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Thyroid Secretion Rate of Albino Rats During Growth, Pregnancy and Lactation

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R. A. MONROE and C. W. TURNER

INTRODUCTION

The thyroid gland is closely associated with almost every organ and tissue in the body. Therefore, a deficiency of the thyroid hormone (hypothyroidism) or an oversupply of it (hyperthyroidism) may reflect itself in demonstrable changes in many organs and functional processes of the body. Most of our knowledge concerning the physiology of the thyroid gland has been inferred from the effects of hypo- or hyperactivity of the gland. One of the most notable effects of varying thyroid function is the accompanying change in basal metabolic rate. Consequently, the thyroid gland has been stressed as a regulator of energy metabolism. It must be remembered, however, that these changes are merely manifestations of the changes which are taking place in all cells of the body. It is obvious, since so many tissues are dependent upon normal thyroid activity for normal functioning, that the thyroid mechanism is one of the most important of the many regulatory mechanisms necessary for maintaining a constant internal environment in a constantly changing external environment.

In recent years, research in the field of thyroid physiology has been given an added stimulus by the development of methods whereby thyroid function can be regulated with relative ease. Not only is this fact of clinical importance, but also it has been found that artificially induced hypo- and hyperthyroidism affect the productive processes of livestock. For example, the administration of thyroidally active substances to cows causes a marked increase in milk and milk-fat production. On the other hand, a hypothyroid condition brought about by the administration of certain goitrogenic agents, such as thiouracil, has been found to enhance the fattening of swine, fowls and other domestic animals.

To administer these compounds effectively, it is first necessary to have a clear picture of the normal thyroid activity. For example, to administer thyroprotein to an animal most effectively, it is essential to know the normal state of activity of the thyroid gland under given physiological and environmental conditions. Otherwise, one might easily give too much or too little of the hormone, since the effective dosage range of so potent a substance is necessarily short.

Until recently, however, an accurate, comprehensive study of the normal rate of thyroid secretion has been impossible because of the lack

of a technique suitable for such a study. A few years ago a method was devised which has made feasible the investigation of the normal thyroid secretion rate under varying physiological and environmental conditions.

It seemed worthwhile, therefore, to undertake a study of the normal thyroid secretion rate in the rat during growth, pregnancy and lactation. Not only would such a study serve as a basis for further investigation, but in addition it would give an interesting insight into the significance of the function of the thyroid gland during these changing physiological states.

ANATOMY OF THE RAT THYROID

Embryology

Early in embryonic life there appear paired outpouchings on the lateral wall of the endodermal pharynx. Five pairs of such branchial pouches are present. The third and fourth pouches develop eventually into the parathyroid glands. Closely associated with this process is the development of the thymus glands, the anlagen of which are ventral and medial prolongations of the third pair of pouches.

In young embryos there also appears a small, endodermal outpouching in the midventral pharyngeal wall—the thyroid anlage. This outpocket later becomes a stalked vesicle, the stalk of which (called the thyroglossal duct) terminates in the *foramen cecum* near the tuberculum impar of the tongue. The duct atrophies, but the *foramen cecum* remains a permanent cicatrix. The thyroid anlage is bilobate and, as the duct atrophies, it loses its lumen. The proliferating endodermal cells then separate into nondescript, anastomosing plates or solid cords of tissue (Norris 1916) which migrate caudally. The gland eventually takes up a position with a lobe on each side of the trachea. Later, lumina appear in these plates of tissue. They are the beginnings of the thyroid follicles in which colloid is stored.

Gross Anatomy

The mature rat thyroid consists of two lobes connected ventrally by a thin band of tissue—the *isthmus*. These lobes lie on the lateral surface of the trachea immediately below the larynx and normally cover four or five tracheal rings. From side to side, the thyroid is convex on the external surface and concave on the internal surface, with the trachea fitting into the concavity. Its ventral surface is covered by the infrahyoid muscles, cervical fascia layers and skin. Dorsally are found the cricoid cartilage and trachea, the esophagus, and the pharynx.

In conjunction with the thyroid are the parathyroid glands. These glands, which can be distinguished by their pale color, are closely attached to the antero-lateral surface of each thyroid lobe. Erdheim (1906) and Togofuku (1911) and others have found parathyroid accessories along the neck and imbedded in the thymus glands, which are found just anterior to the heart. On the other hand, Hoskins and Chandler (1925) maintain that parathyroid accessories are rare in the rat. The thyroid itself, moreover, may also have accessories.

The thyroid gland is enclosed by an outer areolar capsule and an inner true capsule. The outer (false) capsule is loosely attached to the gland. The inner capsule is attached intimately to the thyroid by means of trabeculae which extend into the gland, divide it into ill-defined lobules, and support the blood and lymph vessels.

The thyroid has an enormous blood supply. It has been estimated that in relation to its size, it is about five times as vascular as the kidney (Sloan, 1936). The main sources of blood in the rat thyroid are the superior thyroid artery (a branch of the internal carotid, which is a subbranch of the innominate artery) and the inferior thyroid artery (arising from the subclavian via the costocervical trunk). Blood drainage is taken care of by superior and middle thyroid tributaries of the internal jugular vein and by the inferior thyroid vein which usually empties into the vena cava superior but sometimes enters the internal jugular vein instead.

The thyroid is also richly supplied with lymphatic vessels. The lymph is drained into lymph spaces surrounding the individual follicles and is carried successively through interlobular ducts, capsule plexuses, cervical glands, and, finally into the thoracic duct which joins the venous system in the jugulo-subclavian vein.

For innervation, the rat thyroid depends upon branches of the vagus nerve (10th cranial). The nerves of the thyroid are apparently not secretory in action since thyroids transplanted in different parts of the body still function efficiently. However, the rate of secretion may be influenced indirectly by the nerves to the thyroid by means of their effect on the blood supply to that gland.

Histology

It has been stated previously that the thyroid gland is divided into ill-defined lobules. Within these lobules are found the structural units of the gland—the follicles (acini, alveoli, vesicles). In histological sections, these follicles are usually round or oval but may be irregular. Surrounding each follicle is loose, elastic, connective tissue, which contains numerous blood and lymphatic vessels. Nerve fibers form perivascular plexuses and pass to the follicles.

The wall of the follicle is a single-layered epithelium (columnar or cuboidal) which rests directly upon connective tissue. Apparently there is no basement membrane. The follicular cell nuclei are generally large and distinct and the cytoplasm pale. The Golgi apparatus normally lies between the nucleus and the cell lumen. Fat droplets and cytoplasmic vacuoles may also be present.

Occasionally there is found another type of follicular epithelial cell containing cytoplasmic granules which stain like the thyroid colloid. It is thought that this type represents merely another phase of secretory activity.

Within the follicle lumen, forming a conspicuous feature, is a homogeneous, hyaline material called the thyroid colloid. In the usual histo-

logical preparations the colloid is separated from the epithelium by peripheral vacuoles, which have been accentuated by a contraction of the colloid in fixation. Colloid in sections prepared by freezing-drying methods is free from vacuoles and Williams (1937, 1939) reported that follicles in the living condition contained no vacuoles.

Cytology

It has been pointed out in previous sections that the thyroid follicles, which arise from plates of endodermal tissue, are lined with a simple cuboidal epithelium. The similar interfollicular cells, whose significance is still in question, may or may not play a role in the secretion of the thyroid hormone.

The normal, active cell is cuboidal with a round, lightly staining nucleus. These Bensley (1916) designated as "chief" cells. Also present in the normal follicle is a type of cell which is characterized by a low cuboidal form with a nucleus which may be slightly pycnotic. Its cytoplasm stains darkly and is relatively free from secretory precursors. These are the "colloid" cells of Langendorf. It is now thought, however, that both chief and colloid cells are of the same type but in different phases of secretory activity.

Colloid cells contain but few mitochondria; these are in the form of granules and short rods. In chief cells, on the other hand, are found many mitochondria as blunt rods and some as filamentous strands.

The Golgi apparatus is usually found in the cytoplasm between the nucleus and the follicle lumen. Occasionally, however, cells are found with Golgi material in the basal portion.

The interfollicular cells differ in several respects from the cells described above. They are usually larger than those which line the follicles. Their cytoplasm is uniform in appearance and usually is acidophilic. The nuclei are relatively large and nearly always spherical. Rarely a nucleus stains densely and evenly, but in the great majority of cases there is a distinct chromatin network in a light staining nucleus. Zechel (1932) described these cells at some length and suggests three possible functions: (1) the formation of new follicles, (2) the production of colloid, and (3) the inception of follicular destruction.

Thus far, only the normal cytological picture has been considered. Even here, however, differences are noted in the follicular cells, depending upon their relative secretory activity. Consequently, many experiments have been undertaken in order to study the changes in the cell during the secretory and non-secretory phases of the cycle.

It seems to be the concensus of most workers that in an activated gland (e.g., by exposure of the animals to cold or administration of thyrotrophic hormone) the colloid is diminished with a concomitant increase in the number of vacuoles, while the follicular cells proliferate and increase in height. Associated with these changes, Baillif (1937) observed an accumulation of secretion granules in the follicular cells of thyroids of rats which had been exposed to cold. The work of Williams (1939)

seems to confirm these findings. Cramer and Ludford (1926) and Ludford and Cramer (1928) observed an enlargement of the Golgi apparatus in activated mouse and rat thyroids and also stated that there is a definite relationship between the functional state of the gland and the mitochondria. In their experiments, they observed a great enlargement of the mitochondria in the follicular cells of activated thyroids. Okkels (1934) also found that the Golgi apparatus enlarged in active thyroid cells but did not observe an increase in mitochondria except in the cells of follicles which showed a restoration of colloid. This discrepancy may be accounted for in Okkels' fixation technique (Zenker-formol) which is prone to cause fragmentation of the mitochondria. From his findings, Okkels expressed the opinion that the mitochondria are concerned with the formation of the secretion and the Golgi apparatus with its discharge.

In the non-secreting gland, on the other hand, apparently the reverse conditions are true. The follicles are distended with colloid which has been accumulated and stored. The epithelium becomes flattened and there is a diminution of vacuoles in the colloid. The mitochondria are barely visible, and the Golgi apparatus is contracted. It might be of interest to note here, that since he found the Golgi bodies in the basal parts of several cells in this state, Cowdry (1922) postulated that the position of the Golgi apparatus indicated the polarity of the cell. However, due to a lack of supporting evidence this interesting hypothesis must be considered only tentative. In fact, there is some evidence to the contrary, except in abnormal thyroids (Cramer and Ludford, 1926). Probably this phenomenon of reversed position of Golgi bodies is due to a mechanical displacement, if indeed there is a true displacement. Gillman (1934) maintains that "reversal" of Golgi bodies is observed only in single sections—serial sections showing that the "reversed" body is, in reality, only a part of the whole apparatus.

THYROID SECRETION RATE

Unlike the human thyroid gland, which secretes an active principle relatively early in embryonic life, the fetal rat thyroid does not begin to produce its hormone until the 18th or 19th day of gestation (Kull, 1926, Kaan and Hall, 1937; Gorbman and Evans, 1941; and Hall and Kaan, 1942). Since this hormone affects almost all tissues and organs of the body (and the functional well-being of the organism as a whole) a good deal of study has been devoted to the subject of thyroid secretion. Consequently, many methods have been suggested for the determination of the activity in the thyroid gland.

