STUDIES OF THYROID FUNCTION IN PROTEIN DEPLETED ALBINO RATS BY THE USE OF RADIOACTIVE IODINE

by

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ABSTRACT*

The purpose of this investigation was to study the effect of dietary protein on thyroid function in rats fed low and optimal levels of iodine. The parameters studied were: a) the ability of the gland to concentrate iodide, (percent one hour uptake, T/S, Tt/S and Tt/St); b) synthesis of thyroid hormones by autoradiochromatography (MIT + DIT) /(T_3 + T_4); and c) histological condition of glandular tissue; (cell heights and d/n ratio).

Four experiments were performed. In each experiment the rats were randomized into four groups, two of which received supplemental iodine in the diets; the other two groups received low iodine diets. One of the groups which received iodine was fed a protein-free diet whereas the other received 20 percent casein. Of the two iodine-deficient groups one received casein and the other a protein-free diet.

The results showed that, when the basal diet was supplemented with iodine, protein deprivation did not affect, appreciably, the ability of the thyroid to concentrate iodide. However, in all iodine-deprived rats, a significant increase in the iodide concentrating ability was observed; this increased activity appeared to be suppressed by protein starvation in iodine-deficient animals. The suppression effect was not observed when thyroid function was evaluated in the absence of PTU.

Repletion of protein-depleted rats caused thyroid function to return to normal. In severely depleted, iodine-deficient animals, TSH treatment had no effect on thyroid function.

Radiochromatographic analysis of thyroid hydrolysates did not reveal any striking difference in hormone synthesis among the dietary

groups.

In iodine deprivation, marked histologic alterations were observed. Follicle size and cellular heights were significantly increased in thyroids of iodine-depleted but protein-fed rats. Protein depletion resulted in the flattening of epithelial cells and in the reduction of intrafollicular spaces.

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* The following abbreviations are used: MIT for monoiodotyrosine; DIT for diiodotyrosine, T_3 for 3,5,3'-triiodothyronine; T_4 for thyroxine or 3,5,3',5'-tetraiodothyronine; PTU for propylthiouracil; TSH for thyrotropic or thyroid stimulating hormone.

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INTRODUCTION

Iodine was established as an essential element in human nutrition in 1895. Because of the relatively low level of iodine (20-50 mg) (1) in the human body, it has been difficult to study its intermediary metabolism; hence the clarification of the different steps of its utilization was delayed.

Courtois in 1811 first found iodine while preparing nitre from dried seaweed; a few years later, Gay Lussac described the chemical properties of and named this element (1). In 1820, Coindet successfully treated goitrous patients with tincture of iodine. But it was Chatin who, after analyzing foodstuffs and water, concluded that, in spite of the universal distribution of iodine in nature, goiter occurred in areas where a deficiency of this element was prevalent (1).

Soon after Pasteur's success in identifying bacteria as the cause of infectious diseases, physicians were misled into attempting to relate the incidence of goiter to a pathogenic microorganism. As a result of this misconception, Chatin's work was altogether forgotten. However, after Baumaun's isolation of iodine from thyroid gland in 1895, research was resumed in this field (2). Later, in 1920, Marine and Kimball (3) successfully showed that sodium iodide in adequate doses greatly reduced the incidence of goiter in children; similar results were obtained by other workers in various countries.

Kendall and Osterberg (4) in 1919 isolated and named the thyroid hormone, thyroxine; in 1927, a group of investigators at Edinburgh determined the correct chemical structure of thyroxine and synthesized the hormone (5). Gross and Pitt-Rivers in 1952, demonstrated the presence

of another hormone in the thyroid gland, namely triiodothyronine (6).

Even though the discovery of radioactive iodine contributed greatly to the elucidation of the various phases of iodine metabolism, the problem of goiter has survived from the days of Chatin to present. David Marine (3) in 1920 stated that "simple goiter is the easiest of all known diseases to prevent. It may be excluded from the list of human diseases as soon as society determines to make the effort." No matter how ironical the statement may seem, it does not fail to be true today in developing countries. In Lebanon for example, the problem of endemic goiter has been recognized for many years. Although several workers (7, 8), from 1947 on, have demonstrated that iodine therapy will overcome clinical and biochemical signs of goiter, the problem still exists in this country.

The present study relates states of iodine deficiency to dietary protein levels and the reflection of the combined effects of the two variables on the activity of thyroid gland. Basic information is needed on these combined effects since in many endemic goiter areas, protein malnutrition is also a problem. Thus low protein intake in the presence of iodine deficiency may induce a state of abnormal thyroidal function which, together with disturbances due to low protein intake, could conceivably lead to serious complications.

LITERATURE REVIEW[1]

Absorption and Clearance of Iodine

The extrathyroidal iodide pool is the result of iodide absorbed from the gastrointestinal tract and that derived from metabolic degradation of thyroid hormones (9). Irrespective of the chemical form of dietary iodine, it must be reduced to iodide before being absorbed from the intestine (10).

The kidney and the thyroid gland are the two major organs which clear iodide from the pool. Consequently, the rate of iodide removal from the pool is a direct function of the rate of urinary excretion and organification of iodide in the thyroid gland. Renal clearance of iodide is constant at all concentrations of iodide (11); in contrast, the rate of clearance by thyroid gland is inversely proportional to plasma iodide concentration (12).

Biosynthesis and Metabolism of Thyroid Hormones

Thyroidal iodide trapping is an enzymatic, aerobic process which requires energy (13). This is shown by the fact that cyanide, azide and other compounds that interfere with oxidative processes in cells, inhibit the trapping mechanism (13, 14). The accepted hypothesis of the trapping mechanism is that iodide forms a complex with a carrier in the cell membrane. After passage through the membrane, the iodide is released into the sites of high concentration, where, until organification, it

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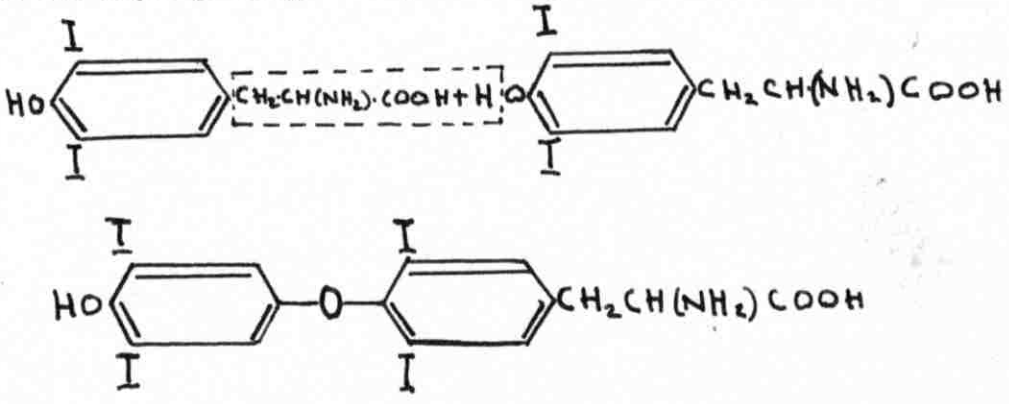
1. Throughout this thesis, the following abbreviations are used: MIT for monoiodotyrosine; DIT for diiodotyrosine, T_3 for 3,5,3'-triiodothyronine; T_4 for thyroxine or 3,5,3',5'-tetraiodothyronine; PTU for propylthiouracil; TSH for thyrotropic or thyroid stimulating hormone; NAD for nicotinamide adenine dinucleotide.

remains as iodide. This hypothesis is based on the principle of active transport and is supported by Wolff (15) and Halmi (16) who presented evidence for this mechanism. In addition, other workers have isolated, from thyroid tissue, a bound form of iodide which was not in equilibrium with extracellular fluid (17, 18). Low temperature (14), anaerobic conditions (19) and compounds like 2, 4-dinitrophenol, that are known to uncouple oxidative phosphorylation, inhibit iodide trapping; that phosphate bond energy is required, becomes evident from these findings (14, 19-21).

The iodide trapped is first oxidized to free iodine (22). Evidence for this oxidation step was demonstrated by experiments which showed that iodide failed to exhibit thyroxine-like action, whereas elemental iodine gave positive results. After oxidation, tyrosyl residues in the thyroglobulin of follicular epithelial cells are iodinated. Tracer studies using I^{131} have shown that the first iodinated amino acid is MIT[1], the level of which gradually declines (23). This decline is followed by an increase in the level of labelled DIT[1]. In the same manner, T_4[1] increaaes as DIT decreases. Thus, it appears that MIT serves as a precursor to DIT which in turn acts as a precursor to T_4.

Oxidative condensation of two molecules of DIT with the exclusion of an alanine side chain has been proposed by Harington (24) as the major pathway of thyroxine synthesis:



However, the formation of T_3[1] could follow two possible routes: a) a reaction similar to that involved in the formation of thyroxine, in which one molecule of MIT is coupled to DIT to form T_3; b) a partial deiodination of T_4 (25). Roche and coworkers favor the view that the former is the probable scheme of T_3 synthesis. They found no direct incorporation of I^{131} into either T_3 or T_4; furthermore, no T_4 deiodinase was detected in thyroid tissue.

The thyroid hormones, T_4 and T_3, are stored as peptide-linked residues within the thyroglobulin. Proteolysis of this protein releases the free hormones which then enter the blood stream (26-28). Also, MIT and DIT are released by proteolysis, but under normal conditions these compounds do not enter the blood stream; they are deiodinated by a deiodinase to form iodide and free tyrosine (29, 30). The iodide thus released is available to be reutilized for hormone synthesis. The circulating thyroid hormones (T_3 and T_4) are carried to the peripheral tissues bound to a plasma protein (31-35). The major hormone carrier is an α-globulin, although some may be bound to a prealbumin (36).

Besides having a significant physiological influence on growth, maturation and differentiation in vertebrates, T_4 has an inhibitory effect on several NAD-dependent dehyrogenases. These enzymes include glutamic, lactic, malic, triosephosphate, yeast alcohol and yeast glucose-6-phosphate dehydrogenase (37). Inhibition of the activity of these enzymes has been ascribed to the fact that T_4 causes mitochondrial swelling, thus allowing leakage of NAD from these structures (38).

