SUMMARY

The function of the thyroid gland has been studied in the experimental nephrotic syndrome. Significant urinary losses of hormonal iodine were found and were dependent on the degree of proteinuria. An increased release of thyroidal hormone appeared to compensate for the urinary losses of hormone. Thyroid function was judged adequate since peripheral utilization of hormone and the rate of oxygen consumption were unaltered in the nephrotic state.

THYROID FUNCTION IN EXPERIMENTAL NEPHROSIS

by

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A thesis submitted to the Faculty
of Graduate Studies and Research
in partial fulfilment of the
requirements for the degree of
Master of Science.

Department of Investigative Medicine
McGill University
Montreal

July, 1960

Suggested title for bound copy:

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ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his director, Dr. Norman Kalant, for guidance and encouragement throughout the period of research and for helpful criticism during the writing of this thesis, and to Dr. J. S. L. Browne, Professor and Chairman of the Department of Investigative Medicine, who acquainted the author with the disciplines of medical investigation.

The author also thanks the research technicians of the Jewish General Hospital for their valuable assistance, the Department of Medicine at the hospital for financial support, and Miss Toba Gantovnick, Miss Ruth Hubner, and Miss Lillias MacIntyre for their work in typing this thesis.

INTRODUCTION

The question as to whether an alteration in function of the thyroid gland exists in human nephrosis was posed soon after it was recognized that nephrosis was a syndrome of renal disease. A relative degree of hypothyroidism was implicated at this time to explain certain similarities between the nephrotic state and hypothyroidism. Although this viewpoint gained limited support from the results obtained following thyroid administration to nephrotic subjects, the thyroid preparations used in these early studies have since been considered to be of questionable and variable potency.

Within the last decade thyroid function has been questioned again subsequent to the observation that the serum level of thyroid hormone was not infrequently reduced in nephrosis. The several studies of human nephrosis have been essentially in disagreement regarding the state of thyroid function in this disease. Nevertheless, it is apparent that basic differences of methods in study make it difficult to clearly define the functional impairment, if present, of the thyroid gland in nephrosis.

Within the same ten year period, an experimental renal disease in rats has been studied extensively, and found to resemble human nephrosis. Since the experimental animal offers a means of control of variables not possible in the human situation, it was deemed advisable to study thyroid function in experimental nephrosis.

This thesis is concerned with a study of the alteration of thyroid function in experimental nephrosis with an attempt to relate the findings to the human disease counterpart.

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I. THE NEPHROTIC SYNDROME.

A. Definition:

The nephrotic syndrome is characterized by four cardinal features, namely: edema, proteinuria, hypoalbuminemia, and hyperlipemia including hypercholesterolemia. The latter three features are fundamental derangements and so long as they persist, the nephrotic syndrome may be considered to exist (1).

B. Clinical Nephrosis:

1. Etiology.

The causes of the nephrotic syndrome are varied. The great majority of cases occur in children as a disease of unknown etiology known as lipoid nephrosis. In adults, the syndrome occurs as a feature of a number of disease states. Thus it is commonly found as a component of diffuse glomerulonephritis, of diabetic renal disease (intercapillary glomerulosclerosis), and of disseminated lupus erythematosus. Less common causes are amyloidosis and secondary syphylis; uncommon causes are certain drugs and toxins, renal vein thrombosis, and constrictive pericarditis (2).

2. Histology.

The kidney is the site of morphological changes in all cases of the nephrotic syndrome, regardless of etiology. With the use of the light microscope, there is seen to be a thickening of the basement membrane of glomerular capillaries and proliferative changes in the capillary endothelium (3). With the use of the electron microscope, it has been demonstrated that the basement membrane in fact consists of (a) attenuated endothelial cytoplasm, (b) the basement membrane

proper, and (c) the epithelial cell foot processes. In nephrosis, there is found to be a fusion of the foot processes to form a continuous layer adjacent to the basement membrane and extensive vacuolation of the cytoplasma (4, 5, 6). Only minor changes are observed in the basement membrane, while proliferation of the endothelium may be present or absent depending on the nature of the underlying disease. This lesion of the epithelial component of the glomerulus is present regardless of the underlying etiology, duration of illness, or the changes observed with the light microscope. It can safely be concluded, then, that this is the basic morphological finding in the nephrotic syndrome (6).

The proliferative changes of the capillary endothelium are characteristic of an underlying glomerulonephritis present in many cases of the nephrotic syndrome. They are evident in adults but not in children (3).

