CONCERNING THE RELATION OF ENVIRONMENTAL TEMPERATURE TO RESISTANCE TO THYROID AND THYROXINE, AND THE CREATINE CONTENT OF THE HEART AND OTHER TISSUES IN EXPERIMENTAL HYPERTHYROIDISM

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Plates 39 and 40

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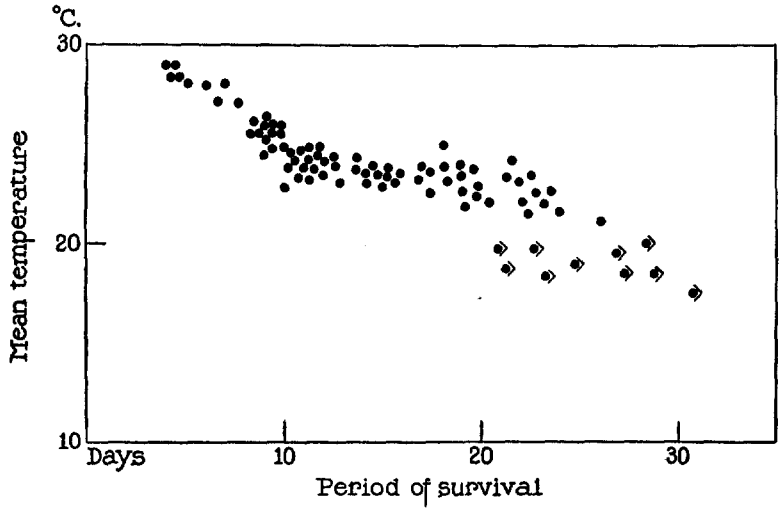
In 1919 Stoland and Kinney (1) published a brief statement concerning the relation of external temperature to the toxicity of administered thyroid. They found that rats kept at 32°C. and receiving 0.2 gm. of desiccated thyroid daily, lived an average of 7.3 days; others at 25°C. lived an average of 22 days, while a third group kept at 18°C. lived more than 32 days. A survey of the literature reveals no other reports specifically concerned with this question, although the more general problem of the relation of the environmental temperature to the structure and activity of the thyroid has been the subject of several investigations (2–9).

The present work is partly the outgrowth of the observation by one of us (10) that rats are much more resistant to thyroid and thyroxine in cool than in warm weather. It is improbable that this simple and obvious relationship has not been frequently observed by experimental workers, and certainly in clinical practice; yet it is remarkable that even in the more authoritative and comprehensive discussions of the physiology, pharmacology and therapeutics of the thyroid hormone, little or no mention is made of the importance of environmental temperature in relation to its tolerance and toxicity. For this reason the present report seems to be justified.

Methods

The rats used in this work were from a pure inbred Wistar strain reared in the laboratory under very favorable conditions. Special attention was given to the

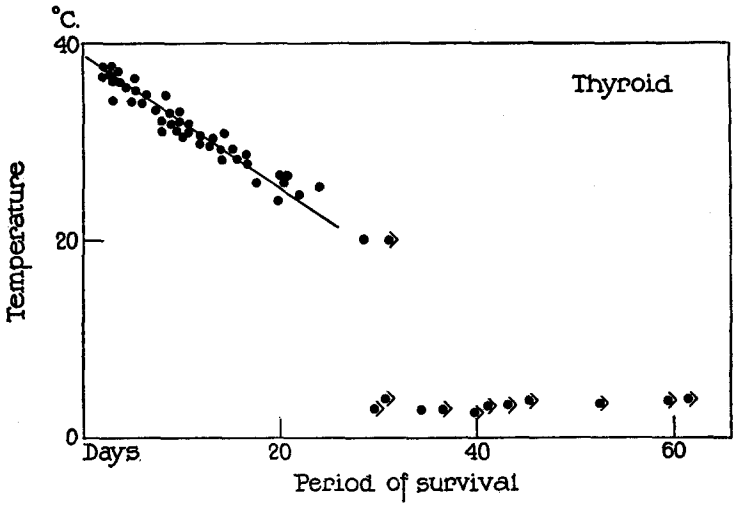
diet, particularly to the vitamin B requirement, as this is said to be greatly augmented in hyperthyroidism (11). One series of animals was observed at room temperature over a period of several months, during which time a daily record was kept of the extreme and mean temperatures. Another series was observed in a thermoregulated incubator at definite temperatures within the range of 20–37.5°C., while a third group was kept in a cold room at a temperature of 4–6°C. As the rat has naturally a high tolerance for thyroid, the daily dose of the desiccated preparation (U. S. P. Lilly) was usually fixed at 250 mg. per 100 gm. of body weight, although in some of the experiments at low temperatures the dose was increased to 1 gm. per rat per day. The thyroxine used was the crystalline, synthetic product