Determination of Secretory Activity in the Thyroid Gland

Qualitative Methods.—On the basis of changes in the thyroid gland accompanying changes in its activity, the following methods have been suggested for observing the functional activity of the gland: (1) observation of the changes in mitotic activity, (2) determination of the average diameter of the thyroid follicles, (3) direct measurement of the follicular

epithelium, and (4) measurement of increased or decreased amounts of iodine (total or "hormonal") in the thyroid gland and/or in the blood. These methods, however, are more useful in measuring the response of the thyroid gland to stimuli (such as temperature, the thyrotrophic hormone, etc.) than they are in the quantitative determination of the thyroid secretion rate. Consequently, other more precise methods have been sought.

Quantitative Methods.—Until recently, all quantitative results were based on experiments conducted by the replacement therapy technique. By this method, the amount of thyroidally active material needed to maintain thyroidectomized animals in a normal state is determined. The best criteria of normality for this type of experiment are the basal metabolic rate and the heart rate. The growth rate also has been used as an index of normality. The results of various investigators employing this technique, however, vary greatly. Fishburne and Cunningham (1938) found that 40 micrograms of thyroxine was needed daily to maintain the normal heart rate of thyroidectomized rats. Similar results were obtained by Meyer and Yost (1939) in regard to the basal metabolic rate. On the other hand, Evans, Simpson and Pencharz (1939) and Rowlands (1942) found that the basal metabolic rate and growth, respectively, could be maintained in thyroidectomized rats by injecting thyroxine at the rate of 5.0 micrograms per hundred grams body weight. The work of LeBlond and Hoff (1944) indicates that the normal secretion rate may be even lower. The data presented in these last three papers agree closely with results obtained by a more recent, and probably more nearly accurate, method.

MacKenzie and MacKenzie (1943) and Astwood (1943) following the discovery of the goitrogenic properties of certain drugs (MacKenzie, et al., 1941; Richter and Clisby, 1942; Kennedy, 1942) have shown that the goitrogenic action of these drugs is due to the prevention of thyroxine synthesis by the thyroid gland. Compensatory thyroid hypertrophy ensues in accord with the thyroid-pituitary balance theory (Fig. 1). These workers state that the goitrogenic drugs do not inhibit the action of preformed endogenous or injected thyroxine.

On the basis of this knowledge Dempsey and Astwood (1943) proposed a new method for measuring the thyroid secretion rate. They suggested that a goitrogenic drug (e.g., thiouracil) be administered simultaneously with graded doses of thyroxine for a suitable period; the amount of thyroxine needed to maintain a normal thyroid weight (i.e., a normal balance between the pituitary and thyroid glands) they considered equal to the normal secretion rate. Of course, the thyroid hormone is probably not thyroxine *per se*. It does seem reasonably certain, however, that thyroxine is the active, prosthetic group of the thyroid hormone molecule, as found in the blood. This method, while it may not measure the actual amount of "hormone" liberated by the thyroid gland, does enable us to measure quantitatively the thyroid secretion rate in thyroxine equivalents.

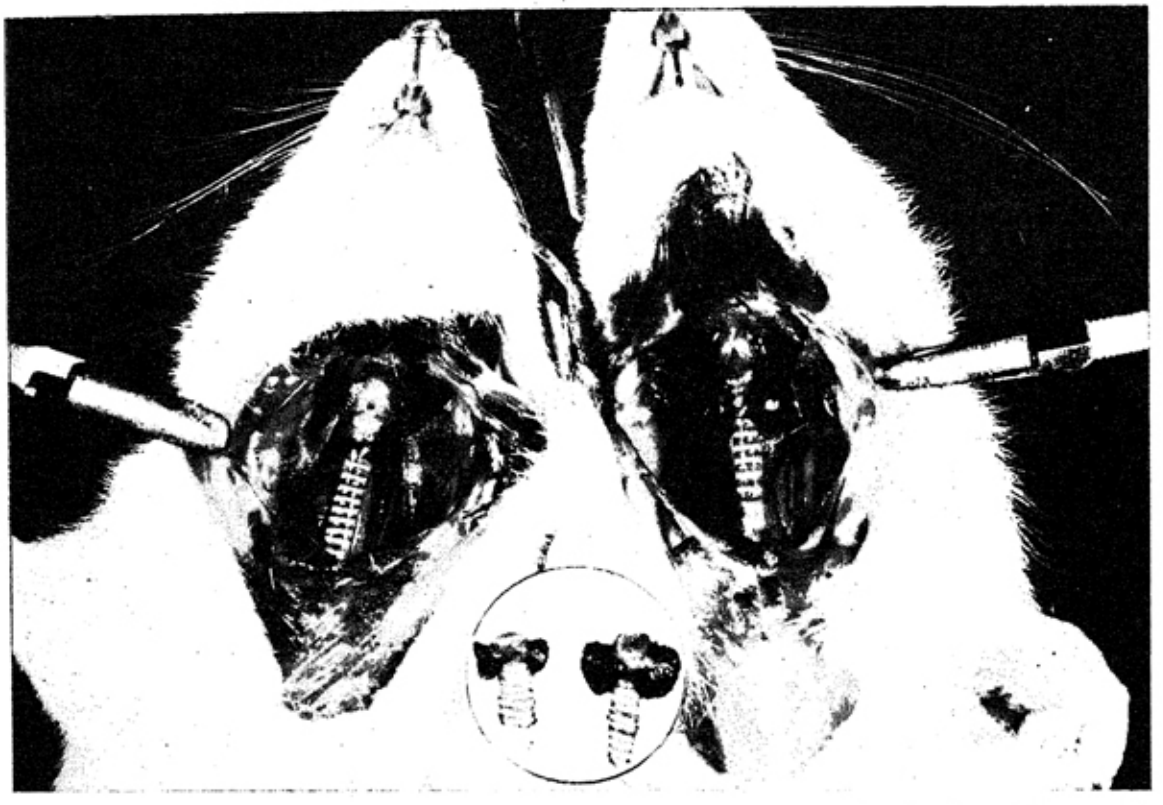

Fig. 1.—Photographs illustrating thyroid enlargement caused by feeding thiouracil.

Several investigators have used this technique recently. Dempsey and Astwood (1943) found that at room temperature (25° C.), the thyroid weight of thiouracil-treated, 100 gram rat was maintained at a normal level by the injection of 5.2 micrograms of l-thyroxine daily. Moreover, they showed that temperature has a marked effect on thyroid activity, at 1° C. it took 9.5 micrograms of thyroxine to maintain the thyroid weight, while at 35° C. it took only 1.7 micrograms. Purves (1943), using the same technique, except for the substitution of rape seed for thiouracil as a goitrogenic agent, found that between 2.0 and 3.0 micrograms of thyroxine per hundred grams body weight would maintain male rat thyroids at normality. However, Astwood and Bissell (1944) studied the problem further and found that 5.0 micrograms of thyroxine per hundred grams body weight represented the normal secretion rate. Reineke, Mixner, and Turner (1945) compared the curve obtained by the Dempsey and Astwood goitrogenic drug technique with that obtained by basal metabolic rate measurements. The curves both showed that approximately 4.8 micrograms of thyroxine was required to maintain normal thyroid weight in thiouracil treated rats of approximately 140 grams body weight. This amount is equivalent to 3.47 micrograms of d, l-thyroxine per hundred grams body weight.

It would appear, therefore, that the normal rate of thyroid secretion by the rat lies within the range of two to five micrograms per hundred grams body weight, measured in equivalent amounts of d, l-thyroxine.

EXPERIMENTAL

The value of knowledge concerning the secretion rate of the thyroid gland seems self-evident. In the past, however, no comprehensive work has been attempted on this problem, except for Schultze and Turner's (1945) extensive study of the fowl, goat and calf. Doubtless the main reason for this want of investigation has been the lack of a suitable, convenient and accurate method for studying the thyroid secretion rate. With the advent of the goitrogenic drug technique, the way was opened for the study of thyroid activity under a variety of conditions. But before one can evaluate intelligently any data concerning the effects of various experimental conditions on thyroid activity, one must first have a clearer understanding of the normal thyroid secretion rate. Conversely, a knowledge of this sort is also essential for an insight into the rate of thyroid function during various physiological activities.

It seemed both feasible and logical, therefore, to attempt a comprehensive study of the thyroid secretion rate of animals under varying physiological conditions. Consequently, several experiments were set up in order to determine the normal thyroid secretion rate of growing, pregnant, and lactating albino rats.

Determination of the Thyroid Secretion Rate in Rats

Methods and Materials.—The experimental animals used were Wistar albino rats of the Missouri strain. They were kept in a ventilated basement room illuminated by diffused sunlight and, from 7:00 a. m. to 5:00 p. m., incandescent lights. The temperature was relatively constant throughout the year, averaging approximately 78° F. The room varied in temperature no more than ±5° F. from summer to winter and no more than ±1° F. during any one experiment.

The following basal ration was fed to all experimental animals:

Yellow corn meal	45	parts by weight
Shorts	15	" " "
Soybean oil meal	15	" " "
Alfalfa meal	10	" " "
Meat scraps (50% protein)	7	" " "
Bran	5	" " "
Bone meal	0.5	" " "
Common salt	1	" " "
Cod liver oil (400 A.O.A.C. units vitamin D per gram)	0.25	" " "

The technique used in determining the thyroid secretion rate varied only slightly from the one suggested by Dempsey and Astwood (1943). Each experimental group was divided into lots. All except the control lot were treated simultaneously with thiouracil and graded doses of d, l-thyroxine. The thiouracil was mixed in the feed (0.1% by weight) and fed *ad libitum*. The d- l-thyroxine was injected subcutaneously as an aqueous solution of the disodium salt.

The thyroxine was prepared for injection in the following manner. Crystalline d, l-thyroxine was dissolved in a minimum amount of N/10 sodium hydroxide and diluted nearly to volume with distilled water. The monosodium salt thus formed was then precipitated by the addition of a small amount of N/10 hydrochloric acid, and the resultant mixture diluted to volume. This was stored in the refrigerator as a stock solution.