Thyroxine has a marked influence on the uncoupling of oxidative phosphorylation and when T_4 is added to mitochondrial preparations, the P/O ratio decreases. The action of T_4 and analogues at low concentrations

is at a specific biochemical locus where it causes the uncoupling of the rate limiting step in the chain of energy yielding reactions (39). Thus, subsequent steps proceed more rapidly and without overall significant loss in efficiency. Upon increasing the concentrations of T_4 and analogues, the inhibiting action spreads to other reactions and the result is an overall decrease of efficiency.

After the hormone has performed its function in somatic cells, a large portion is deiodinated and the iodide thus released reenters the extracellular iodide pool; the remainder of the hormone is excreted. In man, the major excretory route of the iodothyronines is the urine; traces of thyroid hormones are found in feces (40).

Pituitary Control of Thyroid Function

The normal functioning of the thyroid gland is directly governed by the thyroid stimulating hormone (TSH) produced in the anterior lobe of the hypophysis. Any damage to the pituitary gland results in marked alterations of thyroid function (41).

Secretion of TSH is a function of T_4 concentration in blood; as plasma level of T_4 increases, TSH secretion is reduced and vice versa (42). Hypophysectomy (destruction or removal of the hypophysis) results in a reduced I^{131} uptake by thyroid (43); also it depresses the biosynthesis of T_4 more than that of DIT. Furthermore, it was shown that after hypophysectomy, there was a higher ratio of labelled MIT to DIT (44). Taurog (45) demonstrated that in rats, hypophysectomy depressed iodide accumulation, synthesis of iodotyrosines and thyroxine and the discharge of the latter into the blood. Also, thyroid to serum ratio, (T/S) was decreased (46). Treatment of the hypophysectomized rats with

single or repeated doses of TSH restored T/S and I^{131} uptake values to normal.

In a review article on the mode of action of antithyroid compounds, Pitt-Rivers (47) classified these into three major categories according to the mechanism of action: a) inhibition of iodide trapping from the blood; b) inhibition of hormone synthesis; c) inhibition by tissue destruction. Thiocyanates were found to be the major factor in the first category. This property of thiocyanate was established after observing that thiocyanate-promoted goiter could be reversed by iodine administration (48). Compounds which inhibit organic binding (category b) include iodide itself which exerts an inhibitory action when present in excess. Also in this category are sulfonamides and thioureas which produce symptoms in rats (49) similar to those of hypothyroidism. Thiouracil (50, 51) was shown to depress T_4 synthesis from DIT by rat thyroid both in vivo and in vitro; the conclusion was that the drugs interfered in some way with hormonal synthesis (52, 53). That administration of thyroxine overcomes the disorders, shows that iodide uptake continues normally and that organification alone is inhibited. This fact is further substantiated by the observation that when organic binding is blocked by PTU, the T/S ratio is increased greatly and all iodine in the gland is in the form of iodide (17, 18, 52). These above-named agents act by inhibiting the oxidizing enzymes which are involved in iodination of the amino acids. Further evidence for this hypothesis is given by the fact that inhibitors of cytochrome oxidase, (cyanide, azide, sulfide and carbon monoxide) inhibited formation of DIT and T_4 from I^{131} in sheep thyroid slices (53).

It is relevant at this point to mention the goitrogenicity of certain foods. Most notable of the foods containing goitrogens are certain members of the Brassicae family, such as cabbage, turnip, rape and mustard (54, 55). The goitrogenic action of these is similar to that of the agents that inhibit the organic binding of iodine (55). Organic cyanides which are common constituents of Brassicae, are goitrogenic; also, phenylthioureas found in these foods are goitrogenic in rats (56). The cyclized derivative of an isothiocyanate, vinyl-thiooxazolidone is an important goitrogen in rutabaga (yellow turnip); however, upon cooking the goitrogenicity is destroyed (57).

In the third category of agents which interfere with thyroid function is radioactive iodine; excessive doses of this material will destroy the cells of the gland and thus inhibit function (47).

Thyroid Function Under Dietary Restriction

It was previously mentioned that there is a close interrelationship between the anterior pituitary and the thyroid gland. The atrophy of certain glands following starvation and other dietary stresses has been ascribed to a hypoactivity of the pituitary (58). Certain observations made after starvation and hypophysectomy have led some authors to state that starvation has the same effect as hypophysectomy (58, 59). In work with guinea pigs, Stephens (60) found histological evidence to support this latter statement. His conclusions were based on the fact that he found in the thyroid starved animals extremely flat epithelial cells, inconspicuous cytoplasm, small nuclei and the absence

of mitotic figures. Furthermore, injection of the underfed animals with TSH restored to normal the histological condition of the thyroid gland. Because of this positive response to TSH, Stephens proposed that the symptoms of thyroid hypoactivity in inanition were due to a decreased secretion in TSH by the anterior pituitary.

Similar observations of thyroid hypoactivity in rats and mice have been reported by D'Angelo (61) who subjected the animals to acute starvation. In addition, he reported that the TSH content in the blood of starved animals was lower than that of the controls.

In relation to these above observations, Williams (62) observed a lower I^{131} uptake by starved rats; Van Middlesworth (63) reported similar findings in rats subjected to stress conditions like anoxia, nephrectomy and starvation. In addition, Van Middlesworth and Berry showed that PBI^{131} of starved rats was decreased.

Contrary to the results discussed above, Catz (64) reported an increased I^{131} uptake by thyroids of rats starved for 24 hours; there was no histological alteration of the gland. Oral administration of 10 mg of iodide to the fasting animals restored I^{131} uptake to the level observed in the control group. Replacing the caloric value of the diet with pure glucose did not lower I^{131} uptake (64). Similar results were obtained by Storaasli et al. (65). Stenberg and Kusevickij observed a high I^{131} uptake and histologically depressed thyroidal activity in rats fed a diet of 91.8% carbohydrate and low iodine (66).

In a preliminary study in 1952, Aschkenasy and co-workers (67) showed that rats on a 4% protein diet had thyroids in a functional rest with flat epithelium and large vesicles rich in non-vacuolated

colloid. Supplementation of the diet with methionine or cystine did not improve the morphological appearance of the gland. Further, pituitary TSH content of rats on low protein diets was lower than that in the control group. In further experiments, I^{131} uptake measured 6 and 51 hours after injection of radioiodine was higher in rats that received the low protein diet. Also, radioactivity of plasma of these rats was still elevated after 51 hours, due to a stimulation provoked by the prolonged privation of iodine (68). To provide further evidence for increased hormonal I^{131} in the blood, the renal clearance of I^{131} was measured. It was found that, in protein deprived rats, the renal I^{131} clearance was greatly reduced (69). Thus, rats on low protein diets, performed like thyroidectomized rats (70), in which renal clearance of I^{131} was lower than in normal animals. Radiochromatography of thyroid and plasma showed no change in hormonal synthesis (71, 72). Furthermore, the stable iodide content of the protein-free diet used was practically negligible; as a result, I^{127} of thyroid and plasma was very low (73).

In subsequent experiments in which metabolism of iodide was studied, Aschkenasy et al. demonstrated that there was no impairment of hormone synthesis in protein deprivation. Furthermore, I^{131} uptake and iodide concentrating ability of rats in which hormone synthesis was blocked by PTU, were significantly higher in protein and iodine deprived rats (74-76). The same experiments were performed using animals fed diets in which amino acid imbalances were induced. Diets deficient in methionine, cystine, lysine and tryptophan, were unable to cause hyperactivity of thyroid gland similar to complefe protein deprivation (75, 76). However, when tyrosine together with phenylalanine were

withdrawn from the food mixtures, iodine uptake and iodide concentrating ability of thyroids were increased (74, 76) to levels even higher than those caused by protein depletion. According to Aschkenasy et al. (79, 81) the elimination of tyrosine and phenylalanine cancels the "protective" effects of other dietary amino acids; this effect is observed only in the absence of dietary iodine. The same trend was seen when thyroidal activity was evaluated in the absence of PTU injection (76). A higher I^{131} uptake was obtained in the protein-deprived rats, but the values for T/S, Tt/S and Tt/St in this same group were slightly lower due to hormonal release into the blood stream (76).

Repeated injections of TSH to rats on protein-free diet had no effect in raising the I^{131} uptake as they did in hypophysectomized animals (77-79).

All the observations in the work of Aschkenasy et al. lead to the hypothesis that protein deprivation, together with iodine deficiency, induces a hyperactivity of the thyroid gland. Furthermore, these observations tend to invalidate the view that protein starvation results in the functional rest of the gland, or in other words, simulates hypophysectomy.

MATERIALS AND METHODS

Experimental Animals

The experimental animals used were female weanling albino rats of the Sprague-Dawley strain[2]. The rats were received by air at 21-23 days of age and were placed on a stock diet[3] for a recovery period of 8-10 days, or until they reached an average weight of 60 to 80 gr. During the experiments, the animals were individually housed in mesh-bottom cages in an air-conditioned room held at 21 ± 1°C and relative humidity of about 60 percent. The diets were assigned according to a randomized block design (80). Animals received food and distilled water ad libitum; food consumption and weight gains were determined weekly.

Diets

The basal diet had the following composition on an air dry basis:

Ingredients	Percentage
Corn starch	81
Corn oil	10
Non-nutritive cellulose (alphacel)[4]	5
Iodine-free salt mixture[4]	3
Vitamin mixture[4]	1

For the preparation of the protein-rich diets, sufficient purified casein[5] was incorporated, at the expense of corn starch, at a

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2. Obtained from Animal Suppliers (London) Ltd.
3. Obtained from Vitasni Feed Company, Beirut, Lebanon.
4. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.
5. Obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

Table 1

Composition of diets used in various experiments.

Ingredient	Diet I	Diet II	Diet III	Diet IV
		g/1000 gr diet		
Casein	200	200	-	-
Corn starch	610	610	810	810
Corn oil	100	100	100	100
α - Cel	50	50	50	50
I-free salt mixture	30	30	30	30
Vitamin mixture	10	10	10	10

level of 20 percent. Table 1 shows the composition of the four diets that were used in all experiments.

Iodine was added to diets I and III in the form of potassium iodate solution; the appropriate amount of solution was added to a small portion of the diet which was then thoroughly dried in a mechanical convection oven. This portion was then mixed thoroughly with the main bulk of the diet for 60 minutes in a Hobart food mixer. In experiments I and II, sufficient iodate was added to diets I and III to give a calculated level of 25-30 µg/Kcal. This level was increased in experiments III and IV to 41 µg/Kcal[6]. The low iodine diet contained 7 µg/Kcal[6].

The Experimental Period

The experiments were of two major types: (1) protein depletion, and (2) depletion followed by repletion. In the depletion experiments, the duration varied from 16 to 30 days depending on the condition of the depleted animals. The experiments were stopped when the rats could no longer control urination. Repletion was accomplished by incorporating 20 percent casein into the diets of depleted animals; no change was introduced in the dietary iodine levels. The duration of repletion was from 65-75 days or until the rats reached an average weight of 160-190 gr. In all experiments, there were two control groups receiving 20 percent casein; one on adequate iodine, the other on low iodine.

Biochemical Methods

Unless otherwise specified, at the end of each experiment, each rat was given an intraperitoneal injection of one ml of saline solution

6. Analyzed by Boston Medical Laboratories, Inc., Boston, Mass., U.S.A.

containing 10 mg PTU[7]. The PTU was given to block the organification of iodine in the thyroid gland (17, 81). Forty five minutes after PTU administration, the animals were injected with 5 μC of radioactive iodine in the form of carrier-free NaI^{131}[8]. One hour later, the rats were anesthetized with chloroform and exsanguinated by cardiac puncture; a small amount of blood was drawn into heparinized capillary tubes for hematocrit determination[9]. The remainder of the blood was centrifuged at 3500 rpm for 10 minutes, and the serum collected for radioactivity measurements[10].

Both lobes of each thyroid gland were excised, weighed on an Ultra-Matic Precision torsion balance and fixed in 10 percent formalin. These preserved glands were used for measurement of I^{131} uptake and for histological studies.

In one experiment, the effect of TSH on thyroid activity of protein and iodine-depleted rats was studied. TSH was administered by daily intraperitoneal injection over a period of seven days; the daily dose was 2 USP units in one ml of physiological saline solution. The last injection was given 6 hrs prior to PTU injection.

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7. Obtained from the "Sigma" Chemical Co., St. Louis, Mo. U.S.A.
8. Obtained from the Radiochemical Center, Amersham, England.
9. Determinations were performed by the author in the Nutrition Laboratory, School of Medicine, American University of Beirut.
10. The instrument used to measure radioactivity was a well-type, crystal scintillation counter; counting efficiency was 60 percent. The instrument was manufactured by "Baird Atomic Inc.," Cambridge, Mass. U.S.A. Counts were measured at 1750 volts; all counts were corrected for background.
11. Distributed under the trade name "Ambinon", by the Organon Company, Holland.

Radiochromatographic Studies

For chromatographic studies, rats were injected intaperitoneally with 25 μC of I^{131} without a prior PTU injection, so as to follow hormonal biosynthesis in animals on the different regimes (71, 72). Twenty four hours after injection, the rats were sacrificed with chloroform and the thyroid glands removed and weighed as before. Each gland was transferred to a test tube containing 1 ml of a 0.5 percent pancreatin solution at pH 8.6; the glands were incubated under toluene at 37°C for 48 hrs.

At the end of the incubation period, a 10 μl portion of each enzymatic hydrolysate was chromatographed on Whatman No. 1 filter paper, along with known standards of MIT, DIT, T_3 and T_4. The solvent used was the top layer of a mixture of n-butanol, glacial acetic acid and water (4:1:5 v/v). Before the solvent was applied, the chromatogram was placed in the chromatography tank for 2-3 hrs to equilibrate with the atmosphere of the tank. All chromatograms were run with the solvent descending; the solvent was allowed to move to within 4-8 cm of the end of the paper.

The chromatogram was dried in air and developed by spraying with a chloroform-collidine-ninhydrin spray mixture (82). By comparing the positions of the spots representing known MIT, DIT, and $T_3 + T_4$ (which do not separate in the solvent used) the positions of the corresponding compounds from the hydrolysates were located. The chromatograms were cut into 1 cm strips and the radioactivity of each strip was measured. By noting the radioactivity of the appropriate areas of the chromatograms the (MIT + DIT)/($T_3 + T_4$), ratio was calculated as an indication of hormonal synthesis (76).

Histology

For histology, the glands preserved in 10 percent formalin were used. One of the two lobes from each gland was thoroughly washed in running water, dehydrated in an alcohol series and mounted in paraffin blocks; longitudinal sections, 8 micra thick, were obtained using an American Optical Microtome (model 820). Three sections from various parts of the glands were mounted on glass slides. The tissues were stained with hematoxylin and counterstained with 0.25 percent aqueous eosin, followed by washing, dehydration and cleaning. The specimens were mounted then in permount and preserved for microscopic analysis.

Parameters Studied

The parameters used to evaluate thyroid activity were:

a) Weight of thyroid glands: It is well known (83) that, as a result of iodine deficiency, the thyroid gland will undergo a compensatory enlargement; consequently the weight of the gland is an indication of activity.

b) Thyroid uptake of I^{131}: This is an indication of the avidity of the gland for iodine, and uptake values are high in animals deficient in iodine (17, 84, 85). In general, thyroidal uptake is expressed as the percentage of the dose of I^{131} trapped by the gland during a defined period after I^{131} injection.

c) The T/S ratio: This term is defined as the ratio of the radioactivity of 100 mg of thyroidal tissue (calculated after measuring the absolute activity of the two lobes) to the radioactivity of 100 mg of serum (calculated as the radioactivity of 0.1 ml of serum diluted to 2 ml with distilled water). This T/S ratio permits the evaluation of the

ability of thyroid gland to concentrate iodide from plasma (17, 84, 85).

d) The Tt/S ratio: This is the ratio of total radioactivity in the thyroid gland to the radioactivity of 100 mg of serum (75, 78). It is an indication of the relative number of iodide acceptor sites in the thyroid (86) and thus is directly related to the size of the gland.

e) The Tt/St ratio: This is the ratio of total radioactivity of thyroid, to total radioactivity of serum (74, 77). It permits the evaluation of the iodide concentrating ability of the gland per se, rather than radioactivity based on 100 mg of tissue. In other words, it represents the absolute capacity to concentrate iodide. The St is calculated according to the following formula (78):

$$St = \frac{(100 - \text{hematocrit}) \times 5 \times P \times c/m/mg}{10}$$

In the formula, P is the final body weight of the rat, the blood mass of which is considered to be equivalent to 5 percent of the body weight, irrespective of the state of nutrition (87); "c/m/mg" is the radioactivity per mg of serum.

(f) Cell height-h: This parameter refers to the height of follicular epithelial cells. These cells of an activated thyroid increase in height and appear to be columnar, whereas those of a resting or inactive gland appear to be flat and squamous (88).

(g) d/n ratio: This is the ratio of the inner diameter (d) of a follicle to the number (n) of epithelial cells surrounding the follicle (89).

RESULTS AND DISCUSSION

Four experiments were performed; the same four diets were used throughout (Table 1). The second experiment was identical to the first and was performed as a means of checking the results of the first. In three experiments (I, II and IV), thyroidal behavior in all animals was evaluated after hormonal synthesis was blocked with PTU. Only part of the rats of each group in experiment III were treated with PTU so that comparisons of thyroid function with and without treatment could be made. The fourth experiment was designed to study the effect of TSH on thyroid function in rats depleted of both protein and iodine.

Experiment I

Protein Depletion and Repletion

Fifty-two rats were randomly assigned to 4 groups of 13 animals each; the groups received diets as described under Materials and Methods. At the end of 26 days, 7 rats from each group were sacrificed and 6 were left to continue. At this time, the two groups on protein-free diets were given diets containing 20 percent casein, and the other two groups remained on their same regimes. The levels of dietary iodine during repletion were the same as during depletion.

Data concerning weight gains and losses and daily food intake of rats used throughout experiment I are summarized in Table 2. The average initial body weight of rats varied from 87 to 90 gr; the average weight of the depleted groups decreased to 46-47 gr, which represented a weight loss of 46 to 48 percent. The daily food intake was 11 gr in the groups receiving protein and 5-6 gr in the protein-depleted groups. In

the depletion part of the experiment, the duration was 26 days after which repletion was carried on for 44 days.

Thyroid Function in Rats Receiving Diets Supplemented With Iodate

The values for various parameters of thyroid function are presented in Table 3. It is evident that protein depletion resulted in reduction of gland size (7.9 vs. 2.9 mg). When the percent one hour uptake, as well as the other parameters (T/S, Tt/S and Tt/St) are considered, it is noticed that, when iodine was added, protein depletion resulted in a trend of decreased thyroid activity. However, the differences from the control values were not significant. The same pattern in parameters was found in animals after repletion (Table 3) except that the thyroid size of the depleted rats returned to normal after they received diets containing 20 percent casein. No significant difference was observed between average thyroid weights of normally fed animals (11.0 mg) and those of the repleted animals (10.1 mg). This trend in results agrees with the work of Aschkenasy et al. (68, 74, 76).

The T/S ratios, shown in Table 3 for iodine supplemented animals, are high compared to the "normal" value of 25 reported by Vanderlaan (17) and Halmi (81), who used diets containing excess iodine. In the present work, sufficient iodate was added to bring the calculated dietary level to 25 µg/kcal, which is considered optimal (90). As is shown in Table 3, this level prevented thyroid enlargement, but T/S was above normal. Therefore, it was concluded that the level of 25 µg/kcal was somewhat low, and in experiments III and IV, more iodate was added (final level, 41 µg/kcal). As will be seen in Tables 7 and 9, the latter supplementary level resulted in normal T/S values.

Thyroid Function in Rats Receiving a Low Iodine Diet

In iodine deficiency, cells of the thyroid enlarge and, as a result, the gland increases in weight. This increase was observed in the present study, but the gland weights of protein-fed, iodine-deficient animals were not significantly different from those of the controls (Table 3). Probably, the animals were not on the low iodine diet long enough to induce significant enlargement. Previous work in this laboratory showed that a period of seven weeks was needed to produce significant thyroid enlargement in rats fed this particular low iodine diet. A significant increase in thyroid weight was obtained when the animals were given the low iodine diet for 10 weeks. In protein-depleted rats, as in the animals which were fed protein, there was no significant difference between the average thyroid weights of the group receiving supplemental iodate and those of the unsupplemented group (2.9 vs. 3.5 mg).

The values for one hour uptake of radioactive iodine, expressed as percent of dose given, were higher in all iodine-depleted animals, irrespective of dietary protein level; also, the values for T/S, Tt/S and Tt/St followed the same pattern. These results are in agreement with those of other workers (17, 68, 74, 76, 84, 85).

Throughout the present investigation, there was a trend noted; the one hour uptake values were almost always higher in rats receiving dietary protein than in the protein-depleted animals. However, the differences were not significant.

As expected, the iodide concentrating ability in both groups of iodine-deprived rats were high in relation to those groups receiving supplemental iodine (Table 3). However, upon comparing the two iodine-deprived groups, it is seen that T/S, Tt/S and Tt/St were significantly

lower in the protein-depleted animals. The finding of lower T/S and Tt/St values is not in accord with the results of Aschkenasy et al. (68, 74, 76). These workers observed results opposite to those reported here, but perhaps this disagreement can be explained on the basis of the differences in experimental conditions. In the present work, female rats weighing less than 100 gr were used, and were severely depleted of as much as 48 percent of their initial body weight. Aschkenasy, in contrast, used adult male rats and, in his work, depletion was never more than 30 percent. Because of the shrinkage of the thyroid due to severe protein depletion in the present experiment, it may be deduced that the number of iodide acceptor sites was greatly reduced. Thus, in animals in which hormonal synthesis was blocked with PTU, the ability of the thyroids to trap iodide from the plasma was limited and low values were obtained. Furthermore, it has been reported (69) that protein depletion impairs kidney function in rats. Thus the injected I^{131} would tend to remain in the blood contributing to high serum counts and causing lower ratio values.

The low Tt/S values for protein-depleted animals agree with the work of Aschkenasy (74, 76). Tt/S is an indication of iodide acceptor sites in the thyroid and is directly related to the size of the gland (86). Therefore, it can be argued definitely that the Tt/S values were lower in protein-starved animals because of the reduced size of the gland.

If the values for T/S are compared with the "normal" value of 25 (90), it is seen that, in rats that were deprived of both protein and iodine, the value was 77.8 (Table 3), indicating a stimulation of the thyroid gland. However, this value is significantly lower than that of the low iodine control group which received protein (168.8). Hence, the stimulation of thyroidal activity due to protein deprivation reported by

Aschkenasy (74, 76), was not observed in this experiment. The lower values of T/S and Tt/St in Table 3 may give the impression that protein depletion had an effect similar to that of partial hypophysectomy; that is, it caused a depression of pituitary function. This possibility was investigated and will be discussed later.

As it was previously stated, thyroid function in protein-depleted animals did not undergo any significant change as long as the diets were supplemented with iodine (Table 3).

Repletion

The repletion experiment was performed to see if any effects induced by protein depletion could be reversed.

Two groups of rats were fed protein-free diets until they were depleted of 40-50 percent of their initial body weights. At this point, they were given a diet containing 20 percent casein. Iodine levels remained the same; that is, one group received supplemental iodine and the other, the low iodine diet. The two protein-fed control groups remained on their same regimes. The experiment was stopped when the depleted animals had reached an average weight of 170 to 190 gr.

In the groups on low dietary iodine, thyroid weights were significantly higher than in the control group which receivediodine. This increase, which was not noticed in the depletion experiment, was due to the fact that the animals were exposed to the iodine-deficient regime for a much longer period of time (10 weeks vs. 4 weeks).

The values for uptake, as well as for T/S, Tt/S and Tt/St, were significantly higher than the control values in both iodine deficient groups (Table 3). In the repleted animals, the values were

lower than in the animals which received protein (but low iodine) throughout; however these differences between the two low-iodine groups were not significant (Table 3).

All these findings indicate that protein depletion did not seriously affect the thyroid gland; furthermore it appeared that no permanent impairment of pituitary function was induced by protein deprivation.

Since this experiment was the first of its kind performed in this laboratory and since the results obtained were different in some respects from those reported by others, it seemed of value to repeat the work.

Experiment II

Protein Depletion and Repletion

(Repeated)

The data in Table 4 show that the initial body weights of rats used in the second experiment varied from 62 to 94 gr. The duration of the depletion part of the experiment was 24 days; repletion was carried out for 60 days after depletion. As in experiment I, the animals were severely depleted and lost an average of 37 to 41 percent of their initial body weights. The daily food intake on the protein-free diets varied between 4 and 7 gr, compared to 11 and 13 gr for the groups receiving protein. During repletion, the daily food intake varied from 10 to 14 gr.

The data in Table 5 show the results of the thyroid function studies. In general, the results of the first experiment were confirmed by those of the second (compare Tables 3 and 5). One difference, however, was observed in the repleted animals. The prolonged iodine deficiency induced, in the protein-fed controls, significantly larger

thyroids than those of the iodine-deficient, repleted animals (18.52 mg vs. 13.48 mg). Because of this difference in thyroid size, it was not surprising to find a higher value for Tt/S in the control group than in the repleted group (35.88 vs. 24.18). In all other respects, the results were similar to those of the previous experiment, but were still in disagreement with Aschkenasy et al. (68, 74, 76).

In summary, it can be said that the results of experiments I and II indicate that protein depletion in the presence of iodine did not affect thyroid function. However, in the absence of supplementary iodine there was significant activation. Protein depletion, in the absence of iodine appeared to suppress thyroidal activity significantly. Also, it was shown that protein repletion of protein-depleted animals, restored thyroid function to normal.

Experiment III

Thyroid Function in the Absence of PTU Injection

Since the results with PTU-treated rats did not agree with those of other workers, it was felt of value to investigate the same parameters in the absence of PTU treatment. Therefore, experiment III was performed to compare thyroidal function of PTU-treated animals with that of animals without PTU treatment. Fifty-two rats were divided into 4 groups of 13 each and were fed the 4 dietary regimes used in experiments I and II. Five of the animals in each group served as controls, and at the end of the experiment, received PTU prior to I^{131} injection. The remaining 8 animals in each group received no PTU treatment.

The growth data of the animals are presented in Table 6. The

initial body weights varied from 64 to 73 gr and weight loss on protein-free diets varied between 33 and 36 percent. In groups receiving protein, the daily food intake was 7 to 12 gr, whereas depleted groups consumed an average of 4 to 6 gr per day.

Thyroid Function in Rats Treated With PTU

The results of experiment III are summarized in Table 7; in all respects, the rats treated with PTU responded similarly to those in the two previous experiments. In the low iodine groups, the values of parameter studied were increased and those for animals on protein-free and low iodine diets were significantly lower than in rats which received protein.

As discussed previously, the high T/S values (Tables 3 and 5) for rats receiving supplemental iodine were indicative of the inadequacy of the level of iodine in the diets of experiments I and II. Therefore, for the present experiment, the level of iodine supplementation was increased to 41 µg/kcal. As seen in Table 7, this increase in iodine level, brought the T/S values closer to the "normal" value of 25 (90).

Thyroid Function in Rats not Treated With PTU

When uptake and iodide concentrating ability were measured in the absence of PTU, all values were much higher (Table 7) than those presented in the previous tables. These higher values were expected because organification of iodide was not blocked; consequently, the values represent the sum of inorganic trapped iodide plus organically bound iodine (76).

As observed before (Tables 3 and 5), protein depletion, in the

presence of iodine, had no significant effect on thyroid function. However, in the low-iodine groups, it is apparent that iodine deprivation induced a greater activity of the gland. In addition, this activation appeared to be stimulated further by the absence of protein from the diet (Table 7). The percent one hour uptake in the protein-free group was increased but not significantly over that of the group receiving protein. However, the values for T/S and Tt/St in the protein-free group were significantly higher than those observed in the group receiving casein. These results were in direct contrast to those obtained in PTU-treated animals and, therefore, deserve some attention.

The low values for T/S and Tt/St (1735 vs. 2644; and 4.45 vs. 6.81) in the protein-fed, iodine deficient animals indicate that, in those animals, iodide was taken up and the newly-synthesized hormones discharged freely into the blood stream. In the protein-depleted group, thyroidal iodide uptake appeared to be very active and the high values of T/S and Tt/St in those animals may have been due to one of the following possibilities: a) that protein deprivation resulted in impaired synthesis of thyroglobulin; hence the amount of thyroglobulin in the follicles necessary to provide tyrosyl residues for normal hormonal synthesis was reduced; or b) that the glands were so depleted of protein that the rate of synthesis was seriously affected. Since the trapping mechanism would continue to be operative; radioactivity would accumulate in the gland, causing the high values.

Under conditions of no PTU treatment, Aschkenasy et al. (76) reported results opposite to those reported here; that is, decreased values for T/S and Tt/St in protein-depleted animals. Again, the difference in experimental conditions may help to explain this

disagreement.

The results obtained in experiments I and II were confirmed in this experiment in rats treated with PTU, but were not consistent with those obtained in the absence of PTU. In this experiment, the additive effect of protein depletion and iodine deficiency reported by Aschkenasy et al. (68, 74, 76) was observed only in rats not treated with PTU.

Experiment IV

Thyroid Function After

TSH Injections

The results of experiment III seemed to present a paradox. The data reported in Tables 3, 5 and 7, concerning PTU-treated rats, give the impression that protein depletion may have induced depressed pituitary function. However, in the absence of PTU treatment, the thyroids of protein-depleted animals were even more active than those of rats which were only iodine deficient (Table 7). These latter results indicated that protein depletion had not suppressed pituitary activity, but had, in fact, further stimulated it. Because of these contrasting data, it was decided to attempt to resolve the question of whether or not protein depletion in the presence of iodine deficiency, suppressed pituitary activity.

The initial body weights of rats used in experiment IV varied between 112 and 113 gr (Table 8); the average percent loss in body weight of depleted animals varied from 36 to 41. In the groups receiving protein, the average daily food intake was 13 gr and in the protein-depleted groups, 8 gr. The rats were kept on the diets for 30 days.

To investigate the possibility that protein and iodine

deprivation may suppress pituitary function, 10 animals on the protein-free, low iodine diets received 14 USP units of TSH (2 units/day) during the last 7 days of depletion. At the end of the experimental period, the animals were treated with PTU as before and thyroid function was studied.

It was previously stated that any damage to pituitary gland would result in depressed thyroid activity, and that administration of TSH should restore the normal function of the gland. The low values for T/S, obtained in experiments I, II and III, in the groups of rats receiving neither protein nor supplemental iodine, suggested that pituitary function in these animals may have been depressed. If this were true, then the administration of TSH should have resulted in increased values for all parameters in the treated animals. However, the data in Table 9 show that TSH did not induce any change. There was no increase in the uptake or in the T/S and Tt/S values; the T/S was 89.3 in the group receiving protein and 62.0 in the depleted group (Table 9). Similarly, TSH injections did not cause an increase in values for percent one hour uptake or Tt/St.

It would appear from these results that protein depletion did not result in damage to the pituitary gland. These results are in agreement with those of several workers (77, 79). Warter et al. (79) found that, in protein deficient rats there was a slight reduction in TSH content of pituitary until the 12th day of depletion, but after that there was no change. In fact, when expressed in terms of mg pituitary per 100 gr body weight, the pituitary TSH content in depleted groups was higher. Srebnik et al. (91) confirmed these findings. In the present experiment it would appear that the double depletion of

iodine and protein stimulated the pituitary to secrete enough TSH to cause complete saturation of the iodide acceptor sites in the thyroid; as a result of this maximum stimulation, exogenous TSH could not induce any change (77).

Radiochromatographic Studies of Thyroid

No abnormal iodinated compounds were revealed chromatographically, in the hydrolysates of thyroids from rats deprived of protein and iodine. The percent 24 hour uptake of iodine per mg of thyroidal tissue was somewhat higher in the protein and iodine-deprived group than in the iodine-deficient group which received protein (6.5 vs. 5.7 percent).

As an evaluation of hormonal synthesis, the $(MIT + DIT)/(T_3 + T_4)$ ratio was calculated (76); the value for each group is shown in Table 10. In the groups which received similar amounts of iodine, the ratio was more or less the same, indicating no change in hormonal synthesis due to protein deprivation. The same trend was observed in the iodine-deprived groups in which no substantial change was induced by protein depletion. However, the ratios of the iodine-deprived groups were slightly higher than those of rats which received iodine. This latter observation indicates that the levels of MIT and DIT slightly exceeded those in the thyroids of iodine-supplemented animals.

Histological Evaluation of Thyroid Function

For histology, glands were chosen from representative animals on the different dietary treatments. Four peripheral and four central follicles from each thyroid section were randomly selected for

observation. The cellular heights were measured using the calibrated eyepiece of a photomicroscope[12]. The values for the histological parameters studied are presented in Table 11.

It can be seen that, in animals which received sufficient iodine, protein deprivation resulted in a slight reduction of cellular heights. Also, the d/n ratios of protein-depleted rats were reduced; epithelial cells were more or less flattened as the result of protein depletion (Figure 3). None of these changes were significant. In the control groups which received protein and iodine, the follicles were compact and surrounded with cuboidal epithelial cells (Figure 1).

In contrast, iodine deprivation resulted in marked histological alterations. The cellular height of epithelium increased, although d/n was reduced (Table 11); this reduction in d/n was not further magnified by iodine deprivation. The follicles of glands from the iodine-deprived groups were markedly enlarged (Figure 2). The glands of the protein and iodine deprived animals (Figure 4) did not undergo much further change from those observed in protein depletion alone (Figure 3), except that the follicles were almost devoid of colloid, and there was intracellular epithelial infiltration. The histological findings were similar to those reported by Aschkenasy et al. (76).

12. Carl Zeiss, Co., Oberkochen/Wurtt., West Germany.

SUGGESTIONS FOR FURTHER RESEARCH

Since the results of the present investigation did not agree with those reported by others, it would seem of value to carry the work further. It has been explained that the disagreement may be due to differences in experimental conditions. Therefore, it would be of interest to use the same diets and to stop depletion experiments at weekly intervals until severe depletion is accomplished. This type of experiment would demonstrate whether or not, during depletion, thyroidal activity is increased and then suppressed or whether the suppressed activity exists throughout. The results would also indicate the critical stage of depletion at which the alleged suppression would take place.

More refined studies on pituitary activity throughout depletion would be of value, also.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the effects of protein depletion on thyroid function in rats fed diets either deficient or adequate in iodine. Four experiments were performed; in each experiment, 4 groups of rats received the following diets:

1. 20% casein + iodine
2. 20% casein, low iodine
3. No protein + iodine
4. No protein, low iodine

In experiments I and II, thyroid stimulation due to iodine deficiency was depressed by protein depletion; protein deprivation had no effect on thyroidal function in the presence of adequate dietary iodine. Repletion of protein-depleted rats with a diet containing 20 percent casein restored thyroid function to normal.

In experiment III, thyroid function was evaluated in rats with and without PTU treatment. The results obtained without PTU treatment showed that protein depletion further stimulated thyroids which were already activated due to iodine deficiency.

Experiment IV was designed to study the effect of TSH injection on thyroid function of protein and iodine deficient rats. No response to TSH was observed.

Radiochromatographic analysis of thyroid hydrolysates did not reveal any striking differences in hormone synthesis among the dietary groups.

Histological examination revealed that follicle size and cellular heights were significantly increased in thyroids of iodine depleted rats receiving protein. Protein depletion resulted in the flattening of epithelial cells and in the reduction of intrafollicular spaces.

From the results of this study it was concluded that protein depletion appeared to depress thyroid function in iodine-deficient animals. However, this suppression did not appear to be due to depressed pituitary activity.

TABLES AND FIGURES

Table 2

Body weights, losses in weight, and food intake of rats in experiment I.

Diet	Number of Rats per Group	Duration (days)	Average Initial Body Weight (gr)	Average Final Body Weight (gr)	% Loss in Body Weight	Average Daily Food Intake (gr)
			Depletion			
20% protein + iodine	7	26	90	167	-	11
20% protein, low iodine	7		90	158	-	11
No protein + iodine	7		87	47	47	5
No protein, low iodine	7		88	46	48	6
			Repletion			
20% protein + iodine	6	70	88	216	-	11
20% protein, low iodine	6		92	216	-	11
20% protein + iodine[1]	6		89	187	-	9
20% protein, low iodine[1]	6		92	189	-	9

1. Repleted.

Table 3

Effect of protein depletion followed by repletion on I^{131} uptake and iodide concentrating ability of thyroids of rats fed high and low levels of iodine (experiment I)[1].

Diet	Thyroid Weight (mg)	% 1 Hour Uptake	T/S	Tt/S	Tt/St
		Depletion[2]			
20% protein + iodine	7.9 ± 0.88[3]	0.51 ± 0.05	68.9 ± 20.1	5.36 ± 3.5	0.11 ± 0.05
20% protein, low iodine	8.2 ± 0.84	1.03 ± 0.24	168.8 ± 42.1	13.64 ± 5.5	0.29 ± 0.10
No protein + iodine	2.9 ± 0.32	0.32 ± 0.04	42.9 ± 18.1	1.19 ± 0.7	0.07 ± 0.04
No protein, low iodine	3.5 ± 0.56	0.80 ± 0.23	77.8 ± 22.4	2.35 ± 0.9	0.22 ± 0.05
L.S.D. @ 5%	1.1	0.36	54.1	6.93	0.05
		Repletion[4]			
20% protein + iodine	11.0 ± 1.61	0.20 ± 0.09	19.7 ± 4.2	2.09 ± 0.43	0.16 ± 0.08
20% protein, low iodine	13.6 ± 2.57	0.48 ± 0.09	42.2 ± 8.6	5.58 ± 1.13	0.09 ± 0.01
20% protein + iodine[5]	10.1 ± 1.09	0.08 ± 0.06	14.3 ± 5.7	1.43 ± 0.37	0.03 ± 0.01
20% protein, low iodine[5]	14.0 ± 0.92	0.31 ± 0.10	37.6 ± 7.5	4.20 ± 2.10	0.08 ± 0.02
L.S.D. @ 5%	2.3	0.12	12.5	1.15	0.14

1. Rats were treated with PTU prior to I^{131} injection.
2. Values represent average of 7 rats.
3. Standard deviation.
4. Values represent average of 6 rats.
5. Repleted.

Table 4

Body weights, losses in weight and food intake of rats in experiment II.

Diet	Number of Rats per Group	Duration (days)	Average Initial Body Weight (gr)	Average Final Body Weight (gr)	% Loss in Body Weight	Average Daily Food Intake (gr)
			Depletion			
20% protein + iodine	8	24	62	156	-	13
20% protein, low iodine	8		63	147	-	11
No protein + iodine	8		63	37	41	7
No protein, low iodine	8		62	39	37	4
			Repletion			
20% protein + iodine	5	84	95	222	-	13
20% protein, low iodine	5		94	220	-	14
20% protein + iodine[1]	5		96	180	-	10
20% protein, low iodine[1]	5		94	172	-	10

1. Repleted.

Table 5

Effect of protein depletion followed by repletion on I^{131} uptake and iodide concentrating ability of thyroids of rats fed high and low levels of iodine (experiment II)[1].

Diet	Thyroid Weight (mg)	% 1 Hour Uptake	T/S	Tt/S	Tt/St
		Depletion[2]			
20% protein + iodine	9.8 ± 1.40[3]	0.46 ± 0.26	60.1 ± 21.7	5.89 ± 2.97	0.14 ± 0.06
20% protein, low iodine	10.6 ± 3.36	1.17 ± 0.72	179.9 ± 41.8	22.22 ±10.40	0.46 ± 0.17
No protein + iodine	3.0 ± 0.77	0.36 ± 0.14	27.3 ± 10.9	0.81 ± 0.37	0.05 ± 0.03
No protein, low iodine	4.0 ± 1.18	1.03 ± 0.63	75.8 ± 24.3	1.34 ± 0.88	0.20 ± 0.10
L.S.D. @ 5%	1.7	0.49	39.1	5.78	0.09
		Repletion[4]			
20% protein + iodine	9.9 ± 1.63	0.24 ± 0.10	49.0 ± 13.8	3.62 ± 0.68	0.04 ± 0.02
20% protein, low iodine	18.5 ± 3.75	2.46 ± 0.42	205.4 ± 74.4	35.88 ± 6.80	0.57 ± 0.14
20% protein + iodine[5]	9.0 ± 1.88	0.64 ± 0.24	65.4 ± 29.4	12.10 ± 4.30	0.18 ± 0.10
20% protein, low iodine[5]	13.5 ± 1.33	2.04 ± 0.77	156.4 ± 36.5	24.18 ± 7.30	0.49 ± 0.14
L.S.D. @ 5%	3.8	0.48	72.9	9.68	0.15

1. Rats were treated with PTU prior to I^{131} injection.
2. Values represent average of 8 rats.
3. Standard deviation.
4. Values represent average of 5 rats.
5. Repleted.

Table 6

Body weights, losses in weight and food intake of rats in experiment III.

Diet	Number of Rats per Group	Duration (days)	Average Initial Body Weight (gr)	Average Final Body Weight (gr)	% Loss in Body Weight	Average Daily Food Intake (gr)
			With PTU treatment			
20% protein + iodine	5	16	73	119	-	8
20% protein, low iodine	5		66	104	-	7
No protein + iodine	5		64	43	33	4
No protein, low iodine	5		64	42	34	6
			Without PTU treatment			
20% protein + iodine	8	24	72	130	-	10
20% protein, low iodine	8		73	155	-	12
No protein + iodine	8		73	47	36	6
No protein, low iodine	8		73	48	34	6

Table 7

Effect of PTU on I^{131} uptake and iodide concentrating ability of thyroids of protein depleted rats fed high and low levels of iodine (experiment III).

Diet	Thyroid Weight (mg)	% 1 Hour Uptake	T/S	Tt/S	Tt/St
		With PTU treatment[1]			
20% protein + iodine	7.3 ± 1.26[2]	0.20 ± 0.10	35.7 ± 12.7	2.64 ± 1.00	0.05 ± 0.04
20% protein, low iodine	7.2 ± 1.30	0.73 ± 0.29	93.8 ± 13.8	6.86 ± 2.40	0.19 ± 0.08
No protein + iodine	3.8 ± 0.69	0.26 ± 0.07	29.5 ± 9.7	1.14 ± 0.45	0.07 ± 0.03
No protein, low iodine	3.8 ± 0.71	0.70 ± 0.10	83.2 ± 22.7	3.13 ± 0.99	0.20 ± 0.08
L.S.D. @ 5%	1.6	0.08	23.6	2.18	0.08
		Without PTU treatment[3]			
20% protein + iodine	8.4 ± 1.36	2.25 ± 1.14	219 ± 145	18.1 ± 8.50	0.49 ± 0.34
20% protein, low iodine	10.6 ± 1.91	13.00 ± 6.40	1735 ± 834	190.0 ±68.70	4.45 ± 2.80
No protein + iodine	3.1 ± 0.71	3.09 ± 1.60	338 ± 139	10.6 ± 4.70	0.71 ± 0.29
No protein, low iodine	3.8 ± 0.36	16.33 ± 4.20	2644 ± 901	98.5 ±41.30	6.81 ± 3.30
L.S.D. @ 5%	1.4	3.97	863	69.6	2.17

1. Values represent average of 5 rats.
2. Standard deviation.
3. Values represent average of 8 rats.

Table 8

Body weights, losses in weight and food intake of rats in experiment IV.

Diet	Number of Rats per Group	Duration (days)	Average Initial Body Weight (gr)	Average Final Body Weight (gr)	% Loss in Body Weight	Average Daily Food Intake (gr)
20% protein + iodine	10	30	113	187	-	13
20% protein, low iodine	10		112	180	-	13
No protein + iodine	10		112	72	36	8
No protein, low iodine	10		113	67	41	8

Table 9

Effect of protein depletion on I^{131} uptake and iodide concentrating ability of thyroids of rats fed high and low levels of iodine (experiment IV)[1, 2].

Diet	Thyroid Weight (mg)	% 1 Hour Uptake	T/S	Tt/S	Tt/St
20% protein + iodine	8.5 ± 1.19[3]	0.32 ± 0.05	34.4 ± 12.4	2.96 ± 1.0	0.05 ± 0.03
20% protein, low iodine	12.8 ± 2.26	1.23 ± 0.30	89.3 ± 28.3	11.61 ± 4.2	0.23 ± 0.07
No protein + iodine	4.4 ± 1.47	0.23 ± 0.03	31.6 ± 10.8	1.17 ± 0.8	0.04 ± 0.02
No protein, low iodine[3, 4]	6.3 ± 1.38	0.74 ± 0.23	62.0 ± 24.6	4.16 ± 2.1	0.17 ± 0.06
L.S.D. @ 5%	1.4	0.31	19.7	0.63	0.05

1. Rats were treated with PTU prior to I^{131} injection.
2. Values represent average of 10 rats.
3. Standard deviation.
4. Each rat received 14 USP units of TSH.

Table 10. Thyroidal hormone synthesis as shown by radiochromatographic analysis of thyroid hydrolysates of rats maintained on high protein and protein-free diets with and without iodine supplementation.

Diet	$\frac{MIT + DIT}{T_3 + T_4}$
20% protein + iodine	5.21
20% protein, low iodine	7.66
No protein + iodine	5.45
No protein, low iodine	7.52

Table 11. Evaluation of thyroidal activity in terms of histological parameters in rats maintained on high protein and protein-free diets, with and without iodine supplementation.

Diet	Cell Height (u)		d/n	
	Central	Peripheral	Central	Peripheral
20% protein + iodine	6.2 ± 1.7[1]	4.8 ± 1.2	2.8 ±0.21	3.6 ±0.30
20% protein, low iodine	10.7 ± 2.8	10.2 ± 2.6	2.5 ±0.18	2.8 ±0.24
No protein + iodine	5.5 ± 1.4	3.7 ± 1.0	2.4 ±0.20	2.8 ±0.18
No protein, low iodine	8.8 ± 2.3	8.3 ± 1.8	2.4 ±0.28	2.6 ±0.20

1. Standard deviation.

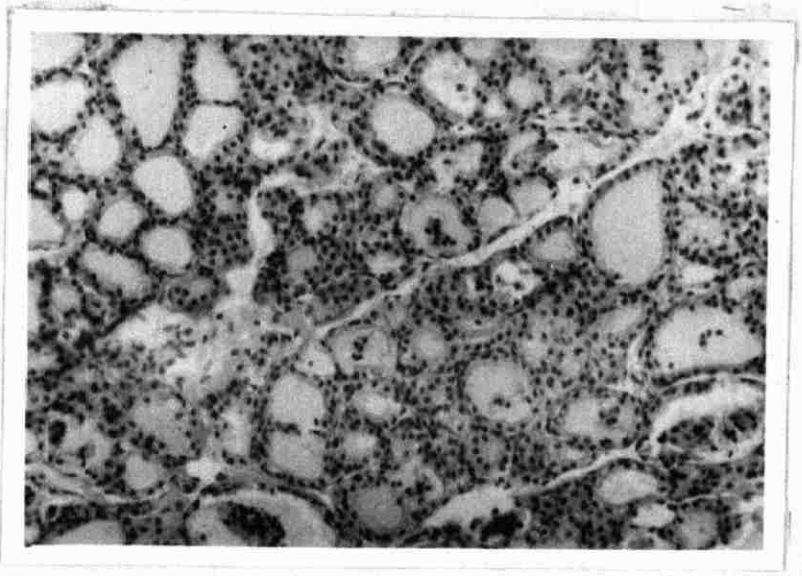

Figure 1

Photomicrograph of a representative section of the thyroid gland of a rat fed a diet containing 20 percent casein and supplemented with iodate (magnification 480x).

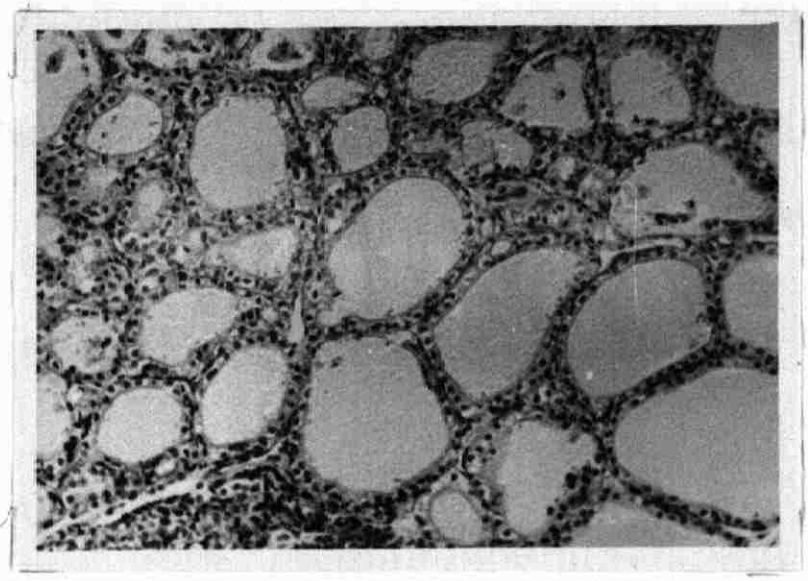

Figure 2

Photomicrograph of a representative section of the thyroid gland of a rat fed a diet containing 20 percent casein without iodate supplementation (magnification 430x).

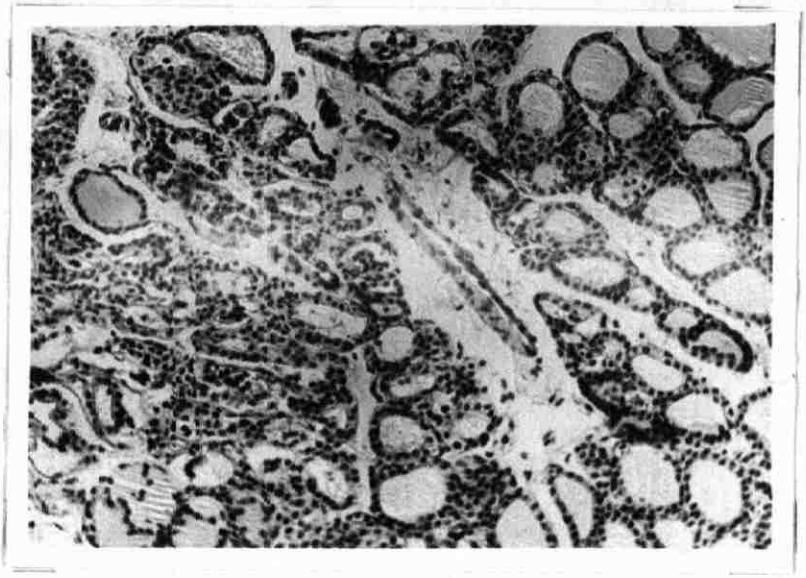

Figure 3

Photomicrograph of a representative section of the thyroid gland of a rat fed a protein-free diet supplemented with iodate (magnification 480x).

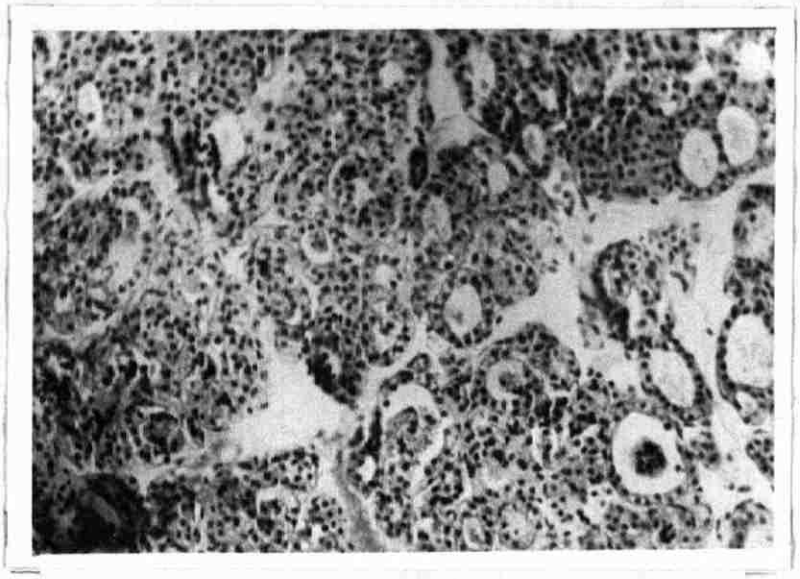

Figure 4

Photomicrograph of a representative section of the thyroid gland of a TSH-treated rat fed a protein-free, low-iodine diet (magnification 480x).

LITERATURE CITED

1. Davidson, S. and R. Passmore. "Human Nutrition and Dietetics", London, E. and S. Livingstone Ltd., (1963).

2. Vannotti, A. "Etude de la Fonction Thyroidienne Avec l'Iode Radioactif", Basel, Benno Schwabe and Co., (1957).

3. Marine, D. and O.P. Kimball. Prevention of simple goiter in man. Arch. Intern. Med., 25:662 (1920).

4. Kendall, E.C. and A.E. Osterberg. The chemical identification of thyroxine.

5. Harington, C.R. and C. Barger. Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J., 21:169 (1927).

6. Gross, J. and R. Pitt-Rivers. The identification of 3, 5, 3'- L - triiodothyronine in human plasma. Lancet, 262:439 (1952).

7. Ciaudo, D., T. Maroun, F. Zebouni and E. Geagea. Le goitre au Liban. La Semaine de Hopitaux de Paris, no 78:1 (1948).

8. Cowan, J.W., S.S. Majjar, R.I. Tannous, Z.I. Sabry and F.S. Simaan. Some observations on goiter in Lebanon II. Amer. J. Clin. Nutr. (in Press).

9. Berson, S.A. Pathways of iodine metabolism. Amer. J. Medicine, 20:653 (1956).

10. Cohn, B.N.E. Absorption of compound solution of iodine from the gastro-intestinal tract with special reference to the absorption of free iodine. Arch. Int. Med., 49:950 (1932).

11. Childs, D.S. Jr., F.R. Keating Jr., J.E. Rall, M.M.D. Williams and M.H. Power. The effect of varying quantities of inorganic iodide (carrier) on the urinary excretion and thyroidal accumulation of radioactive iodine in exophthalmic goiter. J. Clin. Invest., 29:726 (1950).

12. Stanbury, J.B., G.L. Brownwell, D.S. Riggs, H. Perinetti, J. Itoiz and E.B. Del Costillo. "Endemic Goiter. The Adaptation of Man to Iodine Deficiency". Cambridge, Harvard University Press (1954).

13. Freinkel, N. and S.H. Ingbar. Effect of metabolic inhibitors upon iodide transport in sheep thyroid slices. J. Clin. Endocrinol., 15:598 (1955).

14. Slingerland, D.W. The influence of various factors on the uptake of iodine by the thyroid. J. Clin. Endocrinol., 15:131 (1955).

15. Wolff, J. Thyroidal iodide transport. I. Cardiac glycosides and the role of potassium. Biochem. Biophys. Acta, 38:316 (1960).

16. Halmi, N.S. Thyroidal iodide transport. Vitam. and Hormones, 19:133 (1961).

17. VanderLaan, J.E. and W.P. VanderLaan. The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate. Endocrinology, 40:403 (1947).

18. Taurog, A., I.L. Chaikoff and D.D. Feller. The mechanism of iodine concentration by the thyroid gland: its non organic iodine-binding capacity in the normal and propylthiouracil treated rats. J. Biol. Chem., 171:189 (1947).

19. Schachner, H., A.L. Franklin and I.L. Chaikoff. On the in vitro accumulation of inorganic iodide by surviving thyroid tissue with radioactive iodine as indicator. Endocrinology, 34:159 (1944).

20. Freinkel, N. and S.H. Ingbar. The relationship between metabolic activity and iodide concentrating capacity of surviving thyroid slices. J. Clin. Endocrinol., 15:442 (1955).

21. Wolff, J., J. Robbins and J.E. Rall. Iodide trapping without organification in a transplantable rat thyroid tumor. Endocrinology, 64:1 (1959).

22. Droskin, S. The thyroxine-like action of elemental iodine in the rat and chick. Endocrinology, 40:334 (1947).

23. Pitt-Rivers, R. and J.R. Tata. "The Thyroid Hormones", London, Pergamon Press Ltd., (1959).

24. Harington, C.R. Chemistry of thyroxine. II. Constitution and synthesis of desiodo-thyroxine. Biochem. J., 20:300 (1956).

25. Roche, J. and R. Michel. Nature, biosynthesis and metabolism of thyroid hormones.
Physiol. Rev., 35:583 (1955).

26. Laver, W.G. and V.M. Trikojus. Purification of thyroid protease by acetone fractionation.
Biochem. Biophys. Acta, 16:592 (1955).

27. Weiss, B. Peptidase and proteinase activity of beef thyroid tissue.
J. Biol. Chem., 205:193 (1953).

28. Alpers, J.B., J. Robbins and J.E. Rall. The hydrolysis of rat thyroglobulin by thyroidal enzymes.
Endocrinology, 56:110 (1955).

29. Roche, J., R. Michel, O. Michel and S. Lissitzky. Sur la deshalogenation enzymatique des iodotyrosines par le corps thyroide et sur son role physiologique.
Biochem. Biophys. Acta, 9:161 (1952).

30. Roche, J., R. Michel, A. Gorbman and S. Lissitzky. Sur la deshalogenation enzymatique des iodotyrosines par le corps thyroide et sur son role physiologique.
Biochem. Biophys. Acta, 12:570 (1953).

31. Benua, R.S., B.M. Dobyns and A. Ninmer. Triiodothyronine in the serum of patients treated with radioactive iodine.
J. Clin. Endocrin., 15:1367 (1955).

32. Taurog, A. and I.L. Chaikoff. The nature of circulating thyroid hormone.
J. Biol. Chem., 171:439 (1948).

33. Leblond, C.P. and J. Gross. The mechanism of the secretion of thyroid hormone.
J. Clin. Endocrin., 9:149 (1949).

34. Ingbar, S.H. and N. Freinkel. Regulation of the peripheral metabolism of the thyroid hormones.
Recent Prog. Hormone Res., 16:353 (1960).

35. Robbins, J. and J.E. Rall. The interaction of thyroid hormones and protein in biological fluids.
Recent Prog. Hormone Res., 13:161 (1957).

36. White, A., P. Handler, E.L. Smith and D. Stetten. "Principles of Biochemistry", 2nd ed., New York, McGraw-Hill Book Company (1959).

37. Wolff, J. and E.C. Wolff. The effect of thyroxine on isolated dehydrogenases.
Biochem. Biophys. Acta, 26:387 (1957).

38. Emmelot, P. and C.J. Bos. Thyroxine-mediated release of diphosphopyridine nucleotide from mitochondrial dehydrogenases. Exper. Cell Res., 14:132 (1958).