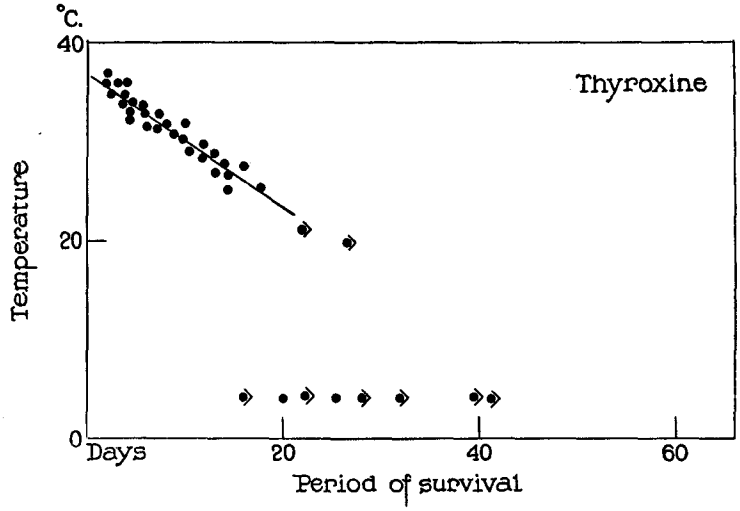
TEXT-FIG. 1. Showing the relation of mean environmental temperature to the survival period of hyperthyroid rats. Solid circles with arrows denote that the animals were alive at the conclusion of the experiments.

of Hoffmann-La Roche. For rats weighing approximately 200 gm., the daily dose was 2 mg., administered subcutaneously. However, essentially the same results have been obtained since with a daily dose of 1 mg. According to the recent observations of Lerman and Salter (12), 0.1 gm. of desiccated thyroid (Lilly, Armour or Lederle) produces approximately the same calorigenic effect in man as 0.3 mg. of pure thyroxine.

The weight of each rat was recorded either daily or every other day. At the termination of each experiment, the organs were removed, weighed and preserved for microscopic study. To add to the data previously reported (10, 13), and for reasons to be considered presently, estimations were made of the creatine content of the myocardium, liver, testes and the muscles of the hind limbs.



TEXT-FIG. 2



TEXT-FIG. 3

TEXT-FIGS. 2. and 3. Showing the relation of constant environmental temperature to the survival period of hyperthyroid rats. Solid circles with arrows denote that the animals were alive at the conclusion of the experiments.

RESULTS

The influence of external temperature on the resistance to thyroid and thyroxine is illustrated by the data charted in Text-fig. 1. As compared with a 4 to 5 day survival period when the mean room temperature was 28–29°C., rats kept at 20°C., or below, were alive at the end of 21 to 31 days. These observations therefore confirmed those of Stoland and Kinney (1). It was apparent, however, that the relationship represented in Text-fig. 1 was only roughly approximate, as there was often a difference of 2–3° between the daily mean and maximum temperatures, and even greater differences over a period of several days. For this reason Text-figs. 2 and 3, representing the data obtained at constant temperatures, give a closer approximation of the relationship of environment to the tolerance for the thyroid hormone.

It should be mentioned that both the incubator and cold room were unavoidably dark, but it is assumed on the basis of several investigations, notably those of Kenyon (9) and Mayerson (14), that the exclusion of light for moderate periods produces no demonstrable effects on the thyroid mechanism.

