THE EFFECT OF THYROID FEEDING UPON OSSIFICATION IN THE WHITE RAT

By

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INDEX.

INTRODUCTION.

The investigation of this problem was begun in the Zoology Laboratory of the University of Kansas, at the suggestion of Dr. Bennett M. Allen, to whom my grateful thanks are due for his constant interest in the work and his many helpful suggestions. I am also indebted to Dr. H. H. Lane for his kindly assistance and advice.

In this paper I shall discuss chiefly the effect on the skeletal development as a result of feeding dessicated thyroids to white rats.

Much work has been done on the effect of the removal of the thyroid gland but up to this time not a great deal has appeared relative to the feeding of the gland and especially to the effect upon the bones.

An incentive to the working out of this problem was the assumption that if the removal of the thyroid gland, as has been shown by several workers, will retard the

development of the bones, then an excess amount of the gland through feeding would cause a precocious development.

LITERATURE.

Hofmeister '92 found as a result of extirpating the thyroid gland of young rabbits that there was a very marked retardation in the proper development of the skeleton and especially the leg bones. He found the least retardation in the skull. The cartilages remained unossified and there was a diminution of the normal cellular proliferation, combined with vesicular swelling and the formation of clefts in the ground substance, and atrophy or even partial destruction of the cartilage cells.

Eiselsberg '95 in removing the thyroid glands from young goats obtained a pronounced shortening of the legs and frontal part of the skull after a period of three weeks feeding. He also noted a high degree of derangement of skeletal growth and retardation of growth in young sheep after thyroidectomy.

Massaglia '07 obtained similar results from the removal of the thyroid glands from dogs, as also did Parhon and Goldstein '09 with cats.

Since the same general results are obtained from herbivorous and carnivorous animals it seems that the results of the removal of the thyroid gland are more or less constant.

Some papers have also appeared relative to the removal of the thyroid gland from lower types of animals and effect upon the bones noted.

Terry '17 reported on the effect of thyroidectomizing Rana pipiens tadpoles and the effect upon their development. The first indication of the effect of the removal of the gland is shown by the fact that the tadpole fails to metamorphose. Body growth continues but the animal remains larval in character. He found that the vertebrae in-

creased in size but failed to ossify. In the long bones the removal of the thyroid gland greatly retarded and apparently stopped both the process of ossification and the process of growth.

Allen '16-'22, as reported in his various papers found that removal of the thyroid gland produced striking inhibitive effects in the development of tadpoles.

Effects of Thyroid Feeding:

Magnus-Levy '95 found that feeding the thyroid gland to an animal may cause loss in weight.

Bircher '10b fed thyroid to young rats and found a retardation in growth and body weight, but an acceleration in the process of ossification.

Gudernatsch '12 found that thyroid administered to tadpoles retarded growth but hastened metamorphosis of the limbs and tail. This work has later been confirmed

in general by West '14, Cotroni '14, Lenhart '15, Romeis '15, Morse '15 and Abderhalden '15.

Hewitt '14 fed thyroid to white rats and noted a loss in weight.

Gudernatsch '15 fed thyroid to white rats and retarded growth and brought about an interference with pregnancy.

The above papers have been selected as typical from among those which have appeared from time to time on the subject.

MATERIAL.

The material used in this work comprised the offspring of the Tyler strain of Albino rats procured from the Wistar Institute. The feeding of the young began at the time of weaning, i.e. when they were about three weeks old.

Records were taken on thirtyfour animals. Weight records were kept and growth curves plotted which show the rate of development.

The food mixture fed was wheat flour, alfalfa flour made from drying and powdering alfalfa leaves, and dessicated thyroids as prepared by Armour and Company, in the ratio of 5:2:1 respectively. For the control animals the same mixture was used except for the absence of thyroid, for which powdered egg yolk was substituted to make up the protein deficiency.

These substances were mixed together

dry in 6-grain capacity gelatin capsules. At the time of feeding this mixture was made into a paste with water and fed fresh each time on clean glass plates. The entire amount was practically always consumed.

TECHNIQUE.

The method of study followed may be divided into four parts, viz., 1. X-ray, 2. Clearing by Schultze's Caustic Potash Glycerin Method, 3. Lundvall's Method, and 4. Microscopical examination of transverse sections through the vertebrae and longitudinal sections through the leg bones and especially the tibia.

In the first method, after it was found that the long bones showed the degree of ossification most clearly, the thyroid-fed and control animals were dissected as soon as the experimental animals died and the limbs and vertebrae were X-rayed. Only the legs from one side of the animal were X-rayed. The pictures were retained for further study and the same legs were cleared as explained in the following paragraph.

Secondly, the X-rayed legs were cleared by being placed in a two per cent solution

of Potassium Hydroxide. They were left in this solution for eight days, after which time they were immersed in a twenty per cent Glycerin solution for two days. The strength of this clearing solution was increased at two-day intervals until the specimens were in a full-strength Glycerin solution where they could be studied and kept indefinitely. After being treated in this manner the cartilage appeared translucent while the bone remained opaque.

The third method was the staining of the bone and cartilage "in toto" by the Lundvall method which briefly stated is as follows: Fixed the specimens in ten per cent Formalin for forty eight hours then placed them in ninety five per cent Alcohol for a similar length of time. Stained with .25 % Toluidin Blue in acid alcohol seven days at forty degrees Centigrade. Decolorized in Acid Alcohol

at forty degrees Centigrade and transferred the material to ninety five per cent Alcohol for several days changing often. Washed specimens in Absolute Alcohol for 24 to 48 hours or longer. Then placed them in two parts Absolute Alcohol and one part Benzol solution for 12 to 24 hours, afterwards reversed the proportions to two parts Benzol and one part Absolute alcohol for 24 to 48 hours. From this the specimens were passed through Benzol into a mixture of four parts Benzol with one of Sulfide of Carbon for preservation. The cartilage remained blue while the bone took on a red color.

The fourth method, which no doubt is of most importance, was a microscopical examination of the bone and cartilage tissues. Both the thyroid-fed and control animals were examined. The fixation fluid used was ten per cent Formalin. After fixation the specimens were placed in a fifteen per cent

solution of Nitric Acid for decalcification.

The leg bones were cut in sagittal sections while the vertebrae were cut in transverse sections. The sections were cut from twelve to eighteen micra in thickness and were stained with Delafield's Haematoxylin with an alcoholic solution of Eosin used as a counterstain.

DISCUSSION.

The animals for this experiment were kept in wire cages in a well lighted and ventilated room and under as hygienic conditions as possible to prevent the development of diseases which might produce abnormal effects on the experiment.

At the time of the beginning of the experiment the young rats were weighed to 1/1000 of a milligram accuracy. The rate of growth was followed and weighings were made every three days. A record was kept and this record is given for the thyroid-fed animals in Table I and for the control animals in Table II.

After the experiments were completed the writer constructed curves of growth which are represented in Graph I and Graph II. These are typical curves for the entire lot, however the ones shown are from only two of the experiments.

Before taking up a discussion of ossification as found in the experiments for this paper it may be well to first discuss the normal ossification of the bone.

Bone tissue makes its appearance relatively late in foetal life. An embryonal type of hyaline cartilage precedes the formation of bone. Practically the entire skeleton, with the exception of the flat bones of the skull and face, is formed from plates of embryonal cartilage. The transformation of these cartilage plates into bone is known as Intracartilaginous Ossification. Each cartilage plate is covered with a layer of embryonal fibrillar connective tissue called the primary periosteum or perichondrium. The outer portion of this layer finally becomes the periosteum, the inner portion contains the osteoblasts which play an important role in bone production.

In most of the long bones the changes in the cartilage take place about the middle of the shaft or diaphysis and also at either end of the shaft. The latter are known as the Epiphyseal Centers.

The cartilage cells enlarge and multiply, arranging themselves in radiating columns. During these changes there is an increase in the intercellular matrix and a deposit there of calcium salts. These are call the Centers of Ossification.

The Epiphyseal Centers appear much later than those in the diaphysis and continue to grow.

Finally as the adult condition approaches, ossification begins in the epiphyses and continues until the growth of the bone is complete, leaving only a line of unossified cartilage called the Epiphyseal Line.

In the vertebrae there are also three centers of ossification, viz., centrum, neural arch and transverse processes.

Ossification proper begins by blood vessels from the osteogenic layer growing into the cartilage in the form of bud-like cords -- periosteal buds -- which are preceded by an absorption of the adjacent cartilage matrix by the osteoblasts .

In regard to the study of the ossification of bone for this paper the writer found the X-ray to be of very great value. The accompanying drawing as found in Plate I was made by means of Bausch and Lomb camera lucida, from the actual X-ray photographs.

After clearing the specimens as has previously been mentioned the process of ossification could also be readily observed. This is shown in the drawings made from the cleared specimens in Plate II.

By staining the bone and cartilage "in toto" by the Lundvall method the same condition of ossification was observed.

Last, but perhaps of most importance, is the microscopic study of the histology of the ossification. A study of Plate III will show very clearly the normal method of ossification as has already been discussed. The drawing includes only a small highly magnified portion of a transverse section of the epiphyseal line of the proximal end of the tibia extending to the periosteum.

Plate IV shows a similar section of the epiphyseal line of a thyroid-fed rat. T These rats were fed every day for thirty-nine days.

Observations were made upon other bones in the body besides the ones illustrated. The skull was studied with reference to the fontanelles but due to the age of the animals they were entirely closed in both the normal and experiment rats.

A study of the vertebrae did not yield any satisfactory results due no doubt

to the reason just given. Bircher, however, observed that animals treated at a much earlier age than the ones for this paper showed a marked advance over the contols in ossification of the vertebrae.

There was an unappreciable degree of difference in regard to the length of cartilage extending from the ends of the ribs to the sternum.

RESULTS.

It was found in the case of the thyroid-fed rats that there was usually a decrease in the weight at first. This was followed by a gradual increase in weight for a period of two or three weeks. Then the animals began to show signs of senescence, the fur became ruffled and shaggy and began to fall out, tetany appeared, and in a few days death occured.

There was always a slow increase in weight but when this increase reached its highest point the decrease to death was very rapid as is shown by the growth curves. The normal curve which continued to rise was much above the curve of the thyroid-fed animals at all times.

The X-ray observations showed a decided difference between the experimental and control animals. The epiphyseal line in the thyroid-fed animals was almost entirely closed while in the normal ones it remained

open as shown in Plate I.

Those specimens cleared in Glycerin showed distinctly that the feeding of thyroid had produced a pronounced effect on the epiphyseal line as illustrated in Plate II.

The study of the histological structure of the proximal end of the tibia as shown in Plates III and IV shows very clearly that thyroid feeding had produced a senile condition in the bones.

The epiphyseal line, as averaged from a number of specimens and measurements, exists in the ratio of 2:5 between the thyroid-fed and control animals, as illustrated in Plate V.

CONCLUSIONS.

From the results of this experiment one may justly conclude:

1. That thyroid feeding produces a rapid advance toward senescence as indicated by weight and external appearances,

2. That the feeding of the thyroid gland to animals produces a precocious ossification of the bones, and

3. That hyperthyroidism may cause an increase in the rate of ossification and thus produce dwarfs.

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TABLE I.

Thyroid-fed Rats. Record of weights of seventeen specimens. The weighings were made every three days. The weights are expressed in grams.

TABLE I.

Spec.	Weighings: 1	2	3	4	5	6	7	8	9	10	11	12	13
1.	23.306	22.856	21.752										
2.	23.730	24.214	25.120	26.715									
3.	25.922	26.352	25.708	25.712	25.213	23.715	25.753	25.413	27.575	29.164	22.016	22.010	
4.	22.926	23.496	21.752	24.617	27.669								
5.	32.673	30.543	31.673	32.883	36.419	38.797	43.106	46.845	48.018	52.526	51.567	50.416	42.426
6.	38.975	37.222	39.365	42.276	44.574	46.302	50.004	55.211	52.917	52.347			
7.	32.633	33.273	35.249	34.149									
8.	34.331	35.499	36.772	36.892									
9.	28.463	30.292	33.033	33.423									
10.	22.587	23.630	24.863	26.841									
11.	25.558	26.352	28.234	28.982	28.024								
12.	43.199	42.756	45.307	46.495	44.294								
13.	25.712	26.750	27.713	28.774	26.511								
14.	22.306	22.206											
15.	36.069	36.987	40.746	39.525	40.623								
16.	34.524	35.599	39.745	39.655									
17.	34.081	34.111	36.882	34.924									

Weight record of Experimental Rats.

TABLE II.

Control Rats. Record of weights of seventeen specimens. Animals were weighed every three days. Weights are expressed in grams.

TABLE II.

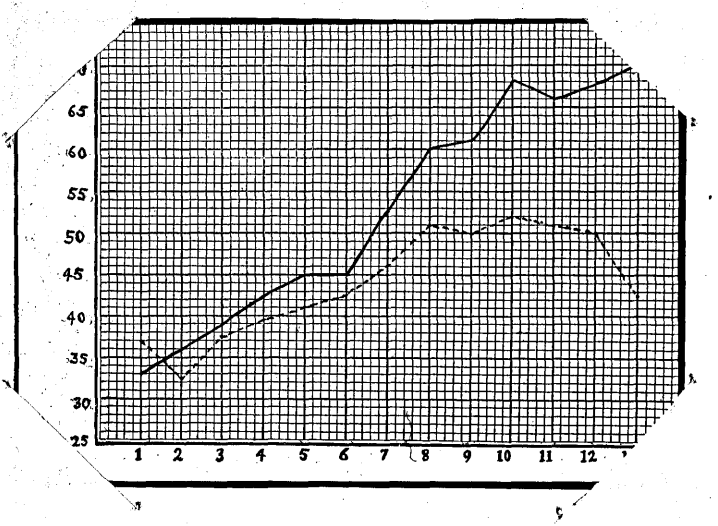
Weighings: 1	2	3	4	5	6	7	8	9	10	11	12	13
1. 24.464	28.563	29.097	34.311	34.221	35.094	37.970	39.558	40.603	41.876	38.895	40.163	
2. 23.864	26.302	26.771	32.103	32.925	35.844	36.720	38.945	39.145	39.605	36.324	38.317	
3. 24.654	27.165	27.709	34.311	35.211	36.942	36.862	39.365	39.305	43.404	37.867	40.003	
4. 22.006	25.745	25.658	31.213	33.463	35.474	36.812	39.005	39.405	40.546	37.057	39.876	
5. 32.243	34.874	37.257	40.305	45.354	45.852	52.953	58.973	62.724	67.778			
6. 36.893	37.122	38.795	40.753	44.644	45.430	53.500	59.023	62.214	68.200	65.063	67.720	70.00
7. 31.713	31.192	35.481	38.796									
8. 36.842	36.942	41.256	44.424									
9. 26.751	27.095	30.435	33.013									
10.22.926	24.764	27.381	29.132									
11.27.813	30.570	32.621	36.822	36.923								
12.35.484	37.122	38.975	42.256	42.406								
13.35.374	37.620	38.625	41.856	42.406								
14.25.765	27.035	28.772										
15.36.822	39.155	41.423	44.164	47.808								
16.37.037	38.407	41.253	43.544	46.252								
17.35.622	36.755	39.725	42.001									

Weight record of Control Rats.

GRAPH I.

Growth curves for one group of Rats. The solid line represents the curve of growth for the control specimens and the broken line represents the curve of growth for the Thyroid-fed animals. Note that there is a decrease in weight at first and then there is a gradual rise in the curve until the end of the third week after which time there is a rather speedy decline to death. The rate for the experimental animals never rises as high as the normal curve.

GRAPH I.



GRAPH II.

Growth curves for another group of Rats.
The solid line indicates the rate of growth
for the control animals and the broken line
represents that for the Thyroidefed specimens.

GRAPH II.

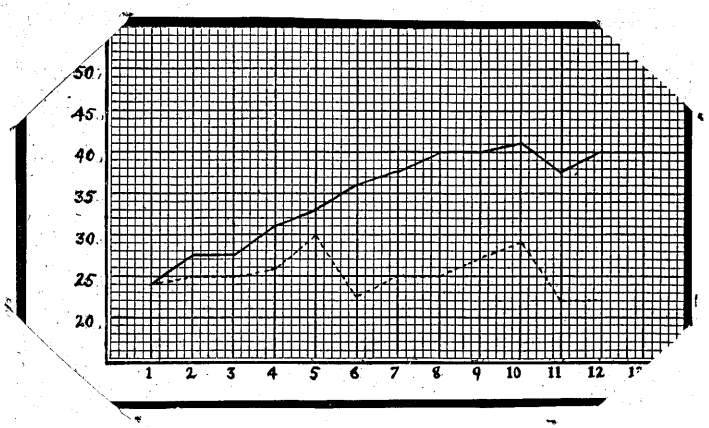


PLATE I.

Drawings made from X-ray photographs of the tibia of White Rats.

a. The tibia from the Control Rat.

b. The tibia from the Experimental Rat.

Note the closing of the Epiphyseal line in the Experimental animal.

PLATE I.

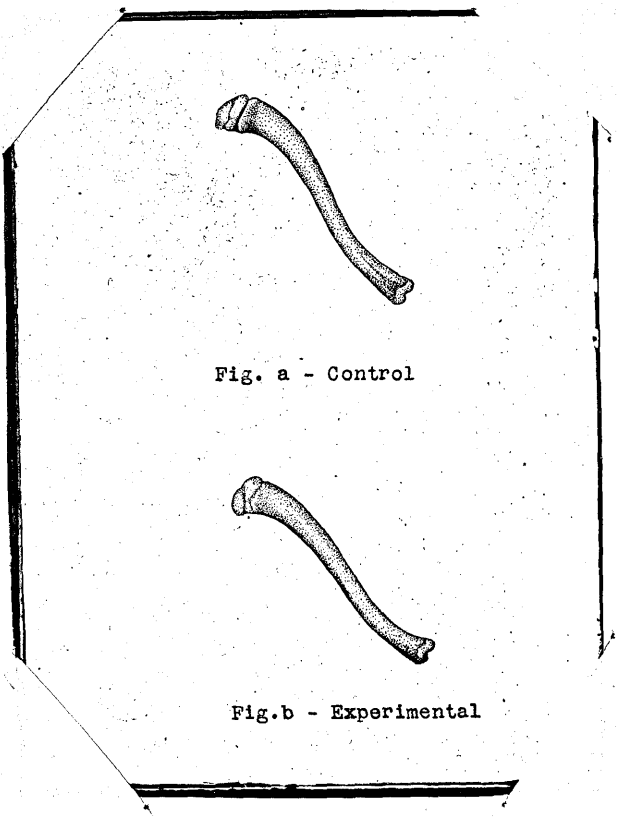


Fig. a - Control

Fig.b - Experimental

PLATE II.

Drawings made from the Cleared legs of White Rats. The difference in the degree of ossification can be clearly noted.

a. Control Rat.

b. Experimental Rat.

PLATE II.

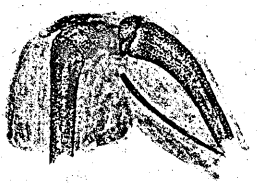


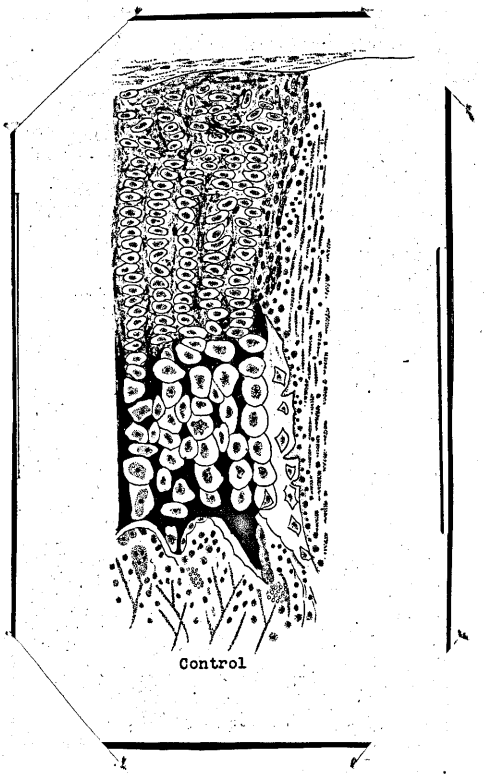
Fig. a - Control

Fig. b -Experimental

PLATE III.

Camera lucida drawing showing the histology of a transverse section highly magnified through the epiphyseal line of the tibia of a Control Rat. Drawn on same scale as Plate IV.

PLATE III.

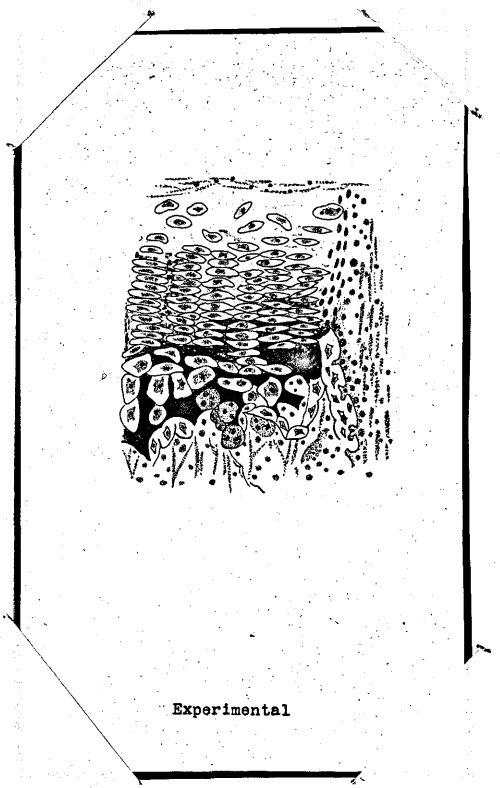


Control

PLATE IV.

Showing the histology of a transverse section through the Epiphyseal line of the tibia of a Thyroid-fed Rat. Drawn on the same scale as Plate III.

PLATE IV.

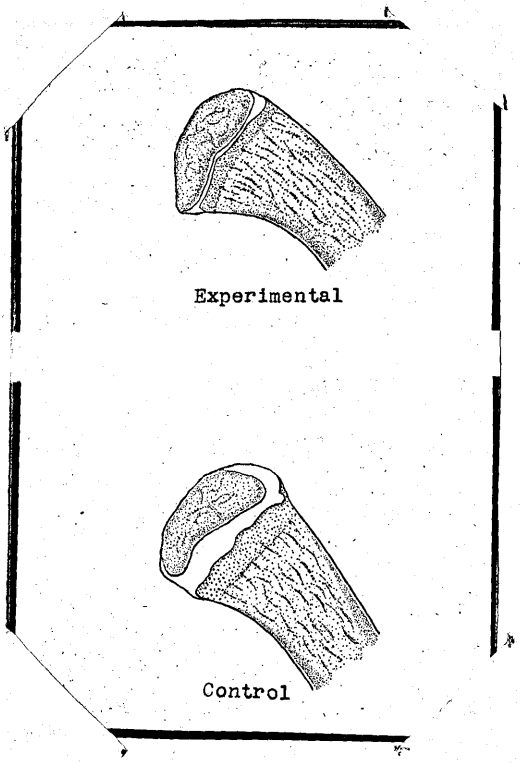


Experimental

PLATE V.

Drawings showing the proximal ends of the tibia of Control and Experimental White Rats used in the experiment. The drawings were made from prepared sections by means of camera lucida as viewed through a compound binocular microscope. Note the closing of the Epiphyseal line in the Experimental specimen.

PLATE V.



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