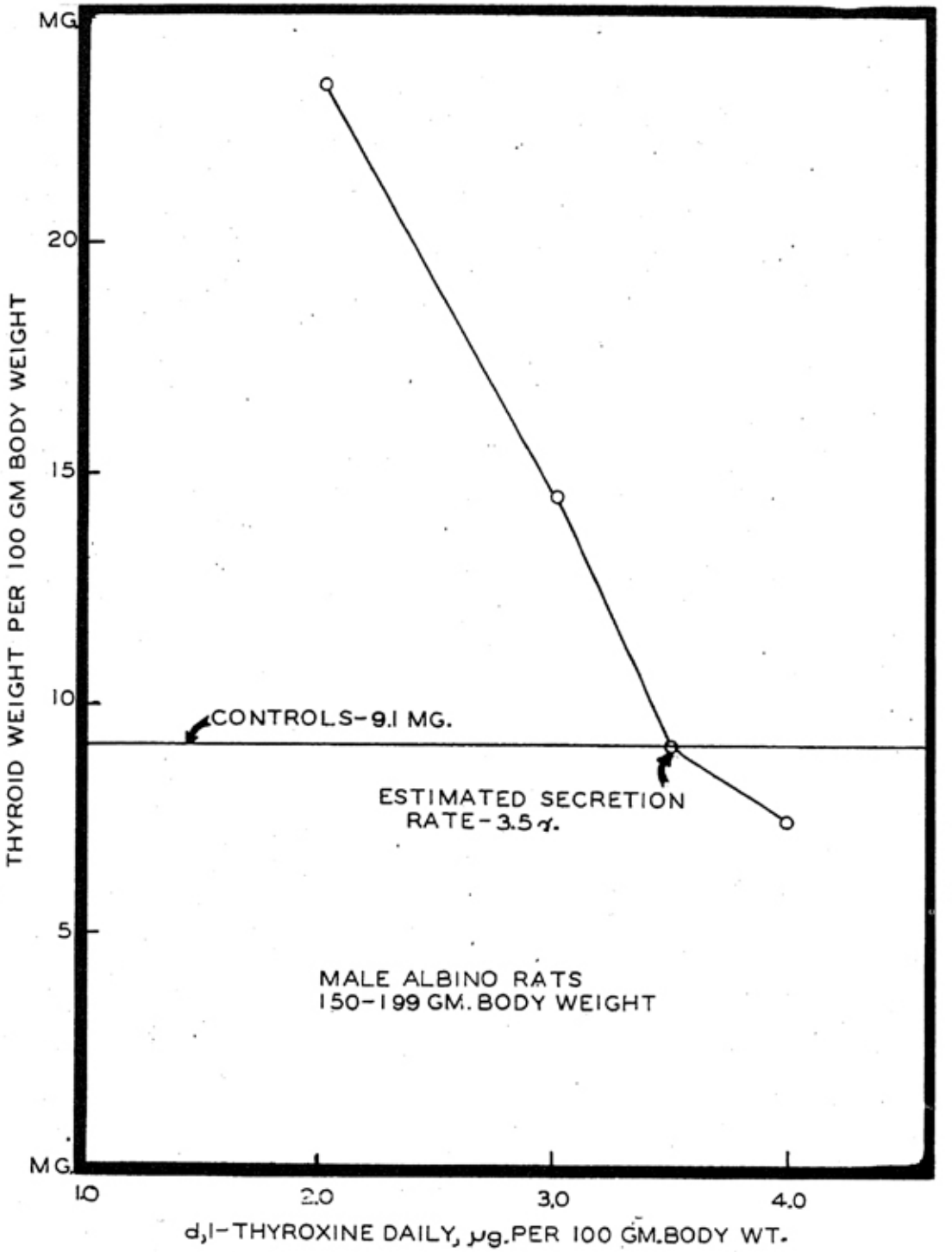

Fig. 2.—Method of plotting data to determine thyroid secrtion rate of growing male rats.

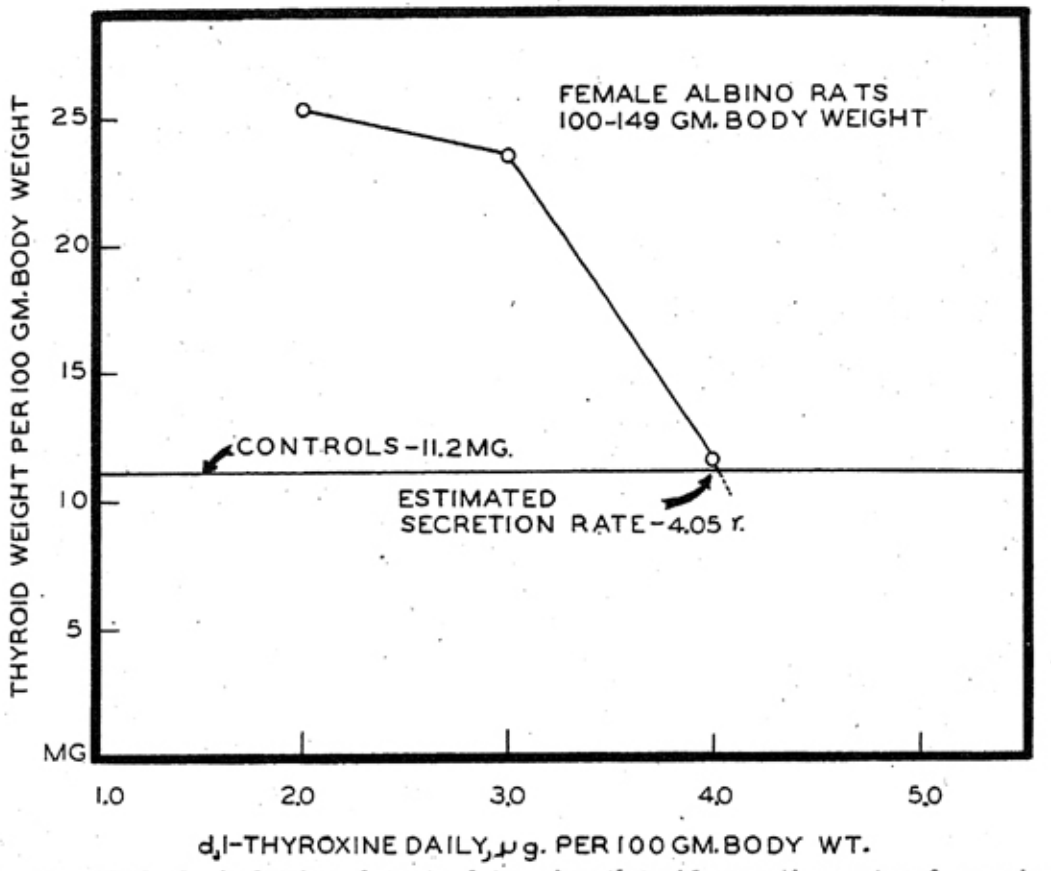

Fig. 8.—Method of plotting data to determine thyroid secretion rate of growing female rats.

As needed for injection, suitable dilutions were made of the stock solution by dissolving the monosodium salt precipitate in a minimum of N/10 sodium hydroxide and diluting to volume with distilled water, thus forming an aqueous solution of the disodium salt of d, l-thyroxine.

The experimental animals were injected daily in proportion to body weight for a two-week period and sacrificed on the 15th day. The body weight of each rat was recorded, along with the respective thyroid weights. From these data, the average thyroid weight per hundred grams of body weight was calculated for each of the lots receiving a different dose of thyroxine within the experimental group. These weights were then compared graphically with a similar figure for the normal control animals. The point on the curve where the thyroid weight of the injected animals was equal to the thyroid weight of the normal control animals was considered to represent the amount of thyroid hormone (in equivalent amounts of d, l-thyroxine) that the rat would secrete normally.

It should be remembered that since l-thyroxine, which is the form naturally produced by the thyroid, is twice as active as the racemic mixture (Reineke and Turner, 1945), the figures obtained in this investigation probably represent twice the amount of thyroxine actually secreted by the thyroid gland.

Growing Albino Rats.—The thyroid secretion rate was determined for both male and female rats at varying body weights (Fig. 2-8). The

rats were divided into weight groups ranging from 50 to 300 grams. Each weight group consisted of rats within the limits of a 50 gram range.

It will be seen that there is a definite sex difference in thyroid secretion rate (Fig. 4, Table 1). Moreover, the nature of the curves obtained by plotting the secretion rates differs for the two sexes.

In Group I (50-99 grams body weight) the males had a thyroid secretion rate equivalent to 3.64 micrograms of d, l-thyroxine per hundred grams of body weight. The succeeding male weight groups did not differ substantially from this figure. The thyroid secretion rate of the males throughout the entire weight range was approximately 3.5 micrograms per hundred grams body weight, expressed in terms of d, l-thyroxine.

In contrast to these figures, the female growing rats showed a higher initial thyroid secretion rate—4.63 micrograms d, l-thyroxine per hun-

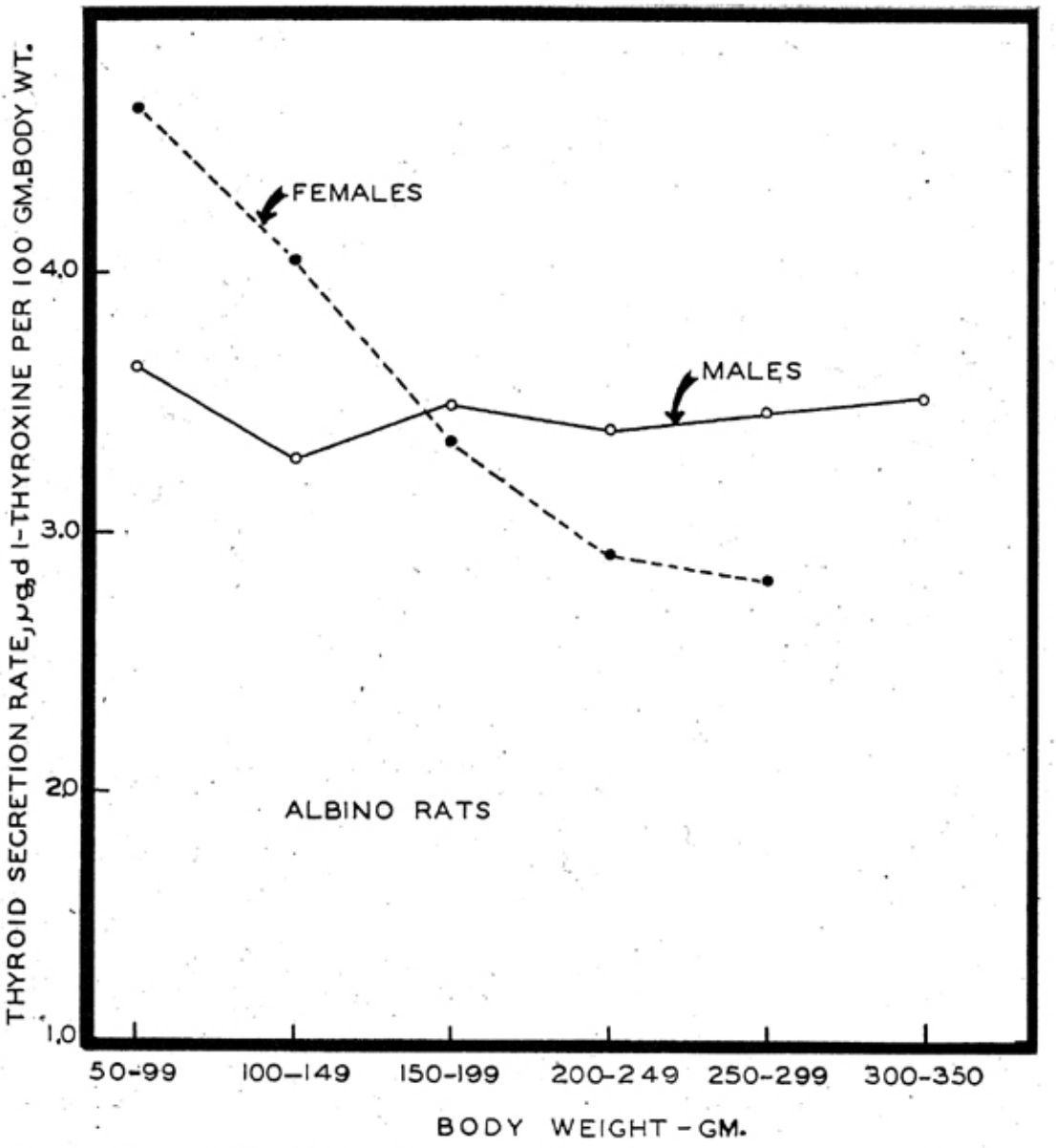

Fig. 4.—Normal thyroid secretion rate of growing rats at various body weights.

Table 1- THYROID SECRETION RATE OF GROWING ALBINO RATS

Growing Male Rats						Growing Female Rats					
Group 1 - 50 - 99 grams body weight											
Thiouracil in feed	Thyroxine injected/100 gm. rat	No. of rats	Avg. body weight	Avg. thyroid weight	Thyroid wt./100 gm. body weight	Thiouracil in feed	Thyroxine injected/100 gm. rat	No. of rats	Avg. body weight	Avg. thyroid weight	Thyroid wt./100 gm. body weight
%	ug.		gm.	mg.	mg.	%	ug.		gm.	mg.	mg.
None	none	21	82.2	7.1	8.6	none	none	17	81.5	6.9	8.5
0.1	2.0	18	85.5	14.3	16.7	0.1	2.0	9	76.2	22.1	29.0
0.1	2.5	6	90.3	10.8	12.0	0.1	3.0	10	76.9	15.2	19.8
0.1	3.0	22	86.1	8.2	9.5	0.1	4.0	20	83.8	9.3	11.1
0.1	3.5	6	78.7	6.9	8.8	0.1	5.0	9	87.2	6.1	7.0
0.1	4.0	19	89.1	7.1	8.0						
Avg. body weight - 85.2 gm. d,l-thyroxine per rat - 3.10 ug. daily d,l-thyroxine/100 gm. body wt. - 3.64 ug. daily						Avg. body weight - 81.1 gm. d,l-thyroxine per rat - 3.75 ug. daily d,l-thyroxine/100 gm. body wt. - 4.63 ug. daily					
Group 2 – 100 - 149 grams body weight											
None	none	21	126.7	11.1	8.8	none	none	18	122.7	13.8	11.2
0.1	2.0	10	128.3	30.2	23.5	0.1	2.0	9	126.3	32.1	25.4
0.1	2.5	10	131.8	17.6	13.3	0.1	3.0	10	127.3	30.0	23.6
0.1	3.0	36	122.3	14.8	12.1	0.1	4.0	12	126.5	14.7	11.6
0.1	3.5	19	125.3	7.5	6.0	0.1	4.5	12	137.8	15.8	11.4
Avg. body weight - 126.9 gm. d,l-thyroxine per rat - 4.18 ug. daily d,l-thyroxine/100 gm. body wt. - 3.29 ug. daily						Avg. body weight - 128.1 gm. d,l-thyroxine per rat - 5.19 ug. daily d,l-thyroxine/100 gm. body wt. - 4.05 ug. daily					
Group 3 - 150 – 199 grams body weight											
None	none	17	173.0	15.8	9.1	none	none	11	178.3	15.0	8.4
0.1	2.0	8	164.9	38.6	23.4	0.1	2.5	11	176.2	21.2	12.0
0.1	3.0	7	167.1	24.2	14.5	0.1	2.75	6	170.7	19.9	11.7
0.1	3.5	8	160.0	14.6	9.1	0.1	3.0	5	183.0	17.0	9.3
0.1	3.75	12	178.5	13.4	7.5	0.1	3.25	7	163.0	14.2	8.7
						0.1	3.5	5	160.0	12.6	7.9
Avg. body weight - 168.7 gm. d,l-thyroxine per rat - 5.90 ug. daily d,l-thyroxine/100 gm. body wt. - 3.50 ug. daily						Avg. body weight - 171.9 d,l-thyroxine per rat - 5.74 ug. daily d,l-thyroxine/100 gm. body wt. - 3.34 ug. daily					

Group 4- 200 - 249 grams body weight

None	none	8	216.1	16.8	7.8	none	none	20	223.0	19.7	8.8
0.1	2.5	8	229.8	24.7	10.7	0.1	1.0	7	223.7	38.5	17.2
0.1	2.75	7	219.9	21.3	9.7	0.1	2.0	7	229.4	30.6	13.3
0.1	3.0	9	215.5	19.2	8.9	0.1	3.0	9	218.4	18.4	8.4
0.1	3.75	7	216.0	14.6	6.8						

Avg. body weight - 219.5 gm.
d,1-thyroxine per rat - 7.46 ug. daily
d,1-thyroxine/100 gm. body wt. - 3.40 ug. daily

Avg. body weight - 223.6 gm.
d,1-thyroxine per rat - 6.53 ug. daily
d,1-thyroxine/100 gm. body wt. - 2.92 ug. daily

Group 5 - 250 - 300 grams body weight

None	none	8	274.4	22.9	8.3	none	none	16	259.9	17.6	6.8
0.1	2.5	8	278.3	30.8	11.1	0.1	1.0	6	263.7	33.7	12.8
0.1	2.75	12	277.8	29.9	10.8	0.1	2.0	6	268.3	27.5	10.2
0.1	3.0	8	272.0	29.0	10.6	0.1	2.5	4	273.3	22.6	8.3
0.1	3.75	6	275.8	19.1	6.9	0.1	3.0	5	265.8	18.6	6.9

Avg. body weight - 275.7 gm.
d,1-thyroxine per rat - 9.54 ug. daily
d,1-thyroxine/100 gm. body wt. - 3.46 ug. daily

Avg. body weight - 266.2 gm.
d,1-thyroxine per rat - 7.51 ug. daily
d,1-thyroxine/100 gm. body wt. - 2.82 ug. daily

dred grams body weight in Group I. In succeeding weight groups, however, the secretion rate declined steadily. In Group III (150-199 grams body weight) the thyroid secretion rate dropped to 3.34 micrograms. Beyond this point, the rate of secretion declined more slowly until, in Group V (250-300 grams body weight) it reached 2.82 micrograms per hundred grams body weight.

However, if the thyroid secretion rate is calculated per rat per day rather than in proportion to 100 grams body weight, it will be seen that the rate of secretion increases with increasing body weight (Table 1). The rate of increase is greater for the male rats than for the females. This relationship may be expressed for the males by the equation Y (thyroid secretion rate) $= 0.041X$ (body weight)$^{0.97}$, and for the females by the equation $Y = 0.422X^{0.51}$ (Fig. 5). It is interesting to note in the males, that the thyroid weight increases in proportion to body weight at

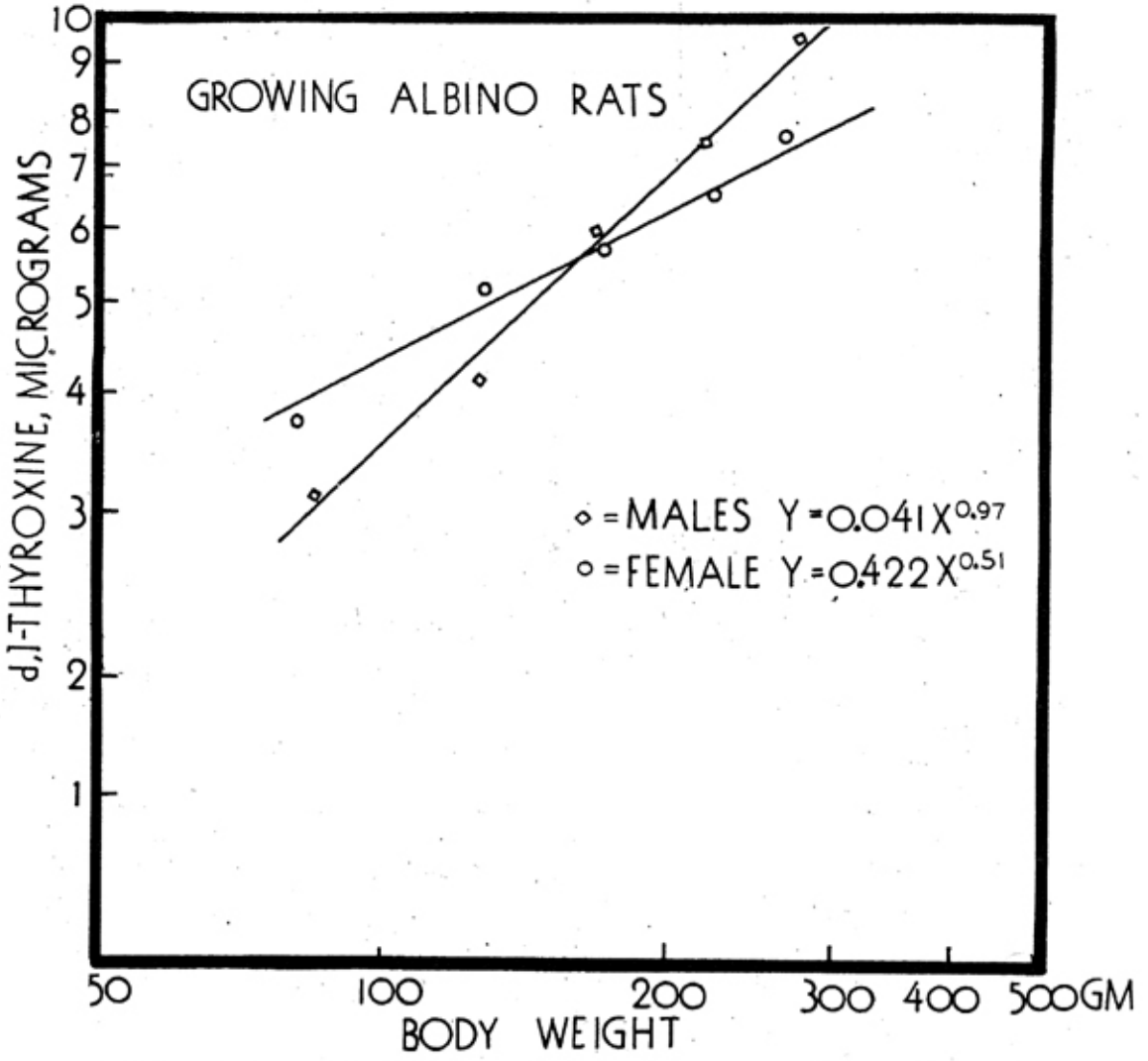

Fig. 5.—Relation of thyroid secretion to body weight in growing rats.

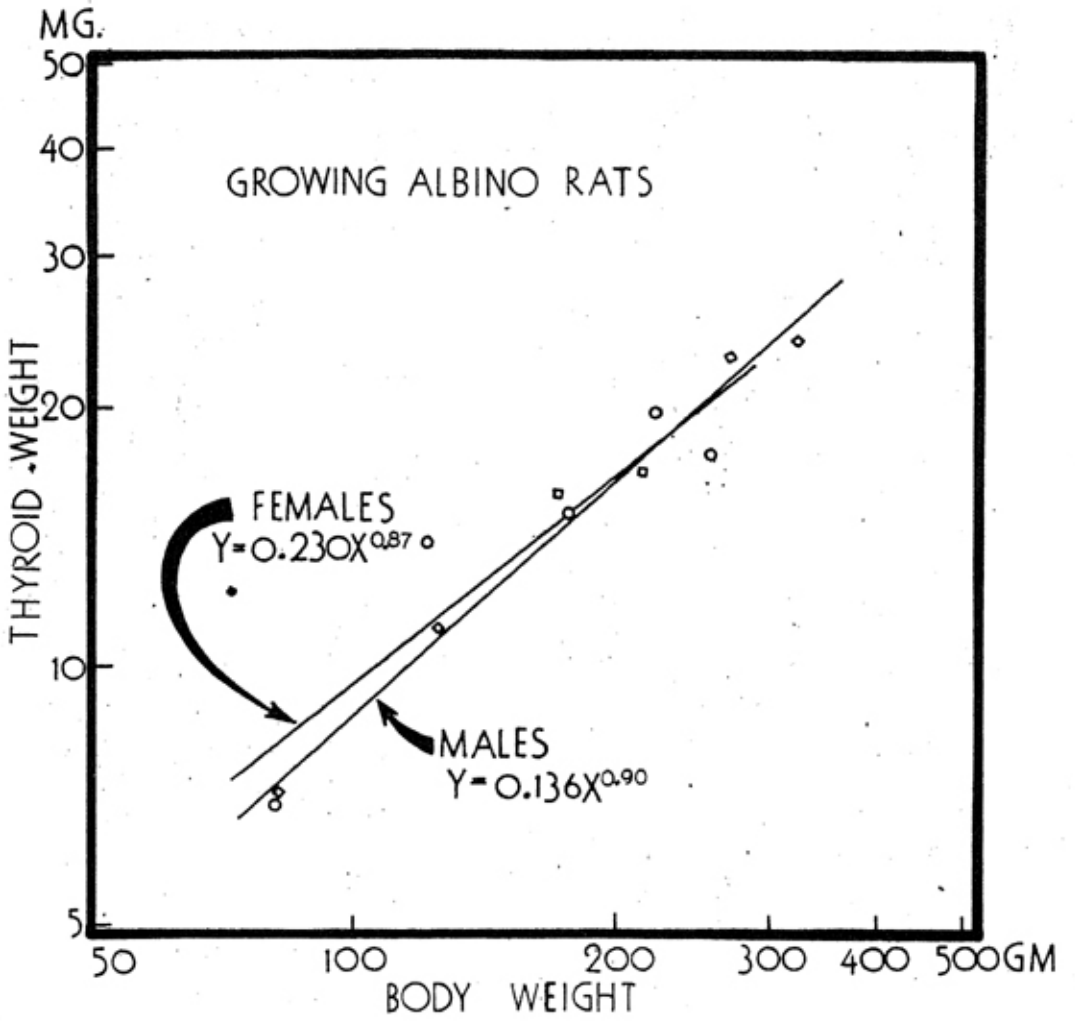

Fig. 6.—Relation of thyroid weight to body weight in growing rats.

approximately the same ratio as does the thyroid secretion rate (Fig. 6). In the females, however, the thyroid weight increases in proportion to body weight at a significantly greater rate than does the amount of thyroid secretion. This relationship (females) may be expressed by the equation Y (thyroid weight) $=$ 0.230X (body weight)$^{0.81}$.

Pregnant Albino Rats.—Opinions concerning the effect of pregnancy on the thyroid gland may be classified into three schools of thought: (1) that which maintains that the thyroid is hyperactive during pregnancy, (2) that which maintains that the thyroid is hypoactive during pregnancy, and (3) that which postulates that thyroid activity does not change during pregnancy.

One of the earliest supporters of the hyperactive theory was Marine (1917). However, his work is by no means conclusive. Bokelmann and Scheringer (1931) assumed that the thyroid was hyperactive during pregnancy, since the same changes in the thyroid gland could be produced by injection of physiologic doses of thyrotrophic hormone. Likewise, Scheringer (1933) showed that the blood iodine picture responded to

injection of adrenalin during pregnancy in a manner similar to injections of the same substance in patients with Graves' disease, in contrast to the response of normal or myxedematous patients. He concluded since the thyroid is hyperactive in Graves' disease, the same must be true during pregnancy.

On the other hand, Knaus (1924a, 1924b) saw histological and physiological evidences of thyroid hypofunction during pregnancy.

In studies showing the percentage of cases in which hyperthyroidism occurs during pregnancy, Clute and Daniels (1930) and Mussey et al. (1926) concluded that pregnancy is not a cause of hyperthyroidism.

More recent studies tend to indicate that the thyroid gland is not substantially affected by pregnancy. Abbott and Prendergast (1936) believe that the percentage of patients with enlarged thyroids during pregnancy is no greater than before pregnancy. They think that the basic factor is lack of iodine rather than pregnancy itself. The work of Hewitt and Van Liere (1941) supports this concept in that they observed no change in the thyroid-body weight ratio of guinea pigs during any stage of pregnancy. Abbott and Prendergast (1936) thought, however, that they did detect histological evidence of a mild increase in physiological activity of the thyroid, especially in the early stages of pregnancy.

Since a convenient technique for measuring the secretion rate of the thyroid gland is now available it seemed of interest to determine whether the thyroid secretion rate of pregnant rats varies from that of non-pregnant rats. Animals of approximately 200 grams body weight were selected and bred at as nearly the same time as possible. Groups of rats were brought into estrus nearly simultaneously by confining them to a large, light-proof cage and subjecting them to alternating 12-hour periods of light (supplied by a 100-Watt bulb) and darkness for four days. These females were then confined with males for eight days before treatment was begun. The rest of the experimental procedure was identical with that used on growing rats, with the exception that each female was examined for fetuses at the time they were sacrificed.

The results show (Fig. 7, Table 2) that these pregnant rats had an average body weight of 190.8 grams and that the estimated thyroid secretion rate of the group was 2.93 micrograms of d, l-thyroxine per hundred grams body weight. This figure does not vary significantly from that of non-pregnant females of the same body weight (Fig. 9).

It would seem, therefore, that pregnancy does not alter the activity of the thyroid gland of the rat.

Lactating Albino Rats.—It would appear from work showing that thyroidectomized animals are capable of being bred and nursing their young that the thyroid gland is not essential for milk secretion (Dragstedt, Sudan, and Phillips, 1924, and Folley, 1938). However, many studies show that thyroidectomy depresses lactation in several species (Graham, 1934; Preheim, 1940; Ralston et al., 1940; Folley, 1938; and Folley, et al., 1942). But at least part of this effect is due to operational shock (Graham, 1934; and Ralston et al., 1940). Nelson and Tobin (1937)

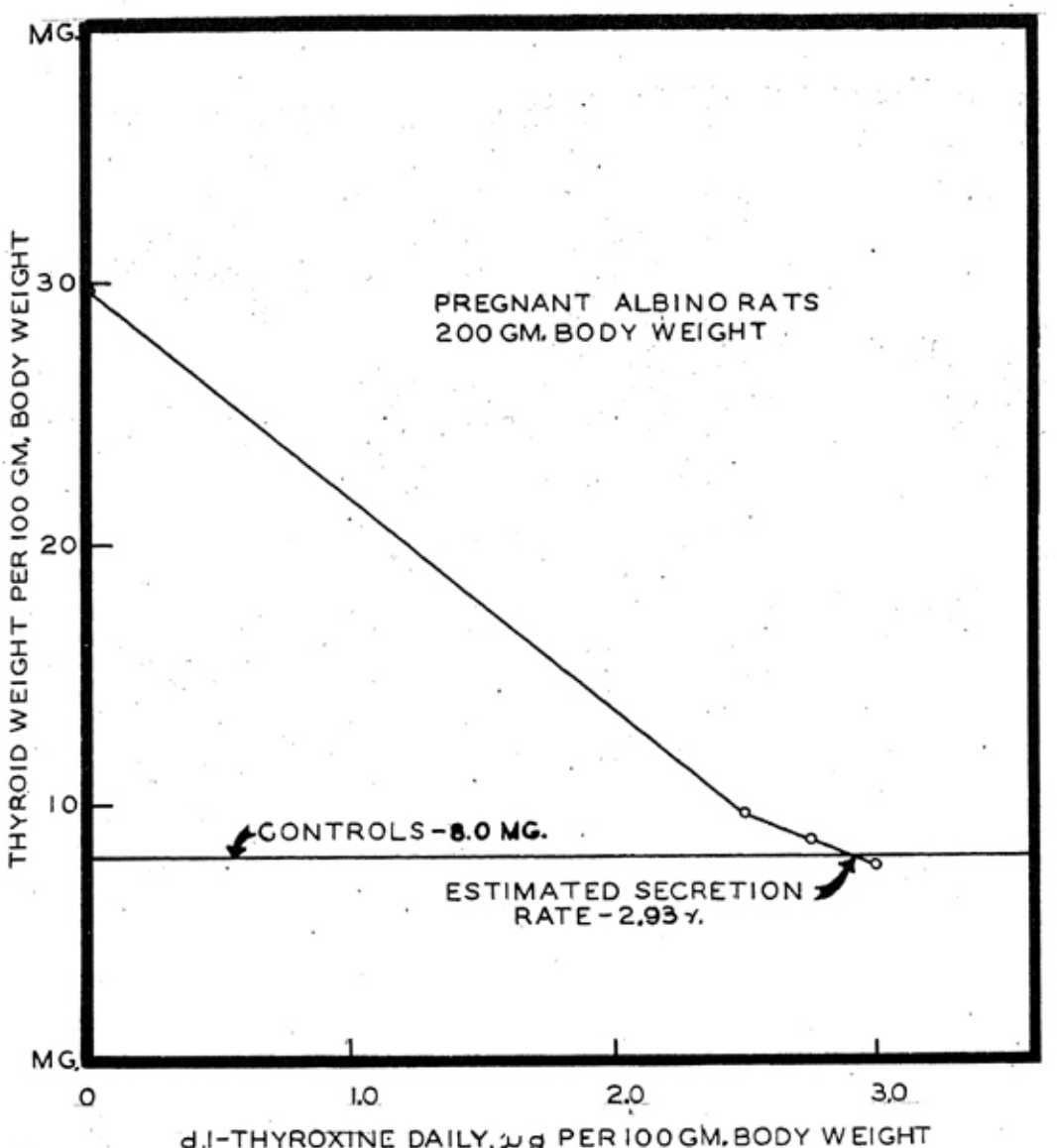

Fig. 7.—Assay to determine the thyroid secretion rate of pregnant rats.

Table 2-THYROID SECRETION RATE OF PREGNANT RATS

Thiouracil in feed	Thyroxine injected/100 gm. rat	No. of rats	Avg. body weight	Avg. thyroid weight	Thyroid wt./100 gm. body weight
%	ug.		gm.	mg.	mg.
none	none	10	207.0	16.5	8.0
0.1	none	6	176.5	52.6	29.7
0.1	2.5	10	185.1	18.0	9.7
0.1	2.75	7	198.3	17.2	8.7
0.1	3.0	9	187.3	14.7	7.9

Avg. body weight - 190.8 gm.
d,l-thyroxine per rat - 5.59 ug. daily
d,l- thyroxine/100 gm. body weight - 2.93 ug. daily

and Nelson (1939), on the other hand, found no difference from normal in the milk secretion of thyro-parathyroidectomized rats, as indicated by the growth of the litters. Folley et al. (1942) think it possible that the differences between their work and Nelson's may be explained on the basis of strain differences in accessory parathyroid tissue, or that Nelson used older rats—the older rats being less sensitive to parathyroidectomy (Dragstedt, 1927).

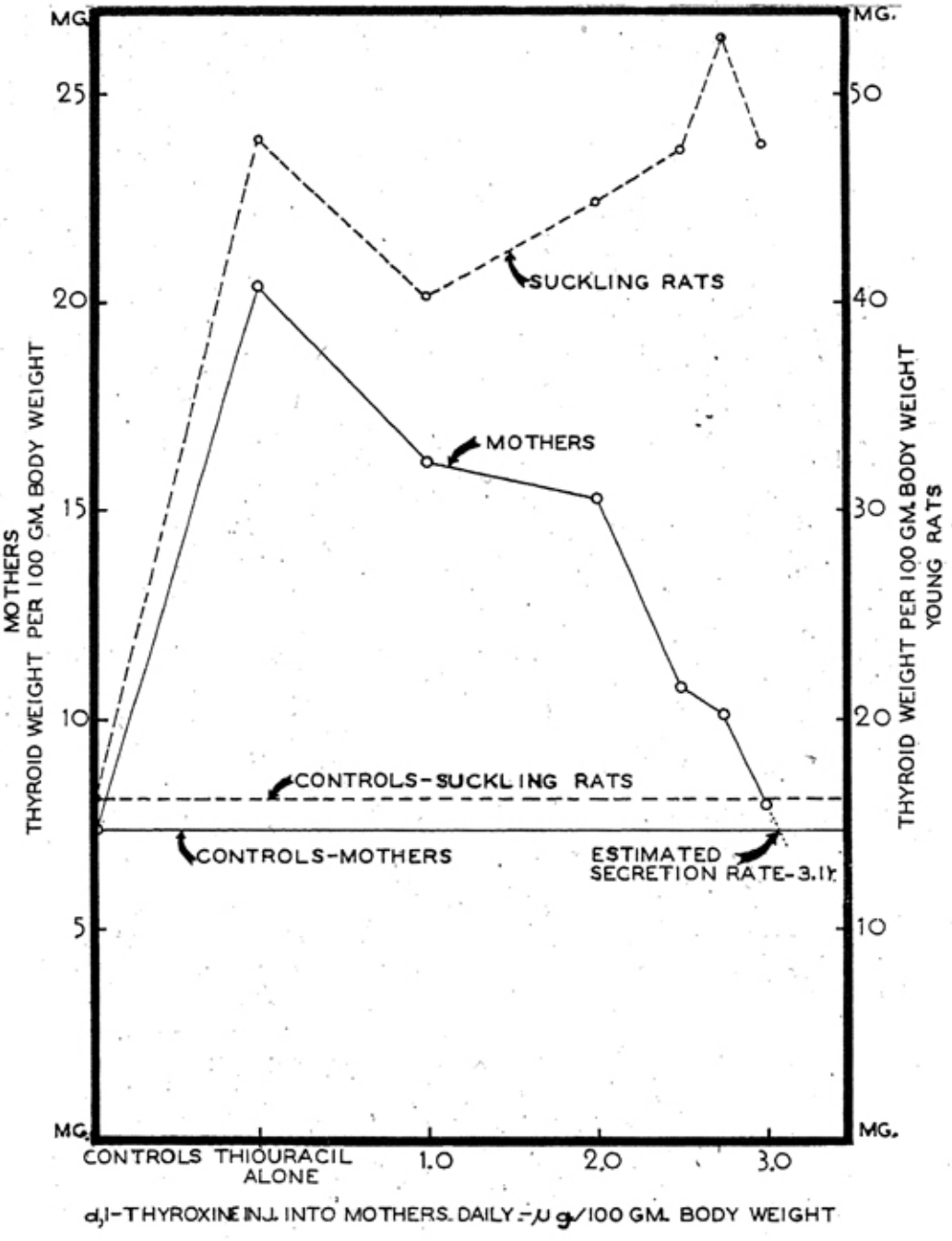

Fig. 8.—Assay to determine the thyroid secretion rate of lactating rats. Dotted line shows the effect of the mothers' treatment on the thyroids of the young.

Table 3-THYROID SECRETION RATE OF LACTATING RATS

Thiouracil in feed	Thyroxine injected/100 gm. rat	No. of rats	Avg. body weight	Avg. thyroid weight	Thyroid wt./100 gm. body wt.
%	ug.		gm.	mg.	mg.
none	none	15	203.1	14.9	7.3
0.1	none	11	214.0	43.7	20.4
0.1	1.0	6	231.5	37.5	16.2
0.1	2.0	6	224.7	34.4	15.3
0.1	2.5	10	194.7	21.0	10.8
0.1	2.75	10	193.5	19.6	10.1
0.1	3.0	11	202.0	16.1	8.0

Avg. body weight - 209.1 gm.
d,l-thyroxine per rat - 6.48 ug. daily
d,l-thyroxine/100 gm. body weight - 3.10 ug. daily

At the other extreme, it is now well known that a slight hyperthyroid condition is beneficial to lactation in some animals. Taking the overall picture, therefore, it seems that although the thyroid gland is not necessary for lactation in the species studied, the amount of milk produced apparently does depend to some extent on the activity of the thyroid. Whether this means that animals which are good milk producers are good producers because they are slightly hyperthyroid is a problem which has yet to be determined.

Granted, then, that a slightly hyperthyroid condition is desirable, there is a question which comes immediately to mind in connection with the present study. Does the thyroid gland become naturally hyperactive during lactation? Hewitt and Van Liere (1941) showed that the ratio of thyroid weight to body weight in guinea pigs does not change in the post-partum period. Ralston et al. (1940) showed that administration of 1.0 mg. of d, l-thyroxine to thyroidectomized, lactating goats produced a heart rate, milk and butterfat yield approximately the same as that of a normal goat. This figure agrees closely with that obtained by Schultze and Turner (1945) for the thyroid secretion rate of normal goats. The latter investigators noted no significant difference between the thyroid secretion rate of goats producing 1.8 pounds of milk daily and non-lactating goats of approximately the same body weight. They did show, however, that higher producing goats (2.8 pounds of milk daily) had a higher rate of thyroid secretion.

In determining the thyroid secretion rate of lactating rats, animals of approximately 200 grams body weight were chosen. These rats were injected daily for 15 days, except for the lots which served as controls and those which received thiouracil alone. These rats were found to have a thyroid secretion rate of 3.10 micrograms of d, l-thyroxine per hundred grams body weight (Fig. 8, Table 3). This amount is not significantly different from that of either normal or pregnant rats of the same body weight (Fig. 9).

On the basis of these findings, it would seem that the rat has no

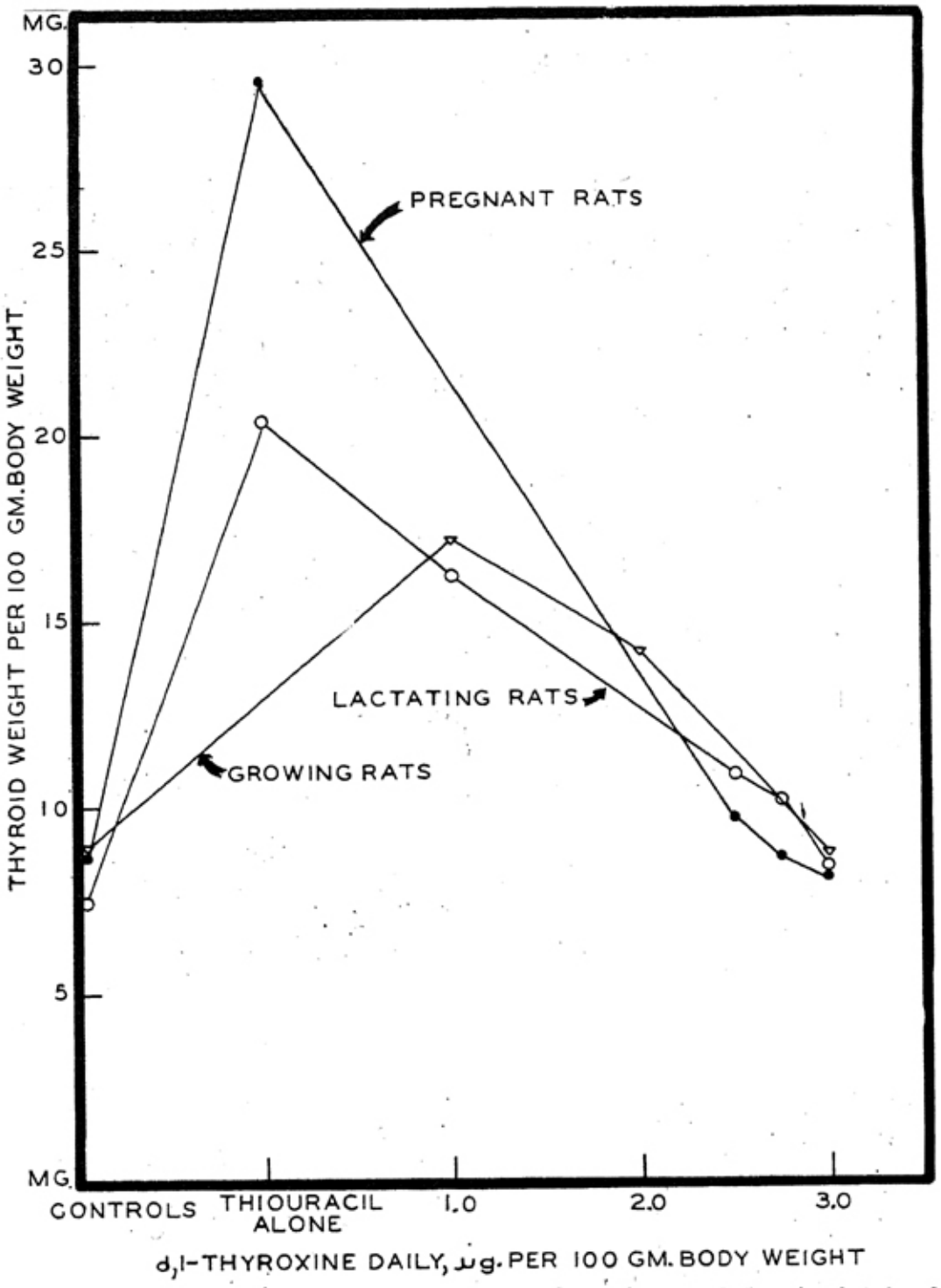

Fig. 9.—Graph illustrating the amount of thyroxine needed to maintain thyroid weight in growing, pregnant and lactating rats.

natural mechanism whereby it can alter the activity of the thyroid to bring about the hyperthyroid condition which we know is desirable for milk secretion in some animals. However, it must be remembered that these data represent the thyroid secretion rate during the last third of lactation. It is possible that the thyroid secretion rate may rise prior to this time and that the figure presented represents a point in its decline.

Effect of Thiouracil and Thyroxine Treatment of Mothers on Growth Rate of Suckling Rats

The study of the thyroid secretion rate of lactating rats presented an excellent opportunity to observe the effect of the treatment which the mothers received, on the suckling rats. The young rats were weighed at intervals throughout the 15-day experiment and were sacrificed, with the mothers, on the 16th day. It can be seen that the growth curves do not differ greatly from one another (Fig. 10). The unexpected gain of the thiouracil group over the normal group may be partially explained by the larger number of fatalities in the litters of the mothers receiving thiouracil (Table 4), thus allowing the surviving young to obtain more milk.