In the range of 20–37.5°C., the relation of temperature to the survival period was found to be almost linear. The tolerance was considerably increased at 20°C., but was especially striking at 4–6°C. Rats receiving large amounts of thyroid or thyroxine survived long periods of exposure in the refrigerator and even gained weight. One rat (290) was alive at the conclusion of the experiment, after remaining in the cold room for 99 days, during which time it consumed a total of 90 gm. of desiccated thyroid and gained 67 gm. in weight.[1]

The normal response to cold is increased metabolism, associated

[1] More recently observations have been made in a cold room maintained at 2–3°C. A proportion of the rats receiving thyroid or thyroxine, as well as their controls, succumbed within a relatively short period (15 to 30 days). In most of these animals loss of weight, edema of the extremities and pneumonia were prominent findings. Not a few chewed their tails and lost blood from hemorrhage. It is possible that the extreme cold, combined with the much higher moisture content of this cold room as compared with the one previously used, may account for the untoward results. As the data obtained in this refrigerator were inconstant, they have not been included in the present report.

with stimulation of thyroid activity. The characteristic changes in the gland are illustrated in Figs. 1 and 3.[2] The acini remain approximately normal in size, but there is an absence of colloid. The capillaries about them are widely distended. There is a piling up of the epithelium and some desquamation. The epithelium is high cuboidal to columnar. The nuclei are apparently normal in size, but stain very densely. These hyperplastic changes, together with the loss of colloid, are generally taken to mean a preponderant excretion from the thyroid gland and, according to Kenyon (9), may be diminished or prevented in rats exposed to cold by the daily administration of small amounts of iodide. In our work this was accomplished even more strikingly and uniformly by the administration of either desiccated thyroid or thyroxine. The glands of these animals showed enlarged acini filled with colloid which stained well (Figs. 2, 4, 5 and 6). The epithelium was flattened by the pressure of the excessive amount of colloid being stored. There was no apparent difference between the glands of animals fed thyroid and those receiving thyroxine.

A conspicuous effect of hyperthyroidism in the rat, induced by the administration of thyroid or thyroxine, is the marked reduction of the creatine content of the myocardium (10), frequently to about 50 per cent of normal. There is apparently a critical level below which the creatine concentration cannot be readily depressed. Thus far, in analyses of more than 200 hyperthyroid adult rats, the creatine concentration of the myocardium was found to be less than 70 mg. per cent only once, while in the majority of cases it was 90–100 mg. at the time of death. Under these circumstances collapse and death are usually sudden in occurrence, frequently following moderate exertion and often with the animal in a sitting posture, suggesting heart failure.

In view of the changes in the creatine content of the myocardium of rats treated with thyroid substance it was logical to inquire whether a similar change accompanied thyroid hyperfunction due to exposure to cold. However, analyses of the hearts of the eight control rats in this group (Table I) yielded values that were within the normal range. Work now in progress indicates that the creatine and creatinine excretion of rats exposed to a low thermal environment approaches levels

[2] The tissues were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and phloxine.

attained by rats receiving thyroid or thyroxine. As the treated animals show marked depletion of the creatine reserves of skeletal and especially of cardiac muscle, it is to be concluded that the replacement of tissue creatine in this form of physiological hyperthyroidism is comparatively effective.

Although Cramer (5) has described a storing of colloid in the thyroid glands of rats and mice kept at higher temperatures than usual, his

