1986

The effects of a four-day treatment with Prophylthiouracil (PTU) on thyroid histology and serum levels of thyroxine (T_4) and triiodothyronine (T_3) in the diabetic mouse C57BL/KsJ (db/db)

Sterling Roulette

THE EFFECTS OF A FOUR-DAY TREATMENT WITH PROPYLTHIOURACIL (PTU) ON THYROID HISTOLOGY AND SERUM LEVELS OF THYROXINE (T_4) AND TRIIODOTHYRONINE (T_3) IN THE DIABETIC MOUSE C57BL/KsJ (db/db)

A Thesis

Presented to the

Faculty of

California State

University, San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

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by

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June 1986

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ABSTRACT

Diabetic and normal mice were injected daily with Propylthiouracil (PTU) at a dosage of 1 µg/gm body weight for four days. Following PTU exposure, serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured and a histological examination of thyroidal tissues performed. Serum T_3 concentrations were greater in diabetic mice in both vehicle and PTU-treated groups when compared to normal mice treated with vehicle or PTU, respectively. No differences were observed between vehicle-treated groups and PTU-treated groups. Serum T_4 concentrations were no different between treatment groups or between phenotypic groups. Serum T_3/T_4 ratio in ng/µg decrease in PTU-treated normal individuals when compared to normal vehicle-treated mice. Morphometric analysis indicates a difference in cellular activity between normal mice treated with PTU and normal mice treated with vehicle. Normal mice treated with PTU were observed to have larger colloids and more columnar follicular cells compared to normal mice treated with vehicle. Diabetic mice treated with PTU exhibited a high level of thyroid activity as determined by follicular histology of the thyroid. Diabetic mice treated with PTU exhibited a reduction in colloid size compared to diabetic mice treated with vehicle. The mechanisms of these differences between normal and diabetic mice to PTU treatment may be due to irregularities in autoinhibition of T_4 in the diabetic mouse.

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Richard Fehn and Cindy Roulette in the preparation of this thesis. Dr. Richard Fehn has proved me with insightful questions about scientific research as well as a logical progression to arrive at the answers. His support went well beyond just the academics of being an advisor to a Masters' Thesis. He was my extra set of hands in all phases of my work such as breeding, dissection, and histology. For all of these reasons and more I am deeply grateful.

I would like also to thank my wife, Cindy Roulette. She was able to follow the countless annotations with their accompanying arrows, erasures, and footnotes into a presentable thesis.

In addition, I would like to thank Dr. Sokoloff and Dr. Mankau for their helpful comments in the editing stages of my work.

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INTRODUCTION

Diabetes mellitus is a disease in which the body does not respond adequately to carbohydrate challenge by the release of, or response to, insulin. Diabetes mellitus classically has been approached from the aspect of a pancreas or insulin dysfunction. Until relatively recently little research has focused on the possible roles of other endocrine glands such as the thyroid on the expression of diabetes mellitus. In the expression of diabetes, particularily those cases exhibiting insulin insensitivity, a thyroid dysfunction is also apparent (1).

Human diabetes has been attributed to a variety of causes such as hereditary factors, viral infection of the pancreatic beta cells and dietary habits (2, 3, 4). The fact that diabetes may stem from a number of anomalies is likely the basis for the variety of different patterns of expression of this disease. There are two broad categories into which diabetes mellitus falls - Type I and Type II (2). Type I diabetes is an inability to produce sufficient quantities of insulin whereas Type II is a lack of tissue response to insulin (5). It is Type II diabetes that this paper will address.

Humans with Type II diabetes exhibit hyperglycemia, polyuria, polydipsia, and a predisposition for obesity. Eighty percent of those individuals with Type II non-insulin dependent diabetes mellitus are categorized as obese at the time of clinical diagnosis of diabetes (2) (6). In addition to this multiplicity of symptoms humans with late onset diabetes also display decreased serum levels of the metabolically active thyroid hormones (7, 8, 9).

The thyroid hormones are important in the maintenance of homeostasis and considered by some to exhibit a permissive action towards other endocrine gland functioning (2, 10, 11). The thyroid gland is composed of groupings of cuboidal epithelium tissue referred to as follicular cells. These follicular cells surround proteinacious masses known as colloid. The functional units of the thyroid gland are the follicular cells which produce the thyroglobulin that is secreted by exocytosis to the interior of the follicle. It is this stored thyroglobulin that is referred to as colloid. Concomitant with the synthesis of thyroglobulin is the organification of iodide and its binding to the tyrosyl moieties within the thyroglobulin molecules. The released iodinated tyrosyl fractions are picked up by endocytosis and carried by lysosomes to the exterior of the follicular cells and released. Once coupled and released these fractions, either triiodothyronine (T_3) or tetraiodothyronine (T_4), are considered the true thyroid hormones which are released directly into the capillary network surrounding the follicular cells (2).

Thyroid hormones directly influence a wide array of metabolic activities (12). These hormones are responsible in a variable degree for thermogenic regulation, protein synthesis, lipid metabolism, carbohydrate metabolism and other endocrine gland functioning. In relation to their thermogenic action the thyroid hormones are able to increase oxygen consumption in mitochondria (13) and increase ATP formation as evidenced by increased Km values in ATPase enzymes (14). ATP utilization is increased in tissues under the influence of thyroid hormones, this is apparently by increasing the number of sodium pumps

(13). T_3, the metabolically active form of the thyroid hormones, is able to directly stimulate protein synthesis (16). T_4, the primary product of the thyroid gland, and T_3 also indirectly influence protein synthesis by causing the phosphorylation of ribosomal subunits (17). Lipid metabolism is influenced by thyroid hormones (18) which directly increase conversion of cholesterol to bile acids (19). This action is thought to arise from an increase in the number of membrane low-density lipoprotein (LDL) receptors (20). The circulation or excretion of other lipids besides cholesterol are also decreased following exposure to T_4 or T_3 (21). There is a growing body of data suggesting a link between thyroid hormones and carbohydrate uptake and metabolism. The research concerning the relationship between carbohydrate metabolism has yielded many conflicting reports. Thyroid hormones have the ability to increase the absorption of hexose sugars in the gut (22). Once these sugars are inside the circulatory system, elevated thyroid hormones appear to increase their tissue uptake. How the thyroid hormones affect sugar uptake by tissues is measured by glucose tolerance tests. Evidence of the thyroid gland's influence on glucose tolerance is controversial. Investigators have noted normal (23), increased (24), or decreased (25) glucose tolerance in hyperthyroid patients and animals. In considering carbohydrate metabolism it is important to look at the role of insulin under these changing thyroid states. One of the primary functions of insulin is to stimulate glucose uptake by cells (2). Thyroid hormones affect insulin by increasing its rate of degradation in rats (26) and man (27). How thyroid hormones affect insulin receptor sites is another area obtaining conflicting reports. Under hyperthyroid

states insulin receptor sensitivity has been shown to increase (28), decrease (29) or remain normal (30). The reasons for the differences in results have not been explained and more research is needed to outline the exact role of thyroid hormones on insulin functioning. Hyperthyroid conditions also affect the insulin producing cells known as the pancreatic beta cells. Researchers examining chronic hyperthyroidal conditions have observed permanent beta cell damage (31, 32, 33). Other endocrine hormones that are influenced by the thyroid are somatotropin and prolactin. Both these hormones are increased in response to thyroid treatment (34, 35).

The observation that thyroid hormones influence a wide array of activities including other hormones is the impetus behind examining the thyroid state in diabetes. Investigators have noted a correlation between hypothyroidism and diabetes. The prevalence of these two conditions occurring together is approximately 1.7% (36) and is in many instances associated with the presence of thyroid receptor autoantibodies (37). In humans with diabetes a serum T_3 and T_4 imbalance is apparent (38). The features of this imbalance are contingent upon the mechanisms involved in causing the anomaly and the state of progression of the disease. In most cases of diabetes in man serum T_3 and T_4 are decreased (39). As yet the causative mechanisms for the changes in serum T_3 and T_4 are unknown. One reason behind our lack of knowledge of this pathological condition is that the exact mechanisms(s) controlling serum T_3 and T_4 in normal systems is also unclear (40). The predominant form of thyroid synthesis in individuals with normal iodine concentrations is through the combining of two

diiodothyronine (DIT) subunits to form T_4 in greater proportion than T_3 (41). Under certain physiological conditions the relative concentrations of T_4 and T_3 shift. Under conditions of iodine deficiency, serum T_3 concentration increases. This increase is due to changes in the synthesis inside the thyroid gland where more of the subunits of (DIT) are combined with monoiodothyronine (MIT) to form T_3. The T_4 to T_3 shift is especially prevalent in endemic goiter patients (42). Changes in deiodinase activity in peripheral tissues are also apparent under certain pathological conditions. The two organs primarily responsible for this conversion are the liver and kidneys (2). In the hyperthyroid state, liver deiodinase activity increases with no change in the kidney deiodinase enzyme kinetics (43). The enzyme 5' monodeiodinase is responsible for converting T_4 to T_3. Under hypothyroid conditions the liver deiodinase activity decreases (44).

The purpose of this study is to examine T_4 to T_3 conversion in diabetic and normal individuals. Because of the inherent difficulties in human experimentation animal models have been developed. A model of human diabetes is available in the diabetic mouse C57BL/KsJ db/db. This strain of mice is quite similar to human Type II diabetes in having abnormally high deposition of fat tissue at 3 to 4 weeks of age that is shortly thereafter accompanied by hyperglycemia, polyuria, and glucosuria (45). The gene responsible for diabetes in this mouse is recessive and the expression of its diabetic character is inherited with complete penetrance in the homozygous state. Heterozygotes remain morphologically and physiologically indistinguishable from normal mice. The weight gains for diabetic (db/db) mice are not as great as that of

obese (ob/ob) mice but they reach a maximum weight of approximately 62 grams (45, 46). At approximately 24 weeks of age, weight tends to decrease as serum glucose homeostasis deteriorates. This is evident by the sharp rise in serum insulin level. The chronically elevated serum glucose in these mice eventually brings about a shift from Type II diabetes to Type I (47) which reflects exhaustion of the pancreatic beta cells.

Concomitant with the manifestations of diabetes in these mice, a thyroidal imbalance has also been observed (48). Diabetic individuals display low serum T_4 (48) and elevated serum T_3 concentrations in the nonfasting state. This high serum concentration of T_3 in the diabetic is quite paradoxical because the diabetic mouse does not appear to respond to these elevated T_3 concentrations by increasing metabolic rate as normal individuals do. The thyroid glands of diabetic mice are also abnormal histologically. The thyroid glands of diabetic mice have quite large colloids that are densely stained by hematoxylin/eosin. The follicular cells in these animals are squamous when compared to the cuboidal shape of follicular cells in thyroid tissue from normal mice (48). The cuboidal shape indicates active synthesis of thyroid hormones whereas squamous follicular cells are considered inactive (2).

This study is to examine what effect blocking T_4 to T_3 conversion has on the thyroid histology and serum concentrations of T_4 and T_3 in diabetic and normal mice. The testing will be performed with diabetic and non-diabetic mice after a four-day exposure of propylthiouracil (PTU). PTU is an agent which blocks the enzyme 5' monodeiodinase in the liver and kidneys but not in the pituitary gland (49). The function of

T_4-to-T_3 conversion in the pituitary is thought to be a controlling mechanism for thyroid stimulating hormone (TSH) output (50). The purpose of blocking T_4 conversion is to determine if diabetic animals are equally responsive to PTU treatments as normal animals are in modulating serum T_4 and T_3 concentration. Differences between diabetic and normal mice in serum T_4 and T_3, if they occur, could lead to clues as to why the thyroid glands of diabetic mice are in a state of imbalance. There are a variety of possible reasons for the serum concentrations of T_4 and T_3 to be abnormal in diabetic mice. One possibility is that the 5' monodeiodinase activity is greater in these mice. This response may be a function of an allosteric mechanism of T_4 whereby lower concentrations of T_4 enhance 5' monodeiodinase activity. Another possibility is that the enzyme is synthesized in higher concentrations in diabetic individuals. If either explanation is correct then PTU treatment could potentially normalize the conversion rate while maintaining TSH output to increase serum T_4. It is also possible that the dysfunction in thyroid balance in diabetic mice is occurring at the level of T_4 and T_3 output from the thyroid gland itself. There is evidence of a shoot loop negative feedback of T_4 on thyroid hormone output (51) though this action is controversial (52). If the serum T_4 negative feedback sensitivity is increased in the diabetic mouse then the thyroid output of this hormone might be lowered. This hypothesis as yet does nothing to explain why serum concentrations of T_3 are elevated in diabetic mice. Increases in serum T_3 concentrations in diabetic animals might be high due to a compensatory measure by the peripheral tissues in increasing conversion rate because

of the lowered T_4 serum concentration. The ability to increase serum T_3 even under times of lowered T_4 concentration is possible especially when considering the over one hundred-fold difference in serum T_4 to T_3 concentration found in normal individuals (2, 52).

In addition to the examination of serum T_4 and T_3 concentrations in diabetic and normal mice following PTU treatment, thyroid histology will also be examined. It is expected with prolonged application of PTU that a normalization of follicular cell shape, size, density and colloid size will occur in the thyroids of diabetic animals. Normalized thyroid glands would be evidenced by colloids decreasing in size and lighter staining to hematoxylin. The follicular cells of the thyroid glands of diabetic mice are expected to change from squamous to cuboidal in response to PTU treatment. The reasoning behind these expectations is that mice treated with PTU will decrease serum T_3 concentrations. If serum T_3 is acting as a negative feedback on T_4 output from the thyroid gland (51), then when the level of T_3 is low the thyroid would release T_4. The release of T_4 from the thyroid would be observable as a decrease in colloid size and staining reaction.

MATERIALS AND METHODS

Experimental animals were maintained in a Scherer controlled environmental chamber at 24°C on a 14 light:10 dark lighting cycle in cages that were 11.5" x 7" x 5" in size. Each cage contained two mice and were provided water and Purina Rat Chow ad-libitum.

PTU (Sigma) was prepared at a final concentration of 1.0 milligram/milliliter in 10 mM phosphate buffered saline (PBS) adjusted to pH 7.6. PTU (50 mg) was mixed with 2.5 ml of 0.1 N sodium hydroxide then vortexed until dissolved. To this solution 1.6 ml of 0.1 N hydrochloric acid was added and the solution was immediately vortexed. The pH was adjusted to 7.6 using 0.01 N Hydrochloric acid and once again vortexed. The solution was serially diluted to a final concentration of 1.0 mg/ml using PBS. Solutions were prepared immediately before injection time and used only once. Because PTU is light-sensitive, solutions were protected from light prior to use. Vehicle consisted of an equivalent solution of PBS without PTU.

Experimental animals received doses of 1 mg of PTU per 100 grams of body weight while control animals received an equal volume of vehicle. Body weights and injection volumes were determined each day before injection. The injections were given intraperitoneally into the lower quadrant of the abdominopelvic cavity alternating between right and left sides on alternate days. The injections were made on four consecutive days at approximately 1900 hours. The four experimental groups were as follows: Group 1 - normal mice plus vehicle; Group 2 - diabetic mice plus vehicle; Group 3 - normal mice plus PTU; and Group 4 - diabetic

mice plus PTU. Each group contained five animals that started the experiment at 12 weeks of age.

The fifth day, following four days of injection, blood samples were drawn by cardiac puncture at 1200 h using a 1 ml tuberculin syringe with a 23 gauge needle. Blood samples were transferred into 1.5 ml Eppendorf centrifuge tubes and allowed to clot for 1 hour at 4°C. After this time the samples were centrifuged at 1700 rpm (800 rpm) for 20 minutes at 4°C. Serum was drawn off, placed in 1.5 ml Eppendorf tubes and stored at minus 20°C until assayed. Serum T_3 and T_4 were determined by radioimmunoassay (Micromedic Systems Inc.™).

Directly following cardiac puncture mice were sacrificed by cervical dislocation and the thyroids removed. Thyroidal tissues were removed by isolating the intact tracheal portion containing the thyroid gland and excising it as an intact unit from the animal. Thyroid glands were placed in Bouin's fixative and stored for two weeks at room temperature prior to histological sectioning. After fixing, thyroids were cleared, embedded in hard paraffin, and sectioned at 5 μm following the procedures outlined by Humasen (53). Cut thyroid sections were floated in a water bath containing gelatin. Once relaxed, these sections were placed onto albumin coated slides and heat fixed for one hour at 54°C. Paraffin removal was performed and stained with Harris' hematoxylin/eosin (53).

The tissues were examined under 400 power and photographed in color using a neutral density filter and Kodachrome™ 200 ASA slide film and in

black and white using a green filter and Plus-X™ 125 ASA film. Both color slides and black and white prints were taken at a magnification of 380x.

A morphometric analysis of thyroid tissue was performed using 3" x 5" black and white pictures of thin tissue sections. A transparency was made to divide each picture into sections. For each colloid in the center most of the four sections ten follicular cells were chosen. Using the uppermost follicular cell as number one and counting in a clockwise direction each cell was measured for cell height and width, nuclear diameter, and cytoplasmic density. A relative ranking was used to determine cytoplasmic density using a one to three plus scoring scale. One plus was designated to be of low density whereas two plus was intermediate and three plus high density. The same protocol was used in the determination of colloid density. The interface between the colloid and follicular cell was also ranked on a three plus scale. In this case one plus indicated tight adherence of the colloid to follicular cells, two plus was for cells with light areas between colloid and follicle cells, and three plus for those colloids where visible space was present between the colloid and follicular cells. This particular index may provide information on the activity of reabsorbtion lacunae for thyroglobulin. Colloid area was measured by determining the length of the longest horizontal measurement and taking the perpendicular midline to measure width.

RESULTS

Serum thyroid hormone concentrations for individual experimental animals are given in Table 1.

Table 1. Data representing nonfasting serum T_3, T_3, and T_3/T_4 concentrations for normal and diabetic twelve week old mice following four days of treatment with either 1 µg/gm body weight of propylthiouracil or with an equivalent volume of saline.

CASE	PHENO	TMT	T3 CPM	T3 ng%	T4 CPM	T4 ug%	T3 ng%/T4 ug%
1	Nml	veh	29623	81	34417	4.4	18.4
2	Db	veh	24532	150	32311	5.1	29.4
3	Nml	PTU	29367	82	33245	4.8	17.1
4	Db	PTU	27879	108	33248	4.8	22.5
5	Nml	veh	27116	112	30198	5.8	19.3
6	Db	veh	25771	126	37231	3.8	33.2
7	Nml	PTU	28461	92	27681	6.6	14.0
8	Db	PTU	23619	170	34148	4.5	37.8
9	Nml	veh	35542	37	31166	5.4	6.9
10	Db	veh	27883	108	33014	4.6	23.5
11	Nml	PTU	30766	71	32542	5.0	14.2
12	Db	PTU	24853	148	33843	4.6	32.2
13	Db	veh	25738	131	36148	4.0	32.8
14	Db	veh	24459	150	24277	8.0	18.8
15	Nml	PTU	27448	107	34768	4.4	24.3
16	Db	PTU	27462	107	29565	4.0	26.8
17	Nml	veh	30598	71	33147	4.6	15.4
18	Nml	PTU	26927	116	31924	5.1	22.7
19	Db	PTU	26224	112	33802	4.6	24.3
20	Nml	veh	31580	63	36082	4.0	15.8
21	Db	veh	28518	98	30714	5.5	17.8

Serum T_3 Concentrations. The serum concentrations of T_3 (Table 2) in diabetic mice were significantly greater than non-diabetic mice for both vehicle and PTU-treated groups. PTU treatment appeared to increase T_3 in both diabetic and normal mice.

Table 2. Nonfasting serum T_3 concentration in normal and diabetic twelve week old mice following four days of treatment with either 1 µg/gm body weight of propylthiouracil or with an equivalent volume of saline. Values indicate means of five individuals in each group ± SD reported in ng%.

PHENOTYPE	TREATMENT: VEHICLE	TREATMENT: PTU
NORMAL	73 ± 27	94 ± 18
DIABETIC	123 ± 20[a**]	129 ± 28[a**]

[a]Indicates the presence of a statistically significant comparison within a treatment group between normal and diabetic mice.

**Indicates significance at < .01 P

Serum T_4 Concentrations. The serum concentrations of T_4 (Table 3) in diabetic mice were less than those of non-diabetic mice in both vehicle and PTU-treated groups. In normal mice, PTU caused a slight increase in T_4 compared to vehicle-treated mice. In PTU-treated diabetic groups, the T_4 concentration appeared to decrease slightly compared to vehicle-treated groups, however this is not supported statistically.

Table 3. Nonfasting serum T_4 concentration in normal and diabetic twelve week old mice following four days of treatment with either 1 µg/gm body weight of propylthiouracil or with an equivalent volume of saline. Values indicate means of five individuals in each group ± SD measured in µg% of T_4. No differences were found between groups.

PHENOTYPE	TREATMENT: VEHICLE	TREATMENT: PTU
NORMAL	4.84 ± 0.74	5.18 ± 0.84
DIABETIC	4.60 ± 0.72	4.5 ± 0.30

Serum T_3-to-T_4 Ratio. The T_3-to-T_4 ratios (Table 4) for normal mice treated with vehicle were not different than diabetic vehicle-treated mice. PTU-treated normal mice exhibited decreased T_4/T_3 ratios compared to normal mice treated with vehicle. The PTU-treated diabetic mice exhibited significantly greater ratios than corresponding normal animals which indicates that PTU has greater influence on normal mice than on diabetic mice.

Table 4. Nonfasting serum T_3/T_4 ratio on a ng/µg basis in normal and diabetic twelve week old mice following four days of treatment with either 1 µg/gm body weight of propylthiouracil or an equivalent volume of saline.

	TREATMENT	
PHENOTYPE	VEHICLE	PTU
NORMAL	27 ± 0.23	18 ± 0.17[b*]
DIABETIC	27 ± 0.06	29 ± 0.06[a**]

[a]Indicates the presence of a statistically significant comparison within a treatment group between normal and diabetic mice.

[b]Indicates the presence of a statistically significant comparison within a particular phenotype between vehicle-treated and PTU-treated mice.

*Indicates significance at ≤ 0.1 P

**Indicates significance at ≤ .01 P

Thyroid Histology. Figure 1 shows a thyroid gland section from a vehicle-treated normal mouse which exhibits cuboidal follicular cells of medium cytoplasmic density and a circular nucleus which indicates a cell undergoing normal synthesis activity. The follicular border of this group was closely adhering to the colloid indicating normal secretion activity. Figure 2 represents a thyroid section from a PTU-treated normal mouse. A subtle increase in the number of cells that were columnar and the number of colloids with lacunae was observed in this group compared to normal mice on vehicle treatment. These observations are indicative of greater follicular cell activity. In addition, the colloids of this figure are larger in size compared to normal vehicle-treated mice. Figure 3 is a section of thyroid gland from a diabetic mouse treated with vehicle. This thyroid tissue has squamous follicular cells with dark staining cytoplasm that is granular in nature. The nuclei are small and pycnotic with wrinkled borders which is indicative of inactive protein synthesis. The colloids in this section are large and exhibit follicular borders that are separated from their colloid. A large colloid represents a thyroid that is storing its products. Figure 4 shows a thyroid section from a diabetic mouse treated with PTU. This section exhibits a normalization of thyroid histology. Follicular cells in this picture are cuboidal and have a cytoplasmic density that is non-granular and lightly staining. The nuclear diameter increased over diabetic groups on vehicle and were circular in appearance. This thyroid histology exhibits a resumption of protein synthesis. In PTU-treated diabetic mice the colloid decreased

in area when compared to diabetic mice thyroid tissue on vehicle and nearly equaled that of normal mice treated with vehicle.

A histological comparison was made between each group from the raw data presented in Table 5. Normal mice that were PTU-treated exhibited a subtle increase in the number of follicular cells that were columnar in nature compared to vehicle-treated normal mice. In addition an apparent increase in colloid size was observed. A possible reason why PTU treatment displayed this change in thyroid histology could be that T_4 is acting to autoinhibit thyroid activity (10). If T_4 is acting to autoinhibit thyroid output, then under the influence of PTU, a treatment that blocks T_4 to T_3 conversion peripherially, it is expected that serum T_4 concentration would increase and hence autoinhibit thyroid hormone secretion. This action on the thyroid would be exhibited as an enlarged colloid. The diabetic mice thyroid histology under vehicle treatment was determined to be inactive and storing colloid material compared to a dramatic increase in activity in PTU-treated diabetic mice exhibiting a significant degree of follicular cell activity and a reduction in colloid size. A tenable reason for this difference is that in the diabetic mice T_4 autoinhibition may be dysfunctional. Vehicle-treated diabetic mice are significantly different from vehicle-treated normal mice. The vehicle treated diabetic mice have inactive follicular cells and are storing colloid whereas normal mice exhibit active follicular cells with normal secretion activity as indicated by colloid size. Normal mice treated with PTU exhibited a slight increase in the number of follicular cells that were columnar in nature and an increased colloid size whereas diabetic mice treated with PTU dramatically

increased follicular activity and reduced colloid size. This response indicates a significant difference between normal mice as opposed to diabetic mice when influenced by with PTU.

Table 5. Morphometric analysis of thyroid glands from non-fasted twelve week old diabetic and normal mice treated with 1 ug/gm body weight propylthiouracil or an equivalent volume of saline for four consecutive days.

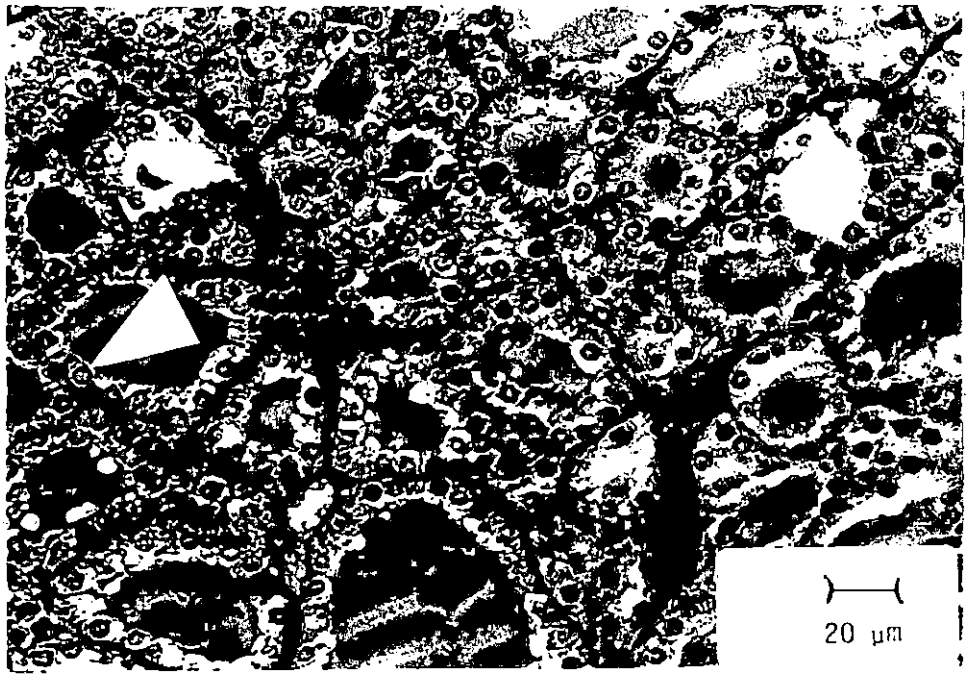
AREA	CHARACTERISTIC	NORMAL		DIABETIC	
		Vehicle	PTU	Vehicle	PTU
Follicle	cell height	.38	.35	.29	.36
	cell width	.36	.34	.30	.38
	cytoplasmic density	1.42	1.57	1.84	1.36
	nucleus diameter	.25	.25	.21	.27
Colloid	area	2.63	2.71	2.78	2.71
	density	2.01	1.90	2.14	3.09
Border	follicle border	1.92	1.81	2.67	1.99

Values for colloid area and follicular cell height, width, and nuclear diameter are based on metric measurements in centimeters taken from 3" x 5" black and white prints photographed at 380x magnification.

Values for follicle border and the densities of colloid and follicular cells are based on a rank scale ranging from 1 to 3.

Figure 1. Thyroid section from a twelve week old normal mouse treated with vehicle which had serum T_3 and T_4 concentrations of 112 ng% and 5.8 µg%, respectively. Normal activity is indicated by cuboidal follicular cells with light staining cytoplasm and circular nuclei. (arrow) 380x

Figure 2. Thyroid section from a twelve week old normal mouse treated with PTU which had serum T_3 and T_4 concentrations of 92 ng% and 6.6 µg%, respectively. Increased activity is indicated by the columnar nature of the follicular cell (black arrow). In addition, note the reabsorption lacunae which also is an indicator of increased activity (white arrow). 380x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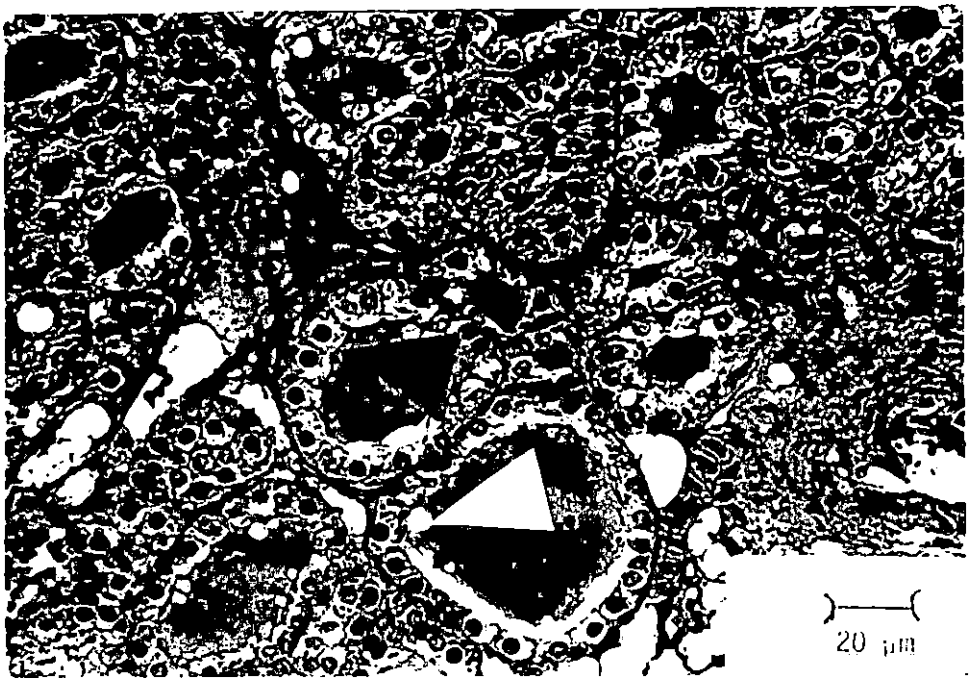
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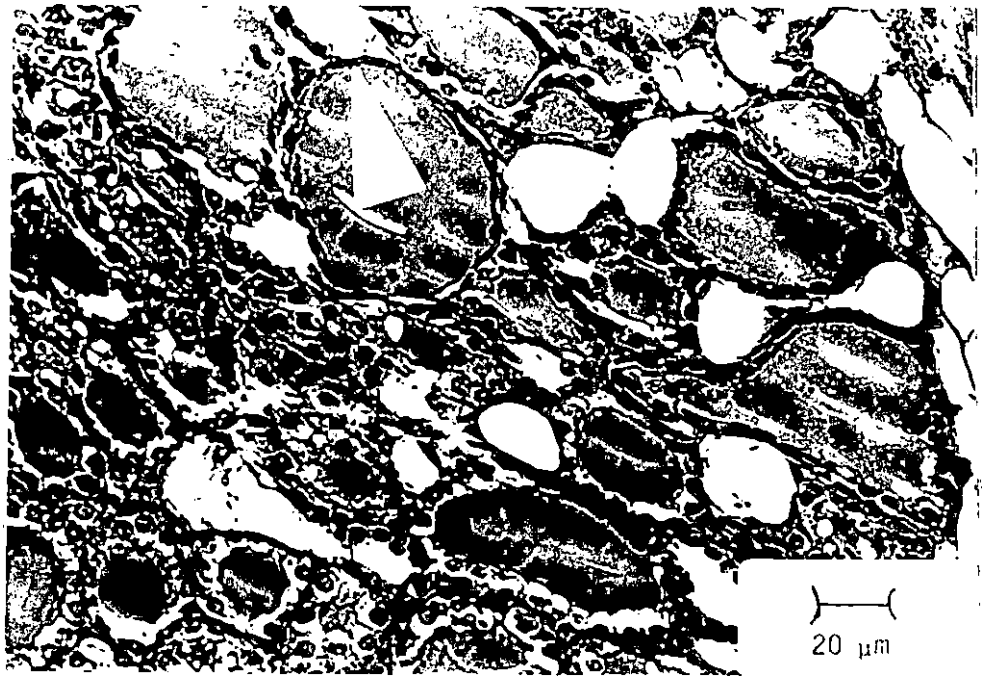
Figure 3. Thyroid section from a twelve week old diabetic mouse treated with vehicle which had a serum T_3 and T_4 concentration of 126 ng% and 3.8 µg%, respectively. Note arrow pointing out squamous follicular cells with small pycnotic nuclei and granular cytoplasm indicative of an inactive thyroid gland. In addition, note enlarged colloid which is caused by increased storage activity of thyroglobulin. 380x

Figure 4. Thyroid section from a twelve week old diabetic mouse treated with PTU which had a serum T_3 and T_4 of 112 ng% and 4.6 µg%. Note arrow pointing out a group of follicular cells that are cuboidal in nature with circular nuclei and light staining cytoplasm. In addition, note the reduction of colloid area. This morphology is representative of thyroid gland under a state of normal synthesis and secretion. 380x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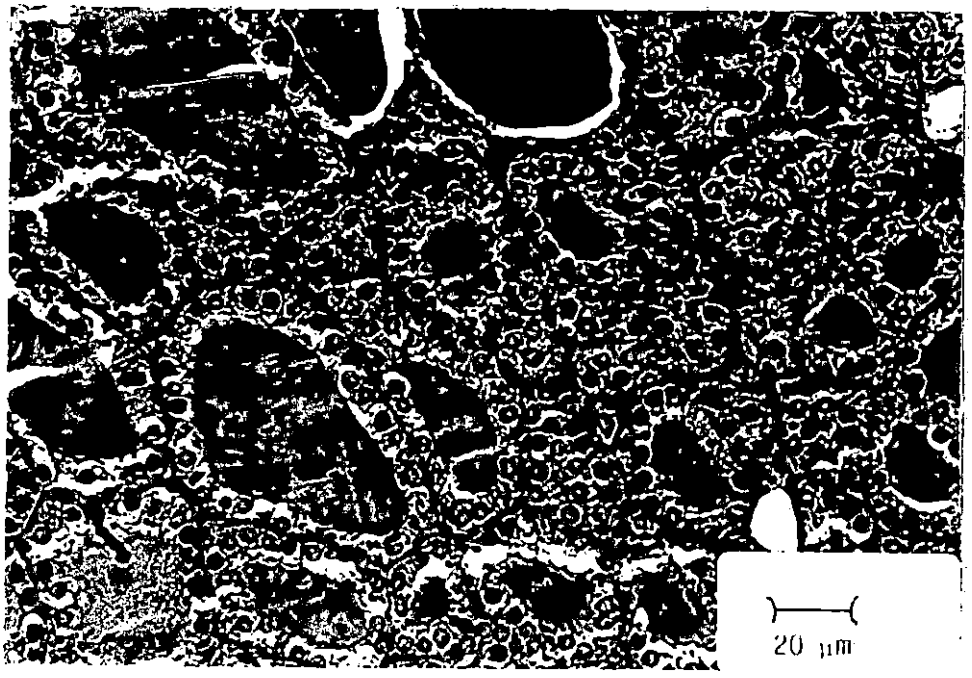
20 µm

DISCUSSION

Serum T_3 concentrations in normal and diabetic mice were slightly higher with PTU treatment than in their vehicle-treated counterparts. The reason for this response is unknown. T_3 was expected to decrease with PTU treatment in normal mice (54,55) and diabetic mice because of the ability of this drug to block the enzyme 5' monodeiodinase, hence, conversion of T_4 to T_3. Serum T_4 exhibited no change in statistical comparisons between phenotypes or treatments. The serum T_3/T_4 ratio was significantly lower in normal mice treated with PTU as compared to normal mice treated with vehicle. This response was as expected because the major source of T_3 is due to peripheral conversion of T_4 which would be blocked by PTU treatment. There was no difference in T_3/T_4 ratios between diabetic vehicle-treated mice compared to PTU-treated diabetic mice. These results indicate that diabetic mice are more resistant to PTU treatment than are normal mice. This conclusion is especially evident in thyroidal histology examination. Histologically, the thyroid glands of normal mice treated with PTU exhibited no appreciable change in follicular cell morphology compared to normal mice treated with vehicle. An apparent increase in colloid size was observed with PTU-treated normal mice compared to vehicle-treated normal mice. In diabetic mice treated with PTU a dramatic change in follicle cell activity was observed compared to diabetic mice treated with vehicle. The diabetic mice treated with PTU exhibited active thyroid tissue with follicular cells which were cuboidal containing circular nuclei whereas in vehicle-treated diabetic mice thyroidal tissue was inactive as indicated by follicular cells which were squamous and contained pycnotic

nuclei. Colloids in diabetic mice treated with PTU were smaller compared to diabetic mice on vehicle indicating an increase in secretion.

Additional evidence that diabetic mice may modulate thyroid output differently than normal mice is provided by studies of both phenotypes in fasting states (54). Serum T_4 in fasting normal mice decreases while in fasting diabetic mice this hormone increases. A possible explanation as to why diabetic mice may increase T_4 output in fasting states and with PTU treatment may be due to altered regulation of the 5' monodeiodinase enzyme but this is only speculation. Serum T_4 in normal mice may decrease in response to fasting states to conserve energy during times of low energy input. This response may not be observable in diabetic mice because of the high serum lipid level (56). The high serum lipid level in diabetic mice may prevent the triggering of an energy conservation mechanism. PTU treatment is similar to fasting conditions in that the tissues of the body are subjected to lower concentrations of T_3, the metabolically active form of thyroid hormones. Without sufficient T_3, the tissues are experiencing a depression in energy utilization. This is based on the fact that decreased T_3 reduces basal metabolic rates (2).

A possible mechanism as to how diabetic mice modulate serum T_4 differently than normal mice may be a function of the proposed autoinhibitory activity by T_4 on the thyroid. In the normal mouse T_4 may function to cause the autoinhibition of T_4 output by the thyroid gland (51). If thyroidal activity was sensitive to serum T_4 concentrations, then, under PTU treatment one would expect a thyroid

gland to undergo storage due to elevated serum T_4 concentration. This response was observed for normal mice. The diabetic mouse autoinhibition may be insensitive to T_4. If the thyroid glands in diabetic mice are insensitive to T_4 negative feedback, then when treating with PTU one would not expect colloid storage. The results in this study indicate T_4 release in diabetic mice treated with PTU. The reason for the release of colloid may be due to the response to lowered T_3 under PTU treatment. Lower T_3 would decrease metabolic rate and simulate an energy deficit. The response of the thyroid to this deficit would be to release colloid. Another possibility for colloid secretion with PTU treatment in diabetic mice is that T_3 may play an inhibitory role towards thyroid output. Under PTU treatment, by lowering serum T_3, the autoinhibitory mechanism in diabetic animals would be reduced allowing for increased thyroid activity and colloid secretion. In addition to the autoinhibitory mechanisms, the thyroid gland is under direct regulation by TSH.

A key to the differences between T_4 sensitivity between diabetic mice and normal mice may rest on the regulatory affect in converting T_4 to T_3. As stated before PTU is not active in preventing T_4 to T_3 conversion in the pituitary and that this conversion is thought to control TSH output (57). Current research suggests that the pituitary deiodinase enzyme is a distinct isoenzyme from the enzyme(s) functioning peripherally (58). This conclusion is based on the finding that the enzyme has different kinetics (59). Further, PTU insensitivity is not due to selective permeability by pituitary cell membranes for T_4 because T_3 does not function to modulate TSH in cell-free extracts (60).