39. Lardy, H.A. and G.F. Maley. Metabolic effects of thyroid hormones in vitro. Recent Prog. Hormone Res., 10:129 (1954).

40. Stanbury, J.B., J.B. Wyngaarden and D.S. Frederickson, ed., "The Metabolic Basis of Inherited Disease", New York, McGraw-Hill Book Co. Inc., (1960).

41. Taurog, A., W. Tong and I.L. Chaikoff. Thyroid I^{131} metabolism in the absence of the pituitary in the untreated hypophysectomized rat. Endocrinology, 62:646 (1958).

42. Umbarger, H.E and B. Brown. Isoleucine and valine metabolism in Escherichi coli. J. Biol. Chem. 233:415 (1958).

43. Morton, M.E., I. Perlman, E. Anderson and I.L. Chaikoff. Radioactive iodine as an indicator of the metabolism of iodine. IV. The effects of hypophysectomy on the distribution of labelled thyroxine and diiodotyrosine in the thyroid gland and in plasma. Endocrinology, 30:495 (1942).

44. Roche, J., G.H. Deltour, R. Michel and E. Velez. Contribution a l'etude du role de l'hypophyse sur la biosynthese de l'hormone thyroidienne. C. R. Soc. Biol., 147:270 (1953).

45. Taurog, A., W. Tong and I.L. Chaikoff. Effects of hypophysectomy on organic iodine formation in rat thyroids. Ciba Found. Coll. on Endocrinol., 10:59 (1957).

46. Halmi, N.S., B.N. Spirtos and R.G. Stuelke. Iodide and thyroidal morphologic response to exogenous thyrotrophin in hypophysectomized rats. Endocrinology, 57:502 (1955).

47. Pitt-Rivers, R. Mode of action of antithyroid compounds. Physiological Revs., 30:194 (1950).

48. Astwood, E.B. The chemical nature of compounds which inhibit the function of the thyroid gland. J. Pharmacol., 78:79 (1943).

49. _____, J. Sullivan, A. Bissell and R. Tyslowitz. Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrinology, 32:210 (1943).

50. A.L. Franklin, I.L. Chaikoff and S.R. Lerner. The influence of goitrogenic substances on the conversion in vitro of inorganic iodide to thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator. J. Biol. Chem., 153:151 (1944).

51. Franklin, A.L., S.R. Lerner and I.L. Chaikoff. The effect of thiouracil on the formation of thyroxine and diiodotyrosine by the thyroid gland of the rat with radioactive iodine as indicator. Endocrinology, 34:265 (1944).

52. McGinty, D.A.and E.A. Sharp. Effect of iodine intake on thyroid iodine distribution and thyroid weight of rats treated with thiouracil and other goitrogens. J. Clin. Endocrinol., 6:473 (1946).

53. Schachner, H., A.L. Franklin and I.L. Chaikoff. The effect of cytochrome oxidase inhibitors on the formation in vitro of thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator. J. Biol. Chem., 151:191 (1943).

54. Marine, D., E.J. Baumann and A. Cipra. Studies on simple goiter produced by cabbage and other vegetables. Proc. Soc. Exp. Biol., N.Y., 26:822 (1929).

55. _____, _____, _____ and A.W. Spence. Further studies on eticlogy of goiter with particular reference to the action of cyanides. Proc. Soc. Exp. Biol., N.Y., 29:772 (1931).

56. Richter, C.P. and K.H. Clisby. Toxic effects of the bitter-tasting phenylthiocarbamide. Arch. Path., 33:46 (1942).

57. Astwood, E.B., M.A. Greer and M.G. Ettlinger. 1, 5-Vinyl - 2-thiooxazolidone, an antithyroid compound from yellow turnip and from Brassica seeds. J. Biol. Chem., 181:121 (1949).

58. Mulinos, M.G. and L. Pomerantz. Pseudo-hypophysectomy. A condition resembling hypophysectomy produced by malnutrition. J. Nutr., 19:493 (1940).

59. _____, _____. Pituitary replacement therapy in pseudo-hypophysectomy. Effects of pituitary in plants upon organ weights of starved and underfed rats. Endocrinology, 29:558 (1941).

60. Stephens, D.J. The effect of thyrotropic principle of the anterior pituitary on the thyroid of the undernourished guinea pig. Endocrinology, 26:485 (1940).

61. D'Angelo, S.A. The effect of acute starvation on the thyrotropic hormone level in the blood of the rat and mouse. Endocrinology, 48:341 (1951).

62. Williams, R., H. Jaffe and C. Kemps. Effect of severe stress upon thyroid function. Am. J. Physiol., 159:291 (1949).

63. Van Middlesworth, L. and H.M. Berry. Iodide metabolism during anoxia, nephrectomy, trauma, avitaminoses and starvation in the rat. Am. J. Physio., 167:576 (1951).

64. Catz, B., I. el Rawi and E. Geiger. Incfeased I^{131} collection by the thyroid of the rat in acute starvation. Am. J. Physiol., 172:291 (1953).

65. Storaasli, J.P., S. Rosenberg and H.L. Friedell. Effect of food and water on thyroid uptake of radiciodine in rats. Proc. Soc. Exper. Biol. Med., 83:748 (1953).

66. Stenberg, A.I. and A.I. Kusevickij. Effect of predominantly carbohydrate diet in iodine deficiency on the development of experimental goiter, with some functional stresses (abstract). Nutr. Abstracts and Revs., 34:730 (1964).

67. Aschkenasy, A., J.P. Benhamou and G.J. Rolland. Effets de la methionine et de la cystine sur la thyroide et sur l'activite thyreotrope des hypophyses de rats partiellement carences en proteines. C.R. Soc. Biol., 146:44 (1952).

68. _____, M.-T. Guerin and R.-A. Guerin. Quelques donnees sur le fonctionnement thyroidien explore a l'aide de l'iode radio-actif chez des rats carences en proteines. C.R. Soc. Biol., 153:1928 (1959).

69. _____, and _____. La clearance renale d'I^{131} chez des rats carences en proteines. C. R. Soc. Biol., 154:561 (1960).

70. Gilbert, J.L. and G. Lange. Renal clearance of I^{131} in hypothyroid and euthyroid rats. Endocrinology, 57:181 (1955).

71. Nataf, B., M. Sfez, A. Aschkenasy and J. Roche. Etude chromatographique du metabolisme d'I^{131} chez des rats carences en proteines. C. R. Soc. Biol., 153:1932 (1959).

72. _____, _____, _____. Nouvelle etude chromatographique du fonctionnement thyroidien dans la carence experimentale en proteines. C. R. Soc. Biol., 155:1232 (1961).

73. Piette, C. and A. Aschkenasy. Retentissement de la carence alimentaire en proteines sur l'iode thyroidien et l'iode protidique plasmatique chez le rat.
C. R. Soc. Biol., 155:982 (1961).

74. Aschkenasy, A., B. Nataf and M. Sfez. Le pouvoir de concentration des iodures par la glande thyroide du fonction de la teneur du regime en proteines et en certains acides amines (acides amines phenoliques et soufres) chez le rat.
C. R. Soc. Biol., 155:986 (1961).

75. _____, _____. Effets exerces chez le rat par la privation exclusive d'acides amines soufres, de lysine ou de tryptophane sur le pouvoir de concentration des iodures par la thyroides en l'absence d'apport alimentaire d'iode.
C. R. Soc. Biol., 155:1914 (1961).

76. _____, _____, C. Piette and M. Sfez. Le fonctionnement thyroidien chez le rat carence en proteines.
Annales d'Endocrinol., 23:311 (1962).

77. Guerin, M.-T. and A. Ashkenasy. Action des injections de thyroxine et d'hormone thyreotrope sur le metabolisme de l'I^{131} chez des rats carences en proteines.
C. R. Soc. Biol., 154:1405 (1960).

78. Aschkenasy, A. Comparaison des effets metaboliques de l'hormone thyreotrope et de la thyroxine en regimes equilibres ou depourvus de proteines.
C. R. Soc. Biol., 155:455 (1961).

79. Warter, J., C. Aron and L. Asch. Activite thyroidienne et fonction thyreotrope hypophysaire au cours du jeune protidique chez le rat.
C. R. Soc. Biol., 152:843 (1958).

80. Snedecor, G.W. "Statistical Methods", 5th ed., Ames, The Iowa State University Press (1962).

81. Halmi, N.S., B.N. Spirtos, E.M. Bogdanove and H.J. Lipner. A study of various influences on the iodide concentrating mechanism of the rat thyroid.
Endocrinology, 52:19 (1953).

82. Corradino, R.A. and H.E. Parker. Magnesium and thyroid function in the rat.
J. Nutr., 77:455 (1962).

83. Axelrad, A.A., C.P. Leblond and H. Isler. Role of iodine deficiency in the production of goiter by the Remington diet.
Endocrinology, 56:387 (1955).

84. Halmi, N.S. Thyroidal iodide trapping as influenced by serum iodide levels and thyrotrophin. Endocrinology, 54:97 (1954).

85. Feldman, J.D. Lab. Invest. 7:103 (1957).

86. Halmi, N.S. Factors influencing the thyroidal iodide pump. Ciba Found. Coll. on Endocrinol., 10:79 (1957).

87. Fried, W., L.F. Pilzak, L.O. Jacobson and E. Goldwasser. Studies on erythropoiesis. III. Factors controlling erythropoietin production. Proc. Soc. Exper. Biol. Med., 94:237 (1957).

88. Maximow, A.A. and W. Bloom. "A Textbook of Histology", 7th ed., Philadelphia, W.B. Saunders Co., (1958).

89. Lever, J. and L. Vlijm. A comparison of the sensitivity of some methods for determining the activity of thyroid glands. Acta Endocrinol., 18:219 (1955).

90. Sharples, G.R., J. Pearsons and G.S. Prato. Production of goiter in rats with raw and with treated soybean flour. J. Nutr., 17:545 (1939).

91. Srebnik, H.H., E.S. Evans and L.L. Rosenberg. Thyroid function in female rats maintained on a protein-free diet. Endocrinology, 73:267 (1963).