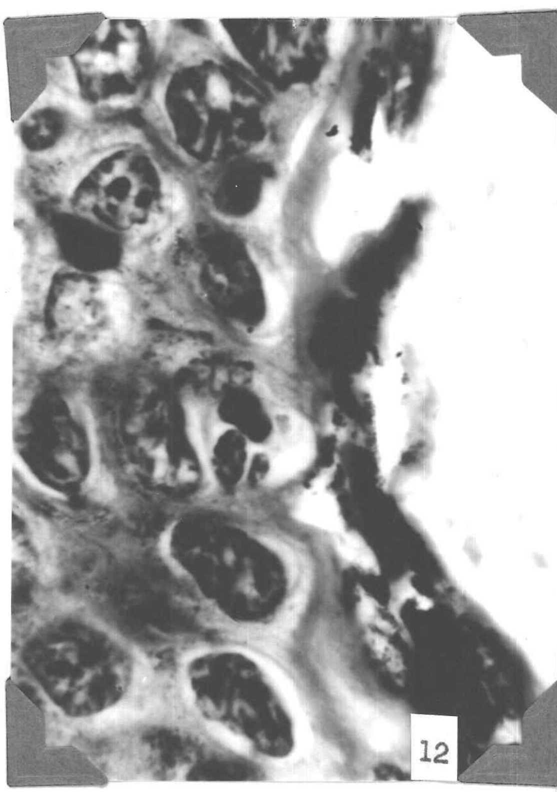

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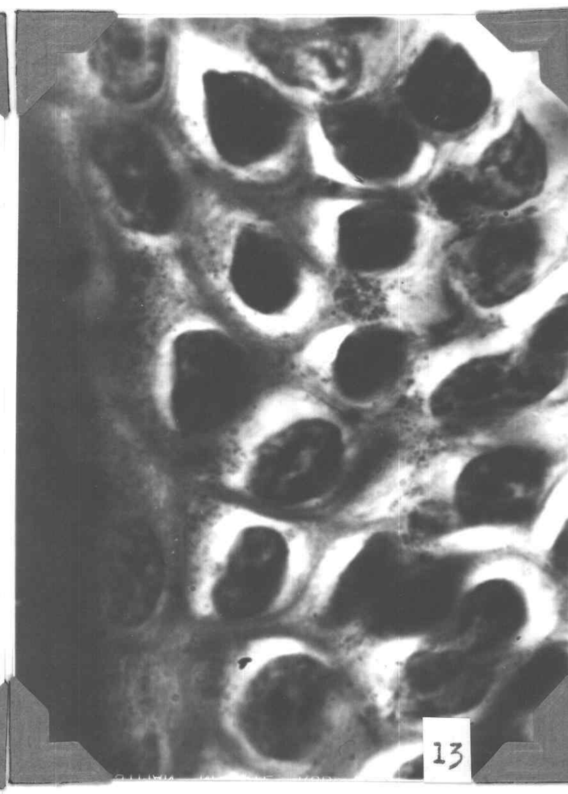

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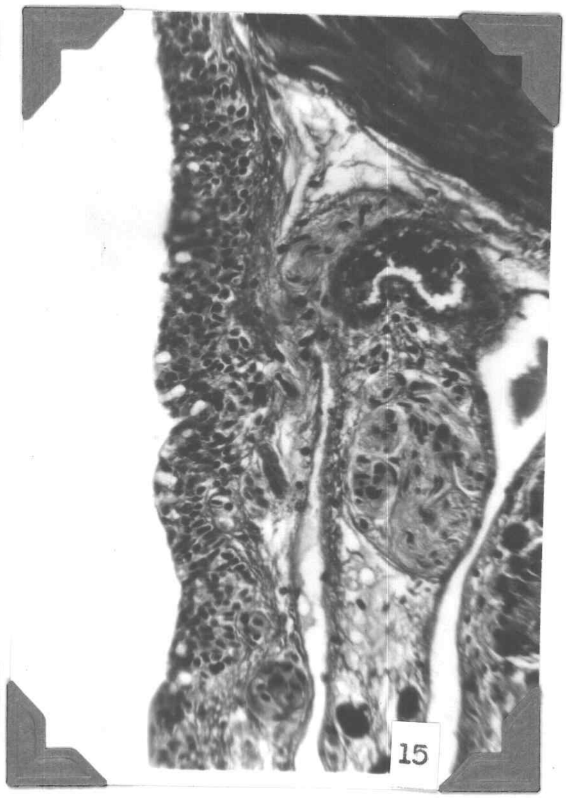
Plate 6

Explanation of figures

Figures 14 and 15 are photomicrographs of sections through the epithelial surface of the tongue. Magnification 500. Figure 15 represents the normal condition of this tissue. Figure 14 was prepared from the tongue tissue of experimental animal MF6 thyroidless 155 days. The goblet cells are greatly increased in number and the epithelial surface is thicker than normal.

Figures 16 and 17. Photomicrographs of sections through testes. Magnification 100. Figure 17 shows a cross-section of a normal testis. Figure 16 was prepared from a testis of experimental animal M3 thyroidless 150 days. There is complete degeneration.



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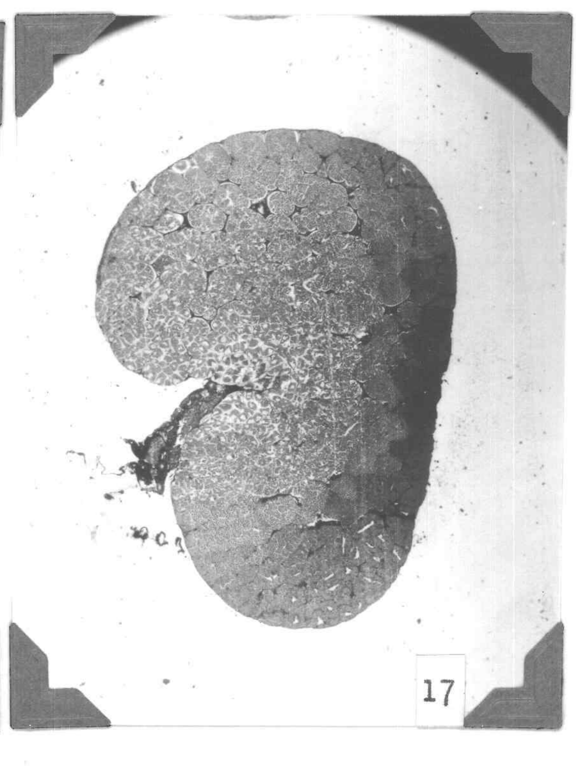

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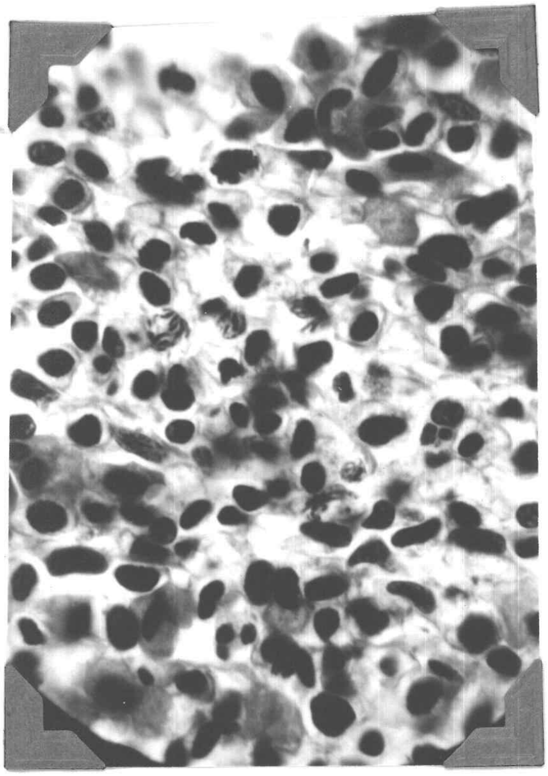
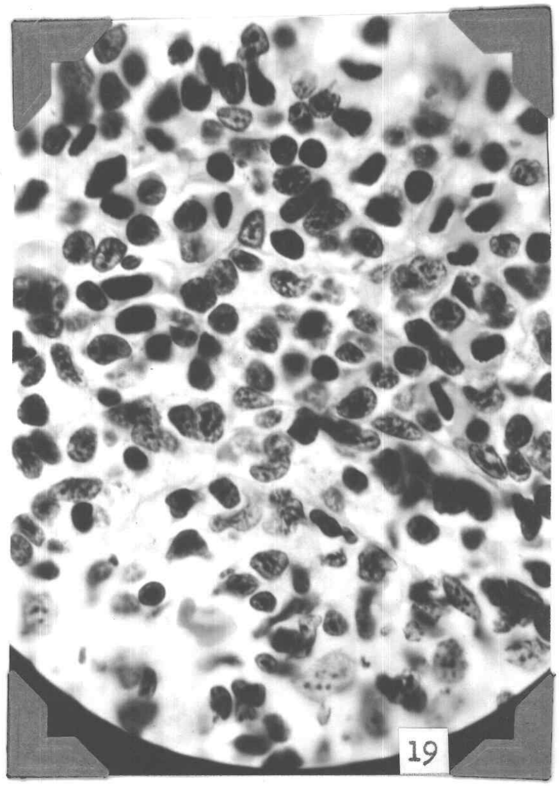
Plate 7

Explanation of figures

Figures 18 and 19. Photomicrographs of spleenic tissue. Magnification 645. Figure 19 represents normal spleen. Figure 18 was prepared from the splleen of experimental animal MF1 thyroidless 20 days. Note the numerous mitoses.

Figures 20 and 21. Photomicrographs of liver tissue. Magnification 500. Figure 21 represents normal liver tissue. Figure 20 was prepared from experimental animal M2 thyroidless 160 days. Note changed appearance of hepatic cells and the numerous large sinusoids. The subserous coat is also much changed.





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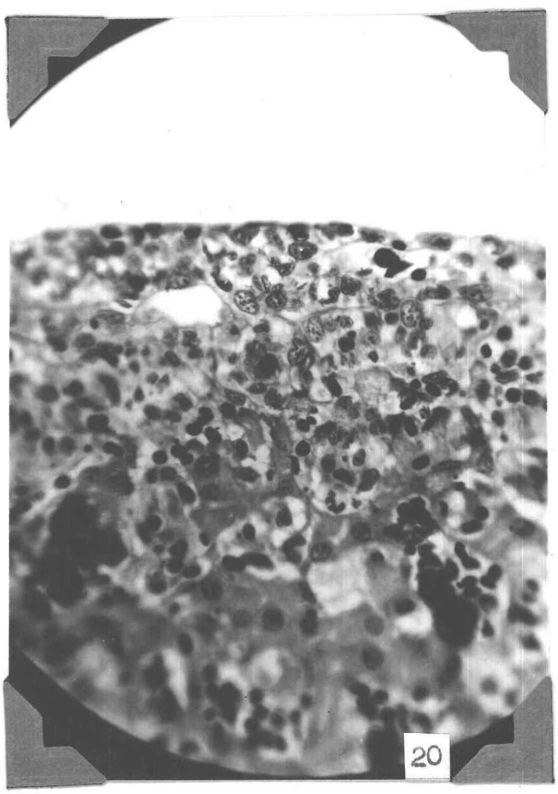

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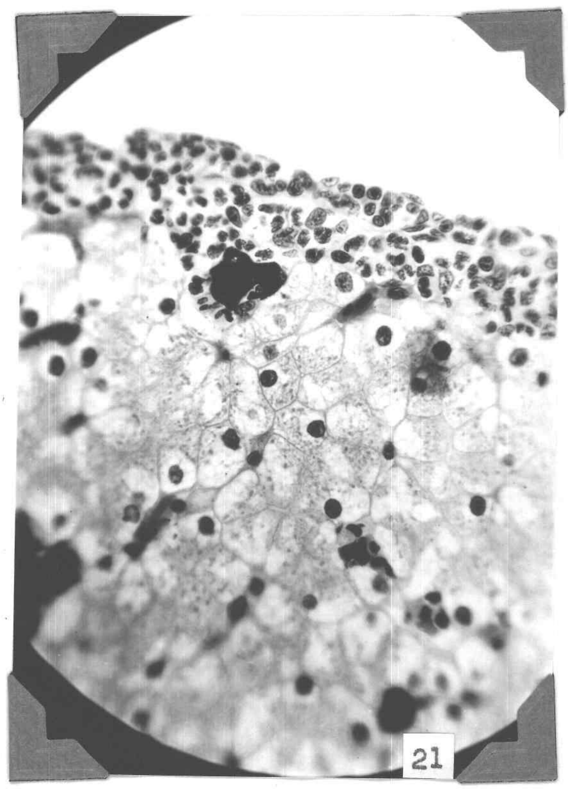

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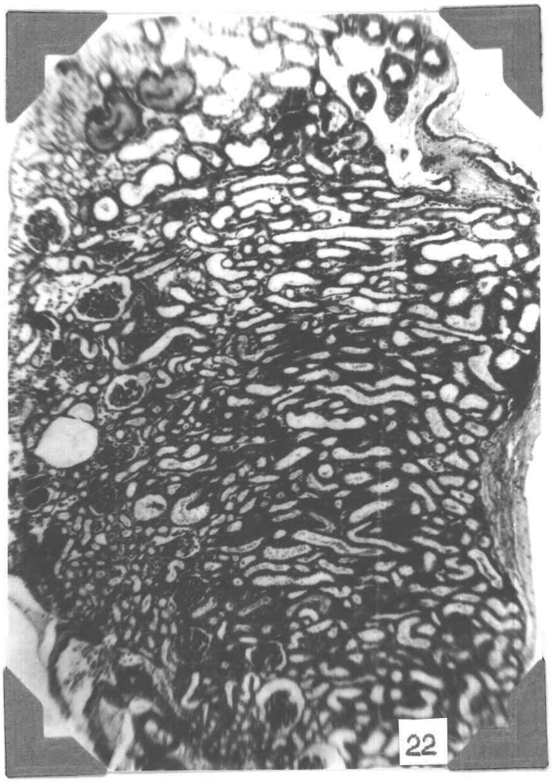
Plate 8

Explanation of figures

Figures 22 and 23. Photomicrographs of kidney tissue. Magnification 100. Figure 23 represents normal tissue. Figure 22 was prepared from a kidney of experimental animal M3 thyroidless 150 days. Note the enlarged secretory and collecting tubules.

Figures 24 and 25. Photomicrographs of Bowmans capsules and contained glomeruli. Magnification 645. Figure 25 represents normal control. Figure 24 was prepared from renal tissue taken from experimental animal M3 thyroidless 150 days. The enlargement of the capsule and reduction in the glomerulus is striking. Note also the worn appearance of cells in tubules.



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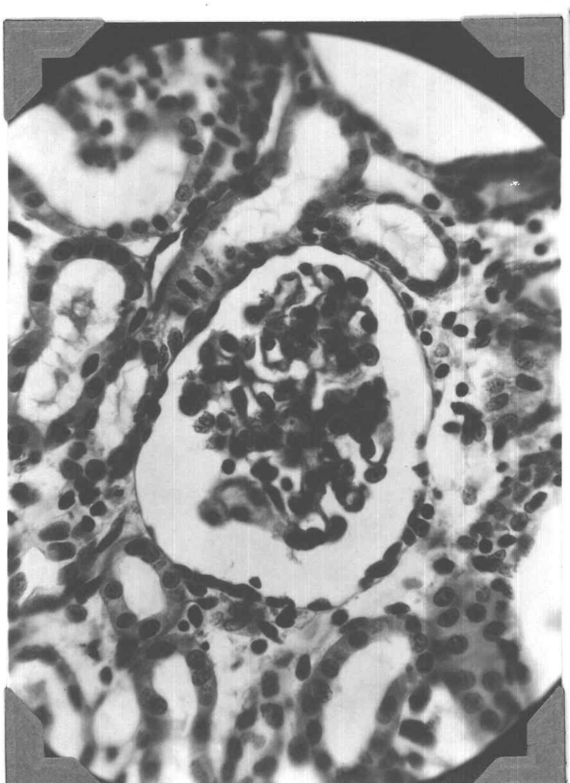

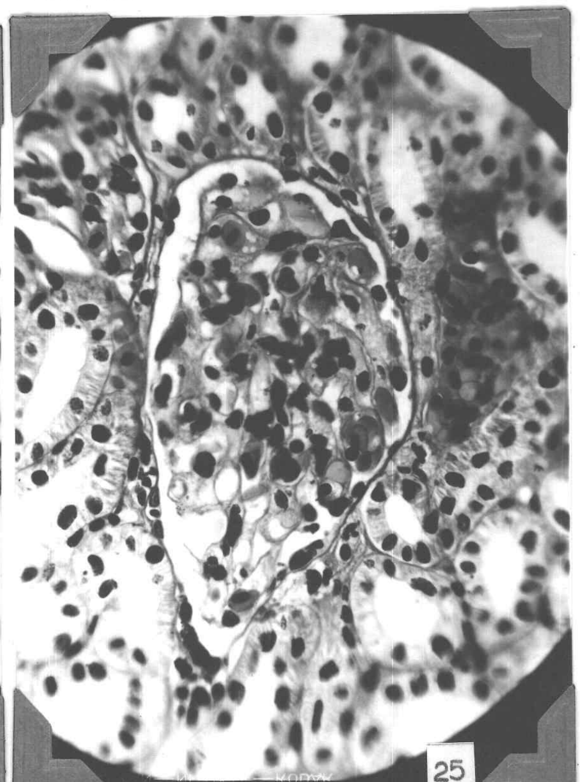

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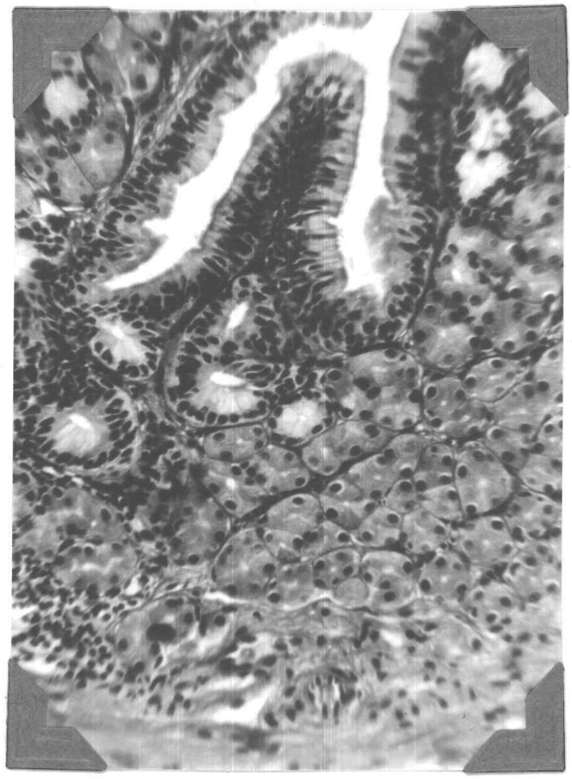
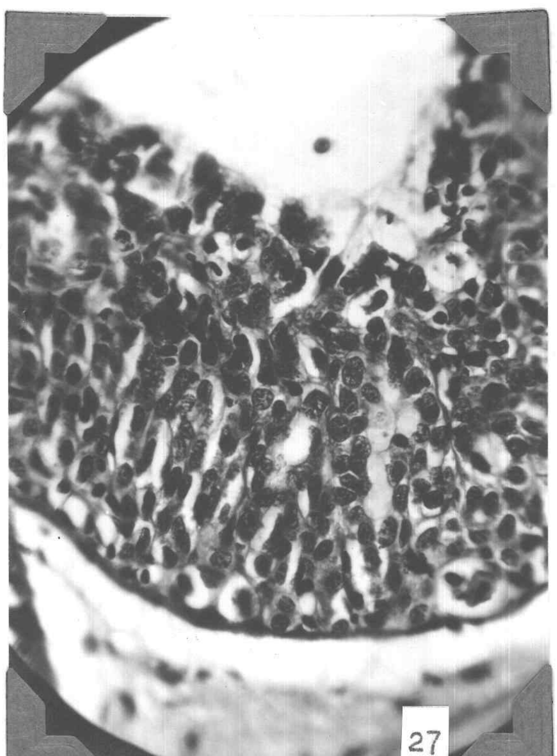
Plate 9

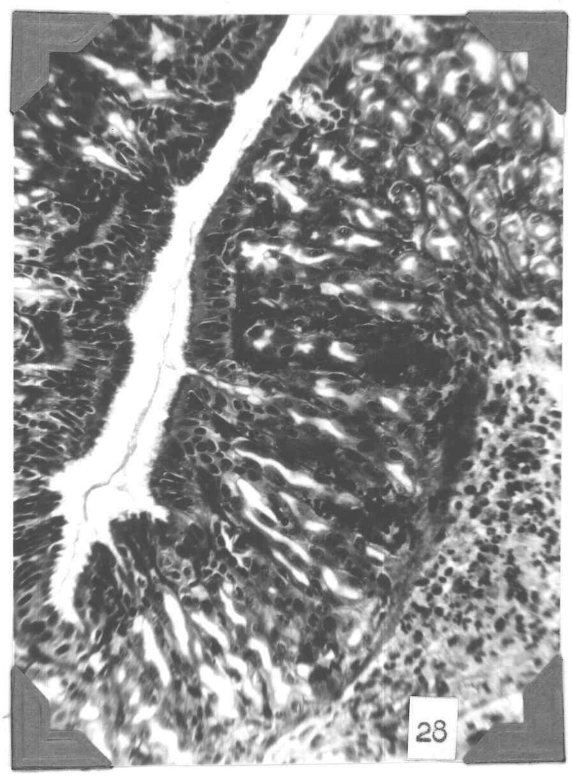
Explanation of figures

Figures 26, 27, and 28. Photomicrographs of the gastric mucosa showing cardiac glands. Figure 26 represents this tissue as it appears in the normal animal. Figure 27 was prepared from tissue taken from experimental animal MF5 thyroidless 46 days. The gastric epithelium is seen to be almost completely disorganized. Figure 28 was prepared from tissue furnished by experimental animal MF1 thyroidless 20 days. Note the worn appearance of the cardiac glands.





27



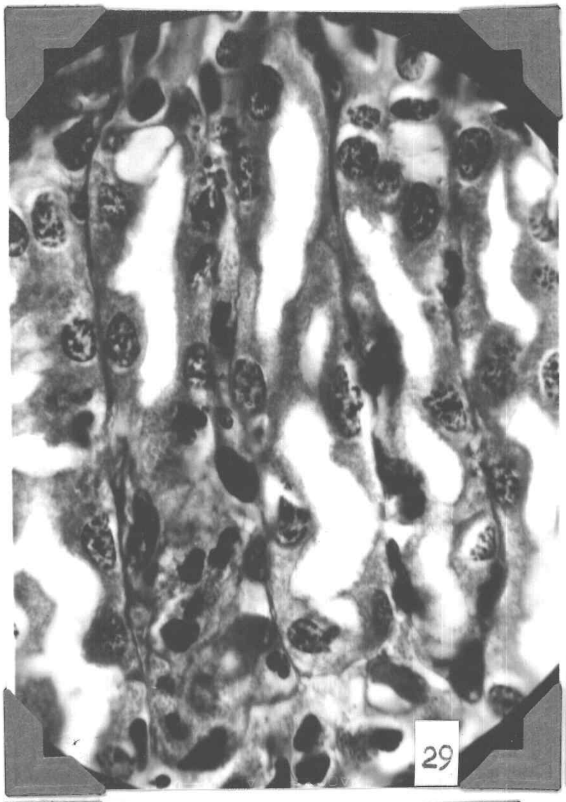
28

Plate 10

Explanation of figures

Figures 29 and 30. Photomicrographs taken from the same material as that used for figures 26 and 28 but at a higher magnification - x 1500. The cardiac glands are seen to be worn away from the lumen of the gland outwards.

Figures 31 and 32. Photomicrographs of tissues taken from the small intestines. Figure 32 shows the normal appearance of the epithelium of this portion of the digestive tract. Figure 31 was prepared from tissue furnished by experimental animal M2 thyroidless 160 days. The goblet cells are more numerous, the cytoplasmic border has become smaller, and there is general structural disturbance.



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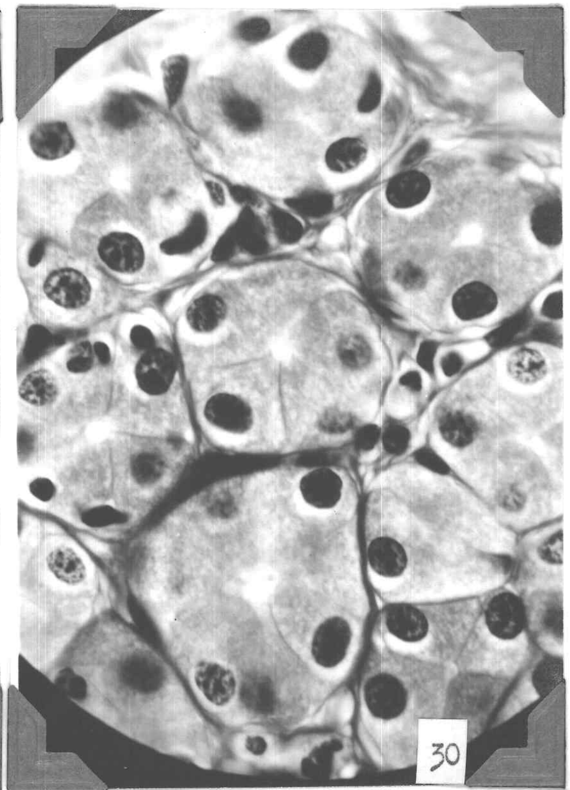

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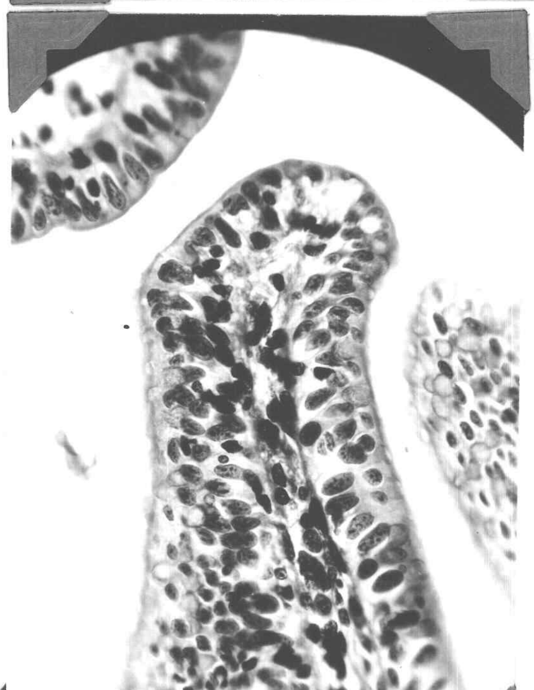

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