Table 4-WEIGHT INCREASE OF SUCKLING RATS WITH MOTHERS RECEIVING THIOURACIL IN FEED AND GRADED DOSES OF THYROXINE

Section 1

Treatment of Mothers		No. of litters	Avg. no. rats in litter		Avg. wgt. per rat in grams (days on experiment)							
Thiouracil in feed	Thyroxine injected per 100 gm rat		start	finish	1	2	3	4	5	6	7	8
none	none	15	9.3	7.9		5.0	6.1	7.2	7.9	8.7	10.6	11.5
0.1	none	11	7.3	4.0	5.7	6.2	7.0	8.2		9.1	11.7	
0.1	1.00 gamma	6	10.0	8.0	5.9	6.5		7.3		8.9	11.8	12.1
0.1	2.00 "	6	7.7	5.7	5.6		6.2	7.2		8.2	10.3	10.5
0.1	2.50 "	10	9.5	7.3		5.4		6.3			10.2	
0.1	2.75 "	10	9.7	8.6		5.4		6.5			9.6	
0.1	3.00 "	11	8.5	7.3	5.6	5.8	6.4	7.8	9.3	9.5	9.9	12.2

Section 2

Treatment of Mothers		No. of litters	Avg. no. rats in litter		Avg. wgt. per rat in grams (days on experiment)							
Thiouracil in feed	Thyroxine injected per 100 gm.rat		start	finish	9	10	11	12	13	14	15	16
none	none	15	9.3	7.9		13.6	14.2			17.1		18.0
0.1	none	11	7.3	4.0	13.3				17.0			20.4
0.1	1.00 gamma	6	10.0	8.0	13.4	13.5	15.4	17.0	18.3		19.6	20.0
0.1	2.00 "	6	7.7	5.7		12.0			14.9		16.4	17.0
0.1	2.50 "	10	9.5	7.3	11.4			13.6		14.4		16.0
0.1	2.75 "	10	9.7	8.6	11.1					14.5		15.1
0.1	3.00 "	11	8.5	7.3	12.9	14.0		14.7		15.3	16.4	18.3

These data may reasonably be questioned on the basis that 15 days are hardly enough to warrant a conclusion concerning the growth rate and also on the basis that body weight may not be an accurate index of growth, for a weight increase may be due to an increase in adipose tissue. In general, however, it may be assumed from these observations that the growth rate of suckling rats, for the first 15 days at least, is not materially affected by thiouracil administration to the mothers. This conclusion is consistent with data reported by Hughes (1944). However, his results show that after this period growth is markedly retarded.

Permeability of the Mammary Gland to Thyroxine and Thiouracil

The passage of thyroxine through the mammary gland tissues into the milk has been the subject of some controversy. However, there has been no direct evidence to show that thyroxine does pass into the milk. On the contrary, most of the work conducted on this problem tends to

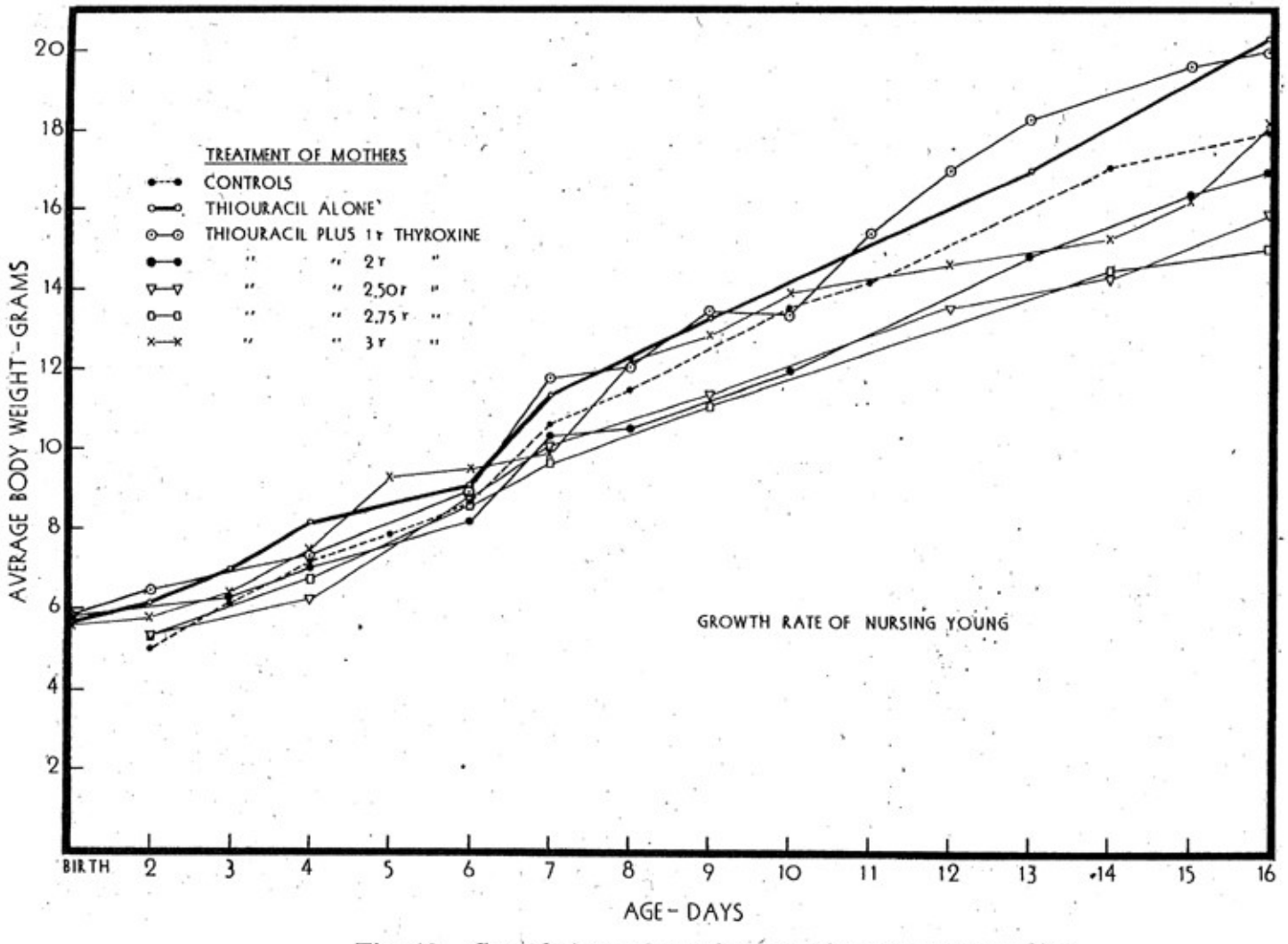

Fig. 10.—Growth rate of nursing rats from treated mothers.

support the view that the mammary gland is not permeable to thyroxine.

Lukacs (1931) reported a retarded growth rate in the young of rats with experimentally produced hyperthyroidism as compared with litters from normal mothers. Since he had previously noted that hyperthyroidism caused a reduction in the growth rate of rats, he concluded that the slower growth rate of the young was due to the presence of the thyroid hormone in the milk. Moreover, he postulated that the amount of hormone present in the milk was proportional to the amount present in the circulation of the mother. Lukacs' work has been criticized by Reineke and Turner (1944) who point out that Lukacs did not consider the effects of hyperthyroidism on milk secretion. Since it is known that excessive hyperthyroidism depresses milk secretion (Herman, Graham and Turner, 1937), it would be expected that the growth rate of the young would be retarded.

Konsulov (1935) supported Lukacs' views when he reported that the administration of thyroidin to a mother caused an increase in the carbon dioxide output of a nursing infant. However, Brody (1942) showed that by decreasing the number of rats in a litter, and thus increasing the amount of milk available per rat, the metabolism of the rats increased markedly. Therefore, the higher rate of milk secretion caused by thyroid administration could account for the reported increase in metabolism without assuming the presence of the hormone in the milk.

As was mentioned above, the great bulk of evidence on this problem points to the probability that the thyroid hormone is not present in the milk. Simpson (1924) noted that when one of a pair of twin lambs or kids was thyroidectomized (leaving the other as a control), the thyroidectomized twin soon showed symptoms of thyroid deficiency, although both twins were fed solely on the mother's milk. Similar observations were reported by Dorff (1934) in human twins, one of which was cretinous.

Reineke and Turner (1941) thyroidectomized kids shortly after birth and observed symptoms of cretinism in a short period of time when the kids were fed goats' milk. However, the kids responded rapidly to administration of thyroprotein. Brody and Frankenbach (1942) also noted that thyroidectomized calves on a cows' milk diet developed cretinism.

In 1944, Reineke and Turner fed milk from cows receiving massive doses of thyroprotein to guinea pigs and thyroidectomized goats. No significant differences could be detected as the result of thyroprotein feeding. No elevation in metabolism was observed by either Reineke and Turner (1944) or Robertson (1944) when milk from thyroprotein-fed cows was consumed by human beings.

In all cases where direct evidence is presented, the results fail to show that the thyroid hormone has passed through the mammary gland into the milk. In the experiments which purportedly show that the thyroid hormone does pass through the mammary gland, all of the effects attributed to the hormone can be shown to be due to the effect of the administration of thyroidal substances on the rate of milk secretion. Mild hyperthyroidism is known to be advantageous to lactation in some species.

On the other hand, both thyroidectomy and extreme hyperthyroidism have a detrimental effect on milk secretion. Consequently, the growth rate of suckling young is not a specific criterion of the permeability of the mammary gland by the thyroid hormone.

The study of the normal thyroid secretion rate of lactating rats presented an opportunity to study the problem of the permeability of the mammary gland to thyroxine still further. A technique was used which differed from those used in all of the previous studies. It was reasoned that if thiouracil passed into the milk, which seemed relatively certain (Hughes, 1944; Williams, Kay and Jandorf, 1944; and Williams, Weinglass, Bissell and Peters, 1944), the thyroids of the young would enlarge. By the same reasoning, if thyroxine passed into the milk at the same time, it would tend to check the thyroid enlargement caused by the thiouracil.

In this experiment the mothers were divided into one control group, one group receiving 0.1 per cent thiouracil in the feed and seven groups receiving graded doses of d, l-thyroxine. The mothers were injected for 15 days of the post-partum period and sacrificed on the 16th day. The young rats were sacrificed at the same time and their thyroids were removed and weighed.

It will be noted that the young from normal mothers had an average thyroid gland weight of 18.1 milligrams per hundred grams body weight (Table 5). The young from mothers fed 0.1 per cent thiouracil in the diet had thyroids weighing 47.9 milligrams per hundred grams body weight. Obviously, the thiouracil passed through the mammary gland in high concentration. However, it is plain that none of the lots of young whose mothers were injected had thyroids which varied significantly

Table 5-THYROID WEIGHT INCREASE OF16-DAY OLD SUCKLING RATS

Treatment of mothers		No. of litters	No. of young rats	Avg. body wt. of young rats	Avg. thyroid wt. of young rats	Avg. thyroid wt. per 100 gm. body wt. of young rats
Thiouracil in feed	Thyroxine per 100 gm body weight					
%				gm.	mg.	mg.
none	none	15	119	17.1	3.1	18.1
0.1	none	10	65	16.9	8.1	47.9
0.1	1.00 gamma	6	48	18.9	7.6	40.2
0.1	2.00 "	6	34	17.6	7.9	44.9
0.1	2.50 "	10	75	15.6	7.4	47.4
0.1	2.75 "	10	86	15.1	7.9	52.3
0.1	3.00 "	10	80	16.5	7.8	47.3
0.1	4.00 "	2	11	17.7	9.2	52.0
0.1	5.00 "	2	10	18.7	7.8	41.7

from this figure, even when the mothers received as much as 65 per cent more thyroxine than is equivalent to the normal thyroid hormone output (Fig. 8, Table 5).

These data are considered conclusive evidence that thyroxine does not pass the mammary gland epithelium and is not secreted into the milk. However, it is obvious that thiouracil does pass into the milk very readily.

The Rate of Thyroid Secretion by Various Animal Species

The work of other investigators concerning the rate of thyroid secretion by the albino rat has been reviewed. It is interesting to correlate also the data which has been determined for various other animal species. Other species studied include White Leghorn cockerels, White Plymouth Rock cockerels, White Plymouth Rock pullets, growing and lactating goats, calves and lactating cows.

A study of the data shows that on the basis of one hundred grams of body weight, the rat has a higher thyroid secretion rate than any of the species mentioned, with the possible exception of some groups of goats (Table 6). The smallest growing goats obviously had a much lower rate of thyroid secretion than that of rats of corresponding size. The two groups of growing female goats, however, had a thyroid secretion

Table 6-THYROID SECRETION RATE OF SEVERAL SPECIES

Animal	Body Weight	Thyroid Secretion Rate as γd, 1-thyroxine/day	Daily Thyroid Secretion Rate as γd, 1-thyroxine/100 gm. body weight	Temperature
		ug.	ug.	F.
White Leghorn cockerels(1)	343.0 gm.	7.55	2.20	82°-86°
	1536.0 gm.	25.00	1.36	82°-86°
White Plymouth Rock cockerels(1)	410.0 gm.	8.10	1.98	82°-86°
	1502.0 gm.	23.00	1.53	82°-86°
White Plymouth Rock pullets(1)	360.0 gm.	8.75	2.43	82°-86°
	1637.0 gm.	26.00	1.59	82°-86°
Growing goats(1)				
Males and females	10.0 kg.	180.00	1.80	50°-80° probable range
Females	20.4 kg.	640.00	3.13	50°-80° probable range
Females	34.5 kg.	930.00	2.70	50°-80° probable range
Lactating goats(1)				
(milk yield 1.8 lbs./day)	43.5 kg.	1000.00	2.29	50°-80° probable range
(mild yield 2.6 lbs./day)	43.5 kg.	1425.00	3.44	50°-80° probable range
Calf(1)	72.6 kg.	1500.00	2.06	50°-80° probable range
Cow (lactating)(1)	454.0 kg.	10,000.00	2.20	50°-80° probable range
Growing male rats	85.2 gm.	3.10	3.64	78°
	126.9 gm.	4.18	3.29	78°
	168.7 gm.	5.90	3.50	78°
	219.5 gm.	7.46	3.40	78°
	275.7 gm.	9.54	3.46	78°
Growing female rats	81.1 gm.	3.75	4.63	78°
	128.1 gm.	5.19	4.05	78°
	171.9 gm.	5.74	3.34	78°
	223.6 gm.	6.53	2.92	78°
	266.2 gm.	7.51	2.82	78°
Pregnant rats	190.8 gm.	5.59	2.93	78°
Lactating rats	209.1 gm.	6.48	3.10	78°

(1) Mo Res. Bul. 392

rate only slightly lower than that of the corresponding growing female rats. Likewise, the lactating goats showed only slight variations from the figures obtained for lactating rats, especially if an average of all lactating goats is taken.

However, there seems to be no question that the rate of secretion by the thyroid glands of all other species studied is considerably lower than that of the rat.

DISCUSSION

In the past many studies have been undertaken in an attempt to determine the activity of the thyroid gland under varied conditions. The methods employed are somewhat less precise than is desirable for investigations of this nature. Consequently, the subject of the rate of hormone secretion by the thyroid gland has been considerably more neglected than its importance merits.

In 1943 Dempsey and Astwood proposed a method based upon the action of certain goitrogenic drugs which seems to answer the need for a convenient and accurate technique for studying thyroid activity. Since the drugs used in this method prevent the process of thyroxine synthesis, this technique is actually superior to thyroidectomy in that the most careful thyroidectomy operations may leave some accessory thyroid tissue. This fact is emphasized by the observation that rats treated with a goitrogenic agent (thiouracil) show a lower basal metabolic rate than do rats which have been surgically thyroidectomized (Dempsey and Astwood, 1943; and Meyer and Ransom, 1945). It seems reasonable to assume that the results of an experiment utilizing the goitrogenic drug technique should be more nearly accurate than one in which thyroidectomized animals are used.

There is another important point in favor of the goitrogenic drug technique. It is difficult to determine the end point in replacement therapy methods, since the indices of normality are, in themselves, variable to some extent. For example, the maintenance of the growth rate of thyroidectomized animals is difficult to determine accurately. Likewise, the heart rate and/or basal metabolism are not delicate measurements of normality. By the use of the goitrogenic drug method, however, the effects of minute quantities of thyroxine can be determined, since the variable factors can be more easily controlled.

Another major consideration concerning this technique is the assumption that the amount of thyroxine required to maintain normal thyroid weight is equivalent to the normal thyroid hormone output. This fact could be assumed to be true, in accordance with known facts concerning the thyroid-pituitary balance mechanism, if it were known that the exogenous thyroxine is utilized as effectively as the naturally produced hormone. It has been shown that thiouracil has no inhibitory effect on the activity of thyroxine (MacKenzie and MacKenzie, 1943; Astwood, 1943; and Astwood, Sullivan, Bissell and Tyslowitz, 1943). Danowski et al. (1946) have suggested that partial destruction of exogenous thyrox-

ine may take place, thus leading to an overestimation of the normal thyroid secretion rate. This suggestion is entirely reasonable. However, it should be recalled that the aim of goitrogenic drug technique is to determine the thyroid secretion rate in equivalent amounts of some thyroidally active substance. Indeed, if Danowski's suggestion is correct, the goitrogenic method can be used to advantage in determining the relative potencies of various thyroidally active compounds.

It might be pointed out at this point that even if this method is found not to measure accurately the amount of hormone produced naturally by the thyroid gland this knowledge would be of little practical importance. From the practical standpoint, the desired knowledge is not concerned with the actual output of thyroid hormone, but rather with the amount of hormone which must be administered to maintain the animal at the desired level of thyroid activity.

Although it is plain that the results obtained in this investigation are consistent within themselves, it may be noted that they differ significantly from the observations of some other workers. Dempsey and Astwood (1943) and Astwood and Bissell (1944), for example, found that the normal thyroid secretion rate of male rats was equivalent to approximately five micrograms of l-thyroxine per hundred grams body weight. On the other hand, Purves (1943) found the normal rate of thyroid secretion for male rats was only between two and three micrograms per hundred grams body weight. Reineke, Mixner, and Turner (1945) obtained a figure in between those reported above. They found that 3.47 micrograms of d, l-thyroxine per hundred grams body weight would maintain a normal thyroid weight in 140-gram rats. The latter figure is very close to that obtained in this study.

The most logical explanation for these variations would be that there are strain differences in the amount of hormone produced by the thyroid. Strain differences might also be postulated, as Danowski et al. (1946) suggest, on the basis of the amount of thyroxine inactivation. An explanation on the basis of strain differences seems the more probable since the animals used in this study and that of Reineke, Mixner, and Turner (1945), whose results agree closely with ours, were of the same strain. Presumably, Dempsey and Astwood (1943) and Astwood and Bissell (1944) used the same strain of rats in their respective studies.

An alternative explanation might be that the techniques of preparing the thyroxine for injection might vary with different investigators. It may make a difference, for example, whether the monosodium or disodium salt was used. Further research on this point should prove interesting.

It might be mentioned that Dempsey and Astwood (1943) reported that they used l-thyroxine in their work. Considering the fact that l-thyroxine is twice as active physiologically as the d, l-form (Reineke and Turner, 1945) and considering, also, the great difficulty in obtaining l-thyroxine, it seems probable in the light of their results, that they were mistaken. The probability that they used d, l-thyroxine seems more likely

since they reported that they used a commercial preparation, and it is known that the common methods of preparation yield the racemic product.

The observations in this study on the rate of thyroid secretion of lactating rats are somewhat surprising in that data reported on the lactogenic and thyrotrophic hormones (Turner and Cupps, 1939) would lead one to expect a rise in thyroid activity at the initiation of lactation. It must be remembered that the data in this study represent the thyroid secretion rate on the 16th day of lactation and, therefore, do not obviate the possibility of a rise in thyroid activity before this time.

SUMMARY

1. The normal daily thyroid secretion rate was determined for growing male and female rats and for pregnant and lactating females. All determinations are expressed in equivalent amounts of d, l-thyroxine injected as the disodium salt.

2. The daily rate of thyroid secretion of growing male rats was found to be approximately 3.5 micrograms of d, l-thyroxine per hundred grams body weight in all the groups studied. When the amount of thyroid secretion per rat per day was calculated, it was found that the amount of hormone output increases with increasing body weight, and can be expressed by the equation $Y = 0.041X^{0.97}$, where Y equals the thyroid secretion rate and X the body weight.

3. The thyroid secretion rate of growing female rats ranged from 4.63 micrograms of d, l-thyroxine per hundred grams body weight in Group I (50-99 grams body weight) to 2.82 micrograms per hundred grams body weight in Group V (250-300 grams body weight). It was found that the amount of thyroid hormone produced per rat per day increased with increasing body weight, but at a slower rate than in growing male rats. This relationship can be expressed by the equation $Y = 0.422X^{0.51}$, where Y equals the thyroid secretion rate and X the body weight.

4. The thyroid weight of growing male rats was found to increase in relation to body weight at the rate $Y = 0.136X^{0.90}$, whereas the corresponding equation for growing female rats is $Y = 0.230X^{0.81}$. In both equations Y equals the thyroid weight and X the body weight.

5. The thyroid secretion rate of pregnant rats weighing approximately 200 grams was found to be 2.93 micrograms of d, l-thyroxine per hundred grams body weight, as compared with 3.23 micrograms per hundred grams body weight (calculated) for non-pregnant females of the same body weight. This difference is interpreted as being insignificant.

6. The rate of thyroid secretion of 200-gram female rats in the 16th day of lactation was determined to be equivalent to 3.10 micrograms of d, l-thyroxine per hundred grams body weight. The thyroid secretion rate of normal female rats of the same body weight was calculated to be 3.09 micrograms per hundred grams body weight.

7. The growth rate of suckling rats was not altered, for the first 15 days at least, by the administration of thiouracil or thiouracil plus graded doses of d, l-thyroxine to the mothers.

8. When thiouracil was fed to the mothers, there was a marked enlargement of the thyroid glands of the suckling young, indicating that thiouracil passes readily through the mammary gland epithelium into the milk.

9. The injection of successively increasing doses of d, l-thyroxine into thiouracil-treated mothers did not check the enlargement of the thyroid glands of the suckling young. Since the thyroxine was injected in doses as high as 65 per cent above the normal requirement of lactating rats, it is assumed that the mammary gland epithelium of the rat is impermeable to thyroxine.

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