One other 5' monodeiodinase has been isolated and is present in cerebral cortex. Being similar to the the pituitary deiodinase it has been proposed that this enzyme may also have a regulatory role, in this case the modulation of thyroid releasing hormone (60,61). Because of the proposed regulatory function of T_4 to T_3 conversion in the pituitary and cerebral cortex is it reasonable to believe that peripheral or thyroidal in situ conversion may have a regulatory function. Evidence of this is provided best in analysis of serum T_4/T_3 ratios. The serum T_4/T_3 ratio is a useful measure to observe both hormone concentrations simultaneously. Comparisons between diabetic and normal mice for serum T_4/T_3 are dramatically different in both vehicle and PTU-treated groups. Normal individuals given PTU treatments displayed decreased T_4/T_3 ratios compared to their vehicle-treated counterpart whereas diabetic mice exhibited no difference between PTU treatment and vehicle treatment groups. The lack of response to PTU-treatment in diabetic mice may be that the concentrations of T_3 and T_4 arising from the thyroid gland itself are different in these animals. As mentioned before, T_4 is the primary product of the thyroid gland and that T_3 is also released but in much lower concentrations (2). If the diabetic mouse is releasing higher T_3 concentrations from the thyroid, these hormone concentrations would be insensitive to a drug which blocks conversion. It may also be possible that diabetic mice, due to their polyuric state (46), are clearing T_4 more rapidly than normal mice. This is doubtful because, though unbound serum concentrations of T_4 are higher than T_3, the solubilities of thyroid hormones are quite low in plasma. Also the

level of free T_4 in normal states is low at 0.5% of the total T_4 present in plasma (10).

Whichever mechanism(s) are operating to account for differences between normal and diabetic mice, whether proposed or not, it is evident from this study that diabetic mice are inherently different in their response to PTU. PTU functions to block peripheral conversion of T_4 to T_3 and to inhibit organification of iodine in the thyroid. Further research to examine how PTU Influences these activities may lead to an increased understanding of the diabetic condition.

REFERENCES

1. Chopra, I. J., 1981. "Triiodothyronines in Health and Disease." Springer-Verlag, N.Y.

2. Falconer, D. S., L. J. P. Duncan, and C. Smith, 1971. A statistical and genetical study of diabetes. I. Prevalence and morbidity. Ann. Hum. Genet. 34:347-349.

3. Craighead, J. E., 1979. The role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus. Prog. Med. Virol., 19:161-167.

4. Craighead, J. E., 1978. Current views on the etiology of insulin-dependent diabetes mellitus. N. Engl. J. Med., 299:1439-1449.

5. DeGroot, L. S., 1979. "Endocrinology" (ed.) vol. 2, Grume and Stratton Publ., N.Y..

6. Mann, G. V., 1974. The influence of obesity on health. N. Engl. J. Med., 178:225-234.

7. Rupp, J. J., A. M. DiGeorge, and K. E. Paschkis, 1955. Hypothyroidism and diabetes mellitus. Diabetes, 4:393-399.

8. Hect, A. and H. Gershberg, 1968. Diabetes mellitus and primary hypothyroidism. Metab., 17:108-123.

9. DeGroot, L. J., and J. Stanbury, 1975. "The Thyroid and its Diseases." N.Y., McGraw-Hill Book Co..

10. Hadley, M. C., 1984. "Endocrinology" New Jersey, Prentice-Hall Inc., pp. 292-312.

11. Oppenheimer, J. H., 1979. Thyroid hormone action at the cellular level. Science, 203:971-979.

12. Kyle, L. J., M. F. Ball, and P. D. Doolan, 1983. Effect of thyroid hormone on body composition in myxedema and obesity. N. Engl. J. Med., 275:12-27.

13. Volfin, P. K., S. S. Kaplay, and D. R. Sandi, 1969. Early effect of thyroxine in-vivo on rapidly labeled mitochondria protein fractions and respiratory control. J. Biol. Chem., 244:5631-5651.

14. Bronk, J. R., 1963. Thyroid hormones: Control of terminal oxidation. Science, 141:816-818.

15. Cole, C. H. and R. W. Waddell, 1979. Alteration in intacellular sodium concentration and ouabain-sensitive ATPase in erythrocytes from hyperthyroid patients. J. Clin. Endocrinol. Metab., 42:1056-1061.

16. Sokoloff, L., P. A. Roberts, M. M. Januska, and J. E. Kline, 1968. Mechanisms of stimulation of protein synthesis by thyroid hormones in-vivo. Proc. Natl. Acad. Sci. USA., 60:652-666.

17. Correze, C., P. Pinell, J. Nunez, 1973. Effects of thyroid hormones on phosphorylation of liver ribosomal proteins and on protein activity, FEBS Lett., 23:87-89.

18. Tulloch, B. R., B. Lewis, and R. T. Fraser, 1973. Triglyceride metabolism in thyroid disease. Lancet, 1:391-396.

19. Kritchevsky, D., 1960. Influence of thyroid hormones and related compounds on cholesterol biosynthesis and degradation: A review. Metab., 9:984-1012.

20. Chait, A., E. L. Beirman, J. J. Albers, 1979. Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultured human skin fibroblasts. J. Clin. Endocrin., 48:887-901.

21. Arons, D. L., P. H. Scheribman, P. Downs, L. E. Brawerman, and R. A. Arky, 1972. Decreased postheparin lipases in Graves disease. N. Engl. J. Med., 286:233-249.

22. Althausen, T. L. and M. Stockholm, 1938. Influence of thyroid gland on absorption in digestive tract. Am. J. Physio., 123:577-590.

23. Andreani, D., G. Menginger, and F. Falluca, 1970. Insulin levels in thyrotoxicosis and primary myxedema: response to intravenous glucose and glucogon. Diabetologia, 6:1-12.

24. Mirsky, I. A. and R. H. Broh-Kohn, 1936. Effect of experimental hyperthyroidism on carbohydrate metabolism. Am. J. Physio., 117:6-17.

25. Shaheen, O., D. W. Morgan, H. G. Wilcox, W. G. Keyes and M. Heimburg, 1982. Modulation by thyroid states of the actions of glucagon and dibutyryl adenosine 3', 5' - monophosphate on metabolism of free fatty acids by isolated perfused rat liver. Endocrinol., 110:1748-1758.

26. Elgee, N. J., R. H. Williams, and N. D. Lee, 1954. Distribution and degradation studies with insulin. J. Clin. Invest., 33:1252-1267.

27. Elrick, H. and C. J. Hlad, 1961. Influence of thyroid function on carbohydrate metabolism and a new method for assessing response to insulin. J. Clin. Endocrinol., 21:287-393.

28. Morecek, R. L. and J. M. Feldman, 1985. Effect of hyperthyroidism on insulin and glucose dynamics in rabbits. Endocrinol., 92:1604-1626.

29. Bar, R. S. and J. Roth, 1980. Altered insulin receptors, insulin resistance, and diabetes mellitus. In: secondary diabetes: the spectrum of the diabetic syndromes. S. Podolsky and M. Viswanathan eds. Raven Press Inc., N.Y., pp. 300-317.

30. Lazarus, S. S. and B. W. Volk, 1952. The estimation of insulin sensitivity by the modified glucose insulin tolerance test. J. Lab. Clin. Med., 39:404-405.

31. Kinash, B., and R. E. Haist, 1985. The influence of the thyroid gland on the islets of Langerhans and the pancreas. Can. J. Biochem. Physio., 33:380-402.

32. Kreines, K. and H. C. Knowles, 1965. Observations in hyperthyroidism of abnormal glucose tolerance and other traits related to diabetes mellitus. Diabetes, 14:740-748.

33. Danowski, T. S., J. V. Boonessi, and C. Moses, 1964. Hydrocotisone and/or dessicated thyroid in physiological dosage. XII: Carbohydrate metabolism during large dose thyroid (Prolod therapy). Metab., 13:739-740.

34. Berelowitz, M., 1982. Somatostatin and diabetes mellitus. In "Diabetes and Obesity." B. N. Brodoff and S. J. Blecher, eds. Williams and Wilkins, Md., pp. 89-94.

35. Honobo, K. S., A. J. Van Herle, and K. A. Kellet, 1978. Serum prolactin levels in untreated primary hypothyroidism. Am. J. Med., 64:782-789.

36. Hect, A., and H. Gershberg, 1968. Diabetes mellitus and primary hypothyroidism. Metab., 17:108-119.

37. Goldstein, D. E., A. Drash, and J. Gibbs, 1970. Diabetes mellitus the incidence of circulating antibodies against thyroid, gastric and adrenal tissue. J. Pediatr., 77:304-306.

38. Fujii, L., T. Akai, S. Tanaka, K. Nadatani, M. Kinoshita, J. Seki and M. Wada, 1981. Thyroid hormone abnormalities in patients with diabetes mellitus. J. Clin. Invest., 4:71-74.

39. Rupp, J. J., A. M. DiGeorge, and K. E. Paschkis, 1955. Hypothyroidism and diabetes mellitus. Diabetes, 4:393-399.

40. DeGroot, L. J. and H. Niepomniszcze, 1977. Biosynthesis of thyroid hormones: Basic and clinical aspects. Metab., 26:665-672.

41. Dunn, J. T., P. S. Kim, A. D. Dunn, 1982. Favored sites for the thyroid hormone formation on the peptide chains of human thyroglobulin. J. Biol. Chem., 257:88-104.

42. Ferguson, D. C. and A. S. Jennings, 1983. Regulation of conversion of thyroxine to triiodothyronine in perfused rat kidney. Amer. J. Physio., 245:220-229.

43. Kaplan, M. M. and R. D. Utiger, 1978. Iodothyronine metabolism in liver and kidney homogenates from hyperthyroid and hypothyroid rats. Endocrinol., 103:156-179.

44. Leonard, L. J., M. M. Kaplan, T. J. Visser, J. E. Silva, and P. R. Larsen, 1981. Cerebral cortex responds rapidly to thyroid hormones. Science, 214:571-573.

45. Coleman, D. L. and K. P. Hummel, 1967. Studies with the mutation, diabetes, in the mouse. Diabetologia, 3:238-248.

46. Coleman, D. L. and K. P. Hummel, 1972. Comparison of the obesity syndromes of obese (ob/ob) and diabetic (db/db) mice. Diabetologia, 8:49.

47. Chick, W. L., R. L. Levine and A. A. Like, 1970. Studies in the diabetic mouse: Glucose tolerance in mice homozygous and heterozygous for the diabetes gene. Diabetologia, 6:257-262.

48. Fehn, R., 1983. Pituitary-thyroid function in the C57BL/Ks db/db mouse. Ph.D Dissertation, University of Arizona.

49. Kaplan, M. M., 1980. Thyroxine 5'-monodeiodination in rat pituitary homogenates. Endocrinol., 106:567-576.

50. Larsen, P. R., T. E. Dick, B. P. Markovitz, M. M. Kaplan, and T. G. Gard, 1979. Inhibition of intrapituitary thyroxine to 3,5,3'-triiodothyronine conversion prevents the acute suppression of thyrotropin release by thyroxine in hypothyroid rats. J. Clin. Invest., 64:117-128.

51. Sugawara, M., R. Lau, H. L. Wasser, A. M. Nelsen, K. Kuma, and J. M. Hersman, 1984. Thyroid thyroxine 5' monodeiodinase activity in normal and abnormal human thyroid glands. Metabol. Clin. Exp., 33:332-336.

52. DuBreuil, A. and A. V. Galton, 1978. Thyroxine studies concerning its intrinsic physiological activity. Acta Endocrinol., 88:87-93.

53. Humasen, G. L., 1967. "Animal Tissue Techniques," 2nd ed. W. H. Freeman and Co. San Francisco.

54. Schimmel, M. and R. D. Utiger, 1977. Thyroidal and peripheral production of thyroid hormones. Ann Intern. Med., 87:760-768.

55. Croxson, M. S., T. D. Hall and J. T. Nicoloff, 1977. Combination drug therapy for treatment of hyperthyroid Graves' disease. J. Clin. Endocrinol., 45:623-630.

56. Wyse, B. M. and W. E. Oulin, 1970. The influence of age and dietary conditions on diabetes in the db mouse. Diabetologia, 6:268-273.

57. Ryder, S., G. M. Gollapudi, and A. Varma, 1980. Relationship of serum T_4 and T_3 to TSH in primary hypothyroidism. Arch. Intern. Med., 140:1290-1291.

58. Silva, J. E., J. L. Leonard, F. R. Crantz, and P. R. Larsen, 1982. Evidence for two tissue-specific pathways for in-vivo thyroxine 5'-deiodination in the rat. J. Clin. Invest., 69:1176-1184.

59. Visser, T. J., M. M. Kaplan, J. L. Leonard and P. R. Larsen, 1983. Evidence for two pathways of iodothyronine 5'-deiodination in rat pituitary that differ in kinetics, propylthiouracil sensitivity, and response to hypothyroidism. J. Clin. Invest., 71:992-1002.

60. Chopra, I. J., 1977. A study of extrathyroidal conversion of thyroxine (T_4) to 3,3',5-Triiodothyronine (T_3) in-vitro. Endocrinol., 101:453-463.

61. Kaplan, M. M., T. S., Visser, K. A. Yaskoski and J. L. Leonard, 1983. Characteristics of iodothyronine tyrosyl ring deiodination by rat cerebral cortical microsomes. Endocrinol., 112:35-41.