TABLE I

Effect of Cold and of Thyroid and Thyroxine on Creatine Content of Rat Tissues

Rat No.	Died (D) or sacrificed (S)	Body weight		Duration of experiment	Creatine per 100 gm. tissue				Weight of ventricles	Creatine in ventricles	
		Initial	Final		Liver	Testes	Muscle	Heart (ventricles)			
		gm.	*gm.*	*days*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*mg.*	
218 ♂	S	160	183	32	19	304	418	125	0.84	1.05	Total of 15 gm. of desiccated thyroid
219 ♂	S	164	165	32	21	354	435	105	0.80	0.84	Total of 60 mg. of thyroxine
220 ♂	S	160	221	32	21	327	454	184	0.75	1.38	Control
255 ♂	S	158	203	29	23	318	370	107	0.95	1.02	Total of 44 mg. of thyroxine
256 ♂	D	151	147	21	35	338	411	76	0.91	0.69	Total of 30 mg. of thyroxine
257 ♂	S	141	204	31	20	293	446	181	0.82	1.49	Control
288 ♂	S	188	238	53	26	—	485	120	1.02	1.22	Total of 45.5 gm. of desiccated thyroid
289 ♂	S	180	197	68	20	297	431	106	1.11	1.18	Total of 64.5 gm. of desiccated thyroid
290 ♂	S	191	258	99	22	329	452	121	1.22	1.47	Total of 90 gm. of desiccated thyroid
	S	223	211	27	20	308	483	177	0.83	1.47	Averages of 6 controls; 3 males and 3 females

experiments were of several hours duration only, or the animals were exposed to the higher thermal environment only a part of each day. In our work, the animals were kept at the higher temperatures continuously for several days. Under these conditions there was no evidence of storage of colloid either in the controls or in the rats treated with thyroid or thyroxine. As illustrated in Figs. 7 and 8, the glands

TABLE II

Effect of High Temperatures and of Thyroid and Thyroxine on Creatine Content of Rat Tissues

Rat No.	Temperature	Died (D) or sacrificed (S)	Body weight		Duration of experiment	Creatine per 100 gm. tissue				Weight of ventricles	Creatine in ventricles	
			Initial	Final		Liver	Testes	Muscle	Heart (ventricles)			
	°C.		gm.	gm.	days	mg.	mg.	mg.	mg.	gm.	mg.	
203 ♂	37.5	S	170	132	3	31	342	578	178	0.525	0.93	Control
204 ♂	37.5	D	170	127	3	48	383	545	126	0.509	0.64	Total of 1.2 gm. desiccated thyroid
211 ♂	37.5	S	168	135	4	30	305	490	206	0.421	0.87	Control
212 ♂	37.5	D	170	135	2	68	358	478	139.5	0.521	0.73	Total of 0.6 gm. of desiccated thyroid
213 ♂	37.5	D	172	140	2	74	308	486	151.5	0.512	0.78	Total of 2 mg. thyroxine
214 ♂	37.5	D	174	135	3	72	320	477	131	0.476	0.62	Total of 4 mg. thyroxine
215 ♀	37.5	S	155	144	4	27	—	491	197	0.463	0.91	Control
216 ♀	37.5	D	155	124	4	68	—	487	144.5	0.474	0.69	Total of 0.6 gm. desiccated thyroid
208 ♂	36	S	165	152	8	27	298	470	167	0.508	0.85	Control
209 ♂	36	D	165	130	2	82	375	491	138	0.520	0.72	Total of 1 gm. desiccated thyroid
210 ♂	36	D	175	136	3	50	310	464	113	0.550	0.62	Less than 1 gm. desiccated thyroid
224 ♂	34.5	D	163	134	3	33	327	528	122	0.466	0.57	Total of 3 mg. thyroxine
225 ♂	34.5	D	164	129	4	52	317	479	109	0.454	0.49	Total of 1 gm. desiccated thyroid
226 ♂	34.5	S	158	151	10	23	274	524	220	0.456	1.00	Control
332 ♂	33	D	229	176	4	37	366	537	121	0.704	0.85	Total of 8 mg. thyroxine
333 ♂	33	S	220	228	14	20	307	497	212	0.600	1.32	Control

appeared edematous, the acini being pushed apart by an unstained material, probably fluid. The acini were either normal in size or somewhat enlarged; the colloid had either disappeared or lost its staining reaction. A number of the acini contained pale granular and fibrillar material. The epithelium was almost entirely cuboidal with a straight inner border, suggesting the presence of some non-staining substance, or fluid, filling the acini. The nuclei were, on the whole, small and dark, almost pyknotic, while the cytoplasm was darker than normal and opaque. In a proportion of the cells, the cytoplasm seemed to have undergone autolysis. These changes, which appeared more degenerative than functional, were essentially alike in the thyroid or thyroxine treated rats and in the untreated controls.

Partly on the basis of the data of Fieschi and Gavazzeni (15), Buell, Strauss and Andrus (16), and on that of a limited number of observations of the changes in phosphocreatine by one of us (10), it may be suggested tentatively that in thyrotoxicosis, the glycogen and phosphocreatine mechanisms are primarily affected and that the depletion of total creatine follows the loss of phosphocreatine. In acute intoxications, such as occur at high temperatures, death from heart failure may thus occur when the phosphocreatine of the myocardium has fallen to a fraction of normal, but before the total creatine content has become markedly reduced. In part this is illustrated by the data in Table II. Values for heart creatine as high as 151.5 and 144.5 mg. were obtained in rats 213 and 216, respectively. It is to be noted at this point that in these experiments the external temperature did not seem to exert any significant effect on the water content of the heart, muscle and testes. Analyses of these tissues yielded results similar to those previously reported.

In addition to the data recorded in Tables I and II, the tissues of 76 rats kept at moderate temperatures were analyzed in connection with the present study. As the results are essentially similar to those previously published, their tabulation in this paper would be superfluous.

Rats exposed to cold apparently withstand 40 to 50 per cent reductions of the creatine concentration of the heart muscle better than rats kept at moderate or warm temperatures. Whether this is due to a loss of the glycogen and phosphocreatine reserves that is relatively

smaller, or to a more adequate cardiac hypertrophy and compensation, or to other factors, remains to be determined. If the administration of thyroid substance is discontinued, the creatine tends to increase.

To illustrate, the observations with rats 252 and 253 may be cited. The former was kept in the cold room and received a total of 36 mg. of thyroxine during a period of 21 says. The injections were discontinued and the rat sacrificed 11 days later. The creatine content of the heart was found to be 144 mg. per cent. On the other hand, rat 253 was in the refrigerator for a period of 21 days before the injections were commenced. During the succeeding 10 days it received a total of 20 mg. Analysis of the heart showed it to contain 97.6 mg. per cent of creatine.

SUMMARY

Experiments have shown that the survival period of thyrotoxic rats is markedly influenced by the environmental temperature, being of much longer duration at low than at high temperatures. Between 22° and 37.5°C., the relation of temperature to the survival period is approximately linear. The tolerance to relatively large doses of thyroid and thyroxine is considerably increased below 20°C., but is especially striking at 4–6°C.

Hyperplasia of the thyroid, which results from exposure to cold, may be prevented by the administration of desiccated thyroid or thyroxine.

The diminished concentration of creatine of the myocardium, which is a conspicuous finding in rats treated with thyroid substance, does not occur in physiological hyperthyroidism resulting from long continued exposure to a low thermal environment.

In rats kept at high temperatures, whether treated with thyroid substance, or not, there is apparently no storage of colloid in the gland. The significance of the relatively high values for heart creatine in these animals at time of death is briefly discussed.

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EXPLANATION OF PLATES

PLATE 39

FIG. 1. Thyroid of rat 220, control in the cold room, showing hyperplasia of epithelium and loss of colloid. Stained with hematoxylin and phloxine. ×240.

FIG. 2. Thyroid of rat 219, which received 60 mg. of thyroxine during 32 days in the cold room, showing storage of colloid. Stained with hematoxylin and phloxine. × 240.

FIG. 3. Thyroid of rat 257, control in the cold room, showing hyperplasia of gland and loss of colloid. Stained with hematoxylin and phloxine. × 240.

FIG. 4. Thyroid of rat 255, which received 44 mg. of thyroxine during 29 days in the cold room, showing storage of colloid. Stained with hematoxylin and phloxine. × 240.

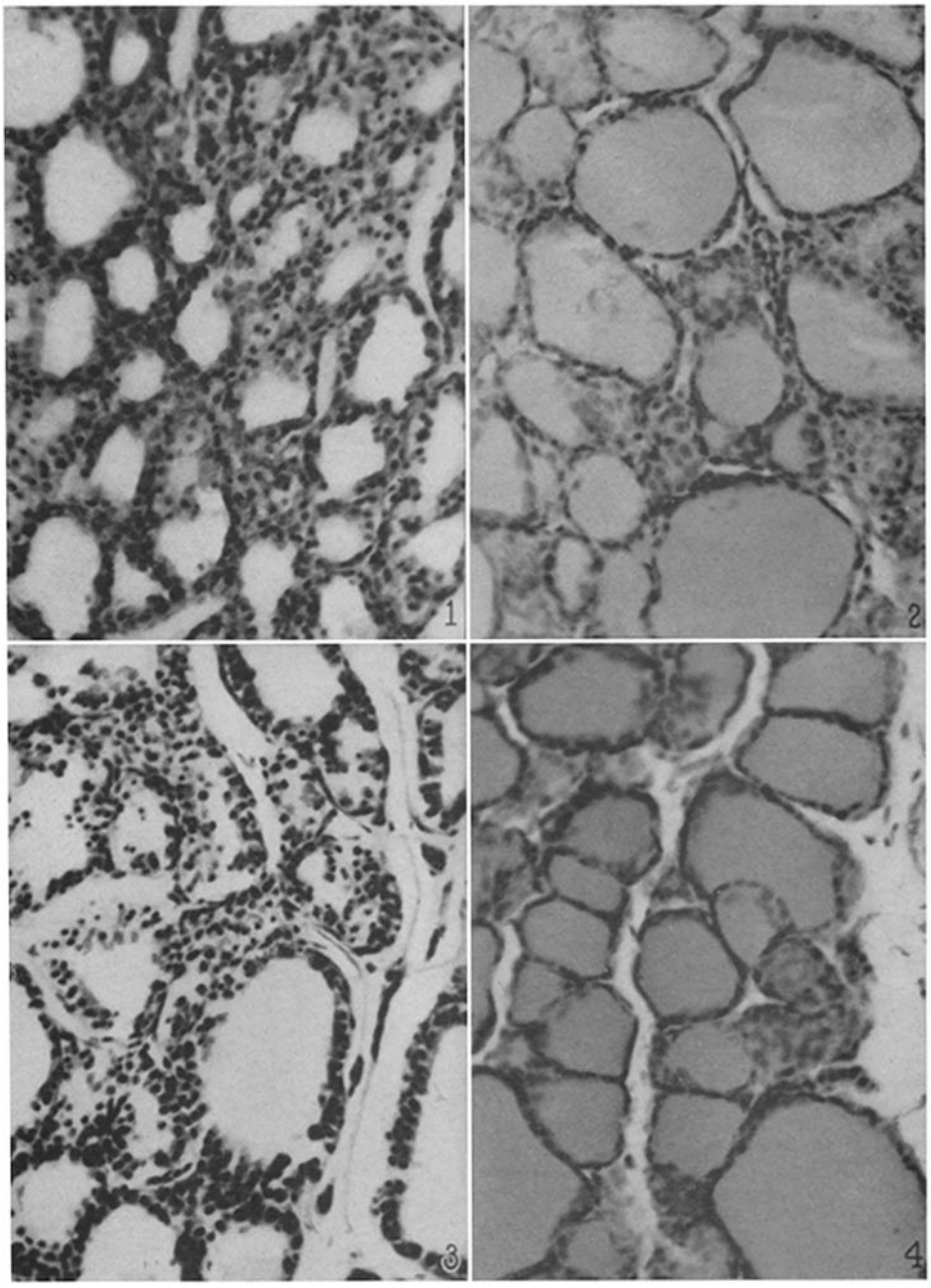
PLATE 40

FIG. 5. Thyroid of rat 289, which received 64.5 gm. of desiccated thyroid during 68 days in the cold room, showing storage of colloid. Stained with hematoxylin and phloxine. × 240.

FIG. 6. Thyroid of rat 290, which received 90 gm. of desiccated thyroid during 99 days in the cold room, showing storage of colloid. Stained with hematoxylin and phloxine. × 240.

FIG. 7. Thyroid of rat 214, which received 4 mg. of thyroxine during 3 days at 37.5°C., showing edema and degeneration of the gland. Stained with hematoxylin and phloxine. × 240.

FIG. 8. Thyroid of rat 215, control in the incubator, showing edema and degeneration of the gland. Stained with hematoxylin and phloxine. × 240.



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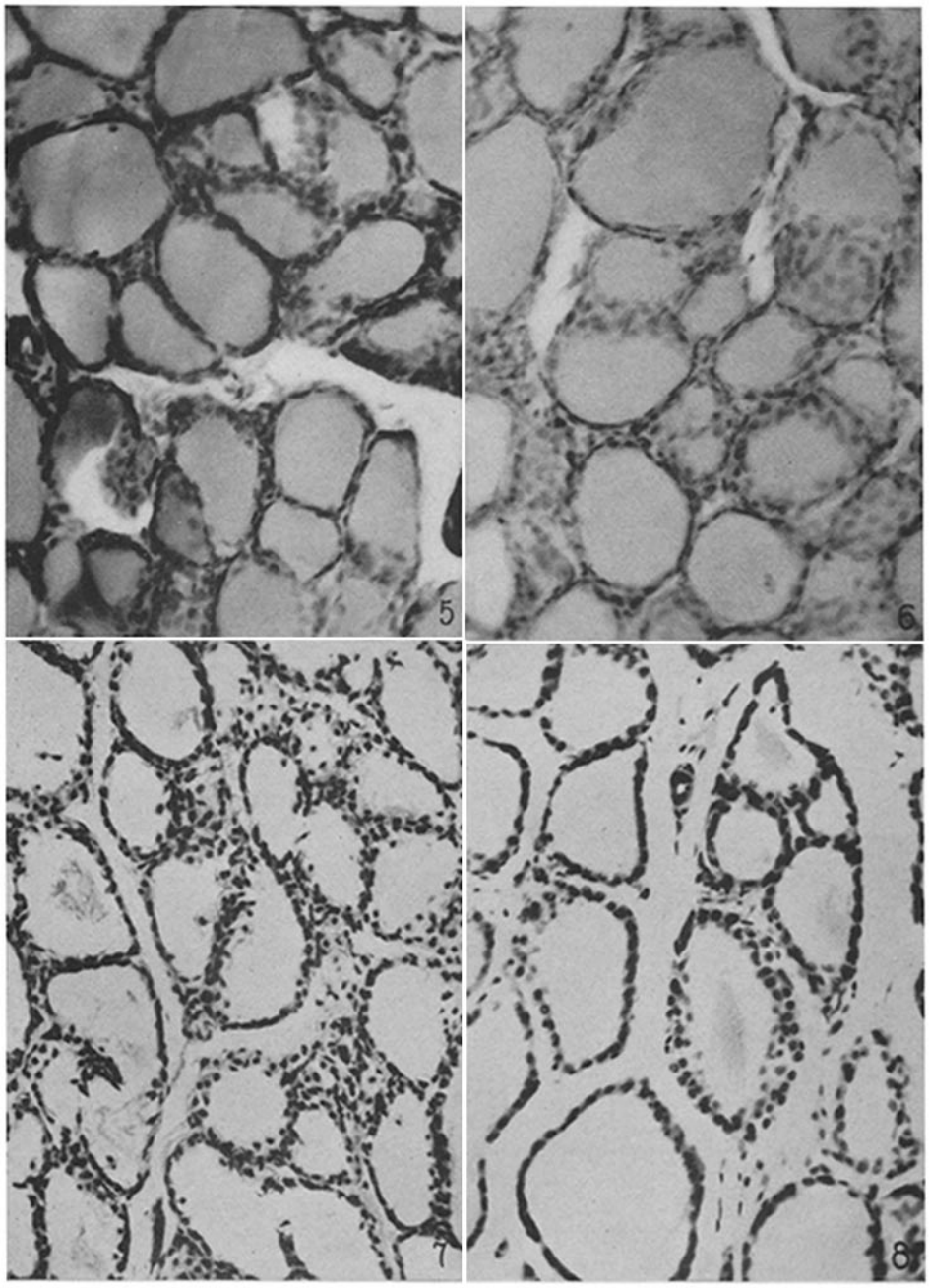

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