3. Clinical Features.

Edema is usually the first recognized clinical feature of the nephrotic state. It is probable, however, that proteinuria has preceded clinical recognition by weeks or months (1, 7). The protein concentration of the urine in some cases of nephrosis may be as high as 60 g. per litre, but usually the nephrotic subject loses from 5 to 20 g. of protein per day (8). Although the quantitative loss of protein is less in children, the severity of loss, relative to body weight, is similar (2). Albumin represents at least 1/2 and commonly 3/4 of the protein in nephrotic urine (8). Urine from nephrotic subjects also contains hyaline and finely granular casts, and frequently, desquamated epithelial cells loaded with fat (2).

The concentration of albumin in plasma is almost always reduced in nephrosis, not uncommonly to 1.0 g. per 100 ml. The concentration of total plasma globulins is variable: alpha- and beta-globulins are almost always increased, while gamma-globulins are usually reduced (8).

The hyperlipemia found in nephrosis affects all three lipid fractions, i.e. glycerides, phospholipids and cholesterol. In general, the blood cholesterol is higher during active edematous states and tends to fall during remissions (1, 7). However, there is no direct relationship between the level of blood cholesterol on the one hand and the level of plasma proteins, degree of edema, or degree of albuminuria on the other (7).

4. Natural History.

The course of the nephrotic syndrome is different in children as compared to adults. In children, nephrosis is usually heralded by the onset of edema. The disease is, in general, punctuated by remissions and exaccerbations and exists without azotemia, significant alteration of blood pressure, or laboratory evidence of renal failure. This disease picture, referred to as "pure" or "lipoid" nephrosis, may last for 2 to 5 years and is followed by healing of the renal lesion (2).

The nephrotic syndrome in adults usually exists in the presence of other manifestations of the underlying disease of which it forms a part. There is commonly impairment of renal function as evidenced by azotemia and hypertension, due to underlying glomerulonephritis. When the latter set of circumstances apply, the term "mixed nephrosis" is used. Remissions and exaccerbation are uncommon. Eventually the nephrotic syndrome is usually replaced by a stage of progressive renal impairment (2).

5. Metabolic Abnormalities.

(a) Protein:

(i) Proteinuria. In the normal state, protein does not appear in the urine in sufficient quantity to be detected by the usual laboratory techniques. Thus, in the nephrotic syndrome, it is necessary to explain the source of the urinary protein as well as the mechanism of loss. There is a preponderance of evidence that in nephrotic subjects the urinary proteins are identical with the plasma proteins in their optical rotation, specific refraction, racemization, osmotic pressure, and immunological properties (9--11), and that the urine proteins do in fact come from the plasma.

It is evident, then, that the presence of excess protein in the urine must depend on some abnormality in the renal handling of plasma protein. It has been suggested that the urinary protein is derived from abnormal plasma proteins which are produced in the course of nephrosis (12). Such a hypothesis, however, is not tenable since the urinary proteins, as determined by their immunological properties (13) and by their electrophoretic mobilities (14), are identical with normal plasma proteins.

Thus the proteinuria must be dependent on the inability of the kidney to handle normal proteins in a normal fashion. In health, proteins appear in the glomerular filtrate (15) and are known to undergo reabsorption (16). There is no evidence that proteins, in the normal or the nephrotic state, are actively secreted by the tubules.

It has been suggested that in the nephrotic syndrome proteinuria might be the result of failure of tubular reabsorption (16). Bradley (1) has estimated that the amount of protein in the glomerular filtrate in the normal state is somewhat less than 25 mg. per 100 ml. Thus, if the concentration in the glomerular filtrate were 20 mg. per 100 ml. and if the filtration volume were equivalent to the normal value of 175 litres per day, it would be expected that total failure of tubular reabsorption of filtered protein would result in a maximum urinary loss of 35 g. of protein daily. However, protein losses greater than this have been frequently observed in nephrosis (7). Consequently, it may be concluded that failure of tubular reabsorption cannot be wholly accountable for the protein losses observed in nephrosis and that some abnormality of glomerular filtration must play a role.

In the normal state, there is a limit to the size of molecules which pass into the glomerular filtrate (17). In nephrosis some of the protein molecules appearing in the urine are above this limiting size. In addition, Squire (8) has demonstrated that the relative amounts of globulins appearing in the urine are inversely related to the molecular weights of these proteins. Such evidence has given rise to the theory that the proteinuria is due to an alteration in the permeability of the glomerular membrane. This membrane has been likened to a sieve in which the pore size is the factor which determines the nature of the proteins lost from the plasma (18). Thus, the protein losses in nephrosis are thought to be due to

changes in the glomerular membrane analogous to an increase in the size of pores.

In summary, the available evidence suggests that proteinuria is due to increased filtration of normal plasma proteins through the glomerular membrane, with a failure of tubular reabsorption of protein, if existent, being of secondary importance.

(ii) Plasma Protein Alterations. The hypoproteinemia in patients with the nephrotic syndrome is thought to be due mainly to massive proteinuria. Plasma protein synthesis has been found to be normal (20, 21) or even increased (19, 20, 90). An increased rate of catabolism of plasma albumin has been suggested as an important additional mechanism in the development of hypoalbuminemia in some patients (21).

Plasma protein concentrations are rarely elevated by albumin infusions or by high protein diets. A greater degree of nitrogen retention may be obtained by dietary means, however. Thus increased nitrogen retention has resulted in nephrotic subjects maintained on diets normal in protein content (22), by increasing the caloric intake while maintaining the nitrogen intake constant (23), and by increasing both the caloric intake and protein intake in commensurate fashion (24).

(b) Lipid Metabolism:

All lipids of human serum are associated with lipoproteins which have characteristic electrophoretic mobilities and are designated as alpha- and beta-lipoproteins. In sera of nephrotic

subjects, the beta-lipoprotein fraction is increased in amount while the alpha-lipoprotein fraction is normal or decreased (8).

Hyperlipemia in nephrosis is not due to excessive absorption of lipids or to a decrease in the rate of excretion of lipid (7). Hiller et al. (25) found that there was no disturbance in actual combustion or oxidation of fat in nephrotic subjects. However, a fat meal resulted in a significantly greater increase in serum fatty acids in nephrotic than in control subjects. These authors felt that there was a disturbed mechanism of transfer of lipid from the blood to the tissue depots. Ling and Liu (26) found an increase in fatty acids as well as a higher degree of saturation of these fatty acids in nephrotic plasma. They felt that such findings were explained by an accelerated transfer of fat to blood from tissue depots in combination with a relative slowness of the liver to deal with (unsaturate) these fatty acids. Stanley et al. (27) demonstrated that there was a decrease in the rate of removal of ingested fatty acids from the circulation in nephrotic subjects.

Epstein (28) noted a decrease in the basal metabolic rate as well as hypercholesterolemia in nephrotic subjects. Since these findings were also observed in hypothyroidism, he implied that hyperlipemia in nephrosis was due to a deficiency of circulating thyroid hormone. His attempts to alter the serum concentration of cholesterol and total lipid with desiccated thyroid, however, were not successful. Rasmussen (29) recently has suggested, though, that the thyroid preparations employed by Epstein were of questionable and variable potency.

Although the cause of hyperlipemia in the nephrotic syndrome is not completely understood, the above experimental findings

indicate that one factor contributing to hyperlipemia is retention of lipid or chylomicrons in the blood plasma.

(c) Edema:

There have been two abnormalities of the blood which have been implicated (1, 7, 8, 30, 31) in an attempt to explain edema formation in the nephrotic syndrome. These are a decrease in the colloid osmotic pressure and a decrease in blood volume. Edema was observed to occur when the colloid osmotic pressure was below 40% of its normal value, or when the serum albumin, which has a colloid osmotic pressure three times greater than globulin (32), was below 1.6 mg./100 ml. (31).

During the period of accumulation of edema fluid and prior to the onset of diuresis, the blood volume is believed to be reduced by 20 to 30% (8). The reduction in blood volume was found to be associated with an increased excretion of aldosterone (33) in the urine and a resultant renal retention of sodium and water. It has been postulated that this mechanism leads to a further increase in interstitial fluid volume (31). Although this appears to be a plausible hypothesis, the suggested mechanism lacks experimental verification.

Other mechanisms have been mentioned. Altered permeability of tissue capillaries (34) has been criticized as a mechanism since such a situation would affect only the rate and not the direction of fluid transfer (35). Robinson's review (36) gives little support to the view that the interstitial tissue exerts an inhibition pressure (8) tending to increase interstitial fluid volume. The existence of an increased amount of urinary

anti-diuretic substance in nephrosis (37) has been questioned from the viewpoint that the technique of measurement may have lacked specificity (38).

(d) Basal Metabolic Rate:

Aub and duBois reported in 1917 (39) that the basal metabolic rate (BMR) was reduced in nephrosis. Other studies at this time (40--43) confirmed this observation. From these and later studies, it has been established that although low BMR values are frequently encountered in nephrotic subjects, it is not an invariable feature of the nephrotic syndrome. Thus, Epstein found that the BMR was below -10% in 60% of his nephrotic subjects and normal or increased in 40% (28). Recant and Riggs (44) found subnormal values in 10 of 15 nephrotic subjects; Ayikama et al. (45), subnormal values in four of nine patients; Fiaschi et al. (46), values below normal in only one of 10 subjects.

Various abnormalities existent in the nephrotic state have been held responsible for the alteration in basal metabolism. Since the measurement of basal metabolism depends on the surface area, it has been suggested that the low values can be accounted for by the increase in surface area occasioned by edema in nephrotic subjects (47). It has been estimated that such a surface area change would introduce a 10% error (48) into BMR estimates. Even allowing for this correction, Moschcowicz concluded that many of the low BMR values in nephrotic patients could not be explained on this basis.

Undernutrition has been implicated to explain the low BMR values. Moschcowicz, however, found low values in nephrotic

subjects on adequate diets and on diets rich in protein (48). It has also been suggested that there is a decrease in the specific dynamic action resulting from inadequate protein intake and inadequate formation of protein (40). Thorough studies on the specific dynamic action are not available, though Moschcowicz reported one case in which the specific dynamic action was normal in a nephrotic subject whose BMR was -30% (48).

Two non-specific features of the nephrotic state have been presented in explanation of the low metabolic rate: the bed-ridden state and anemia. With regard to the former, it is difficult to estimate to what degree the bed-ridden state will lower the BMR, in addition to the other factors to which the nephrotic patient is subject. With regard to the latter, it cannot be said whether anemia, often found in the nephrotic state, is a result or cause of the lowered basal metabolism (48).

Moschcowicz suggested that the reduction in basal metabolic rate was due to a reduction in the conduction, convection, and radiation of heat occasioned by the accumulation of edema in the subcutaneous tissues (48). He supported this hypothesis by indicating that other clinical states with edema or integumentary thickening had low BMR values. Such a hypothesis would not explain the high BMR values occasionally found in left-sided heart failure.

Only one other explanation has been proposed to account for the low BMR values in nephrosis. In the era of 1920-1930, it was felt that there might be a dysfunction of the thyroid gland in nephrosis. This view was forwarded by Epstein who regarded nephrosis as a disease of "disturbed protein metabolism" in which

the thyroid gland was "unable to cope with this metabolic disturbance" (28). In nephrotic subjects with low basal metabolic rates it was found that thyroid extract produced an irregular change in BMR values (7, 28, 47). In general, a rise in the BMR was to be expected with such therapy but there was no consistent relationship between the amount of thyroid extract given and the rise in the BMR. It has recently been suggested, however, that the thyroid extract used by Epstein was of poor quality and questionable potency (29).

II. THE RELATIONSHIP OF THE DECREASED BASAL METABOLIC RATE TO THE THYROID FUNCTION IN THE NEPHROTIC SYNDROME.

Since the determination of the basal metabolic rate is in common clinical usage as a reflection of thyroid secretory activity, the explanation of low basal metabolic rates is most relevant to the problem of the thyroid function in nephrosis.

In myxedema, the BMR is almost always below -25% (49). The BMR in nephrosis has been observed to be as low as -39% (28). Recant and Riggs (44) found values below -25% in seven of 15 nephrotic patients. Although these values are in the myxedematous range, the BMR determinations in nephrotic subjects are believed to be influenced by a greater number of variables than in patients with known thyroid disorders.

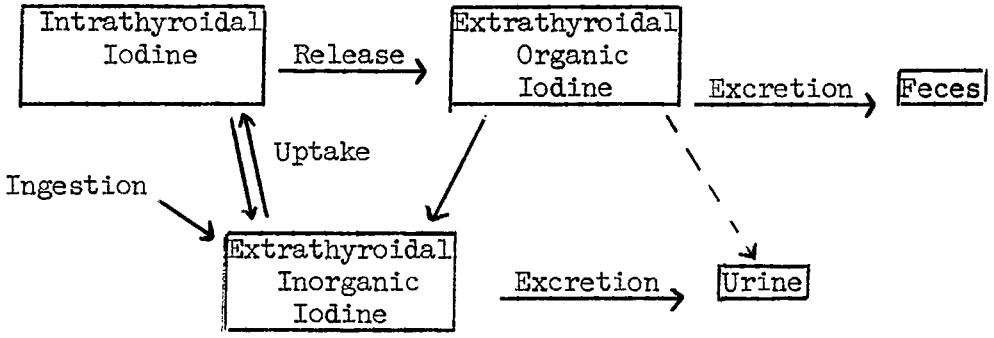
In view of these variables, it is the opinion of the author that the calculated BMR in clinical nephrosis would not reflect thyroid functional activity as closely as in other clinical states. There is no information in the literature specifically relating serum protein bound iodine (PBI) concentrations with BMR values in nephrotic subjects. The author has found that in those studies in which both of these determinations were available for comparative purposes, it was not possible to demonstrate a linear relationship between the serum PBI and the basal metabolic rate largely because of the insufficiency of data. In addition it was not possible to compile data from the various sources because different methods of estimation of surface area have been used to obtain the final BMR values. One cannot state, therefore, whether there is linearity between these two parameters of thyroid function. In addition, the reduction in basal metabolism may be due to the nephrotic state *per se* rather than to a superimposed state of hypothyroidism, or a combination of these features.

III. THE THYROID GLAND - PHYSIOLOGIC CONSIDERATIONS.

A. Iodine Metabolism:

1. Introduction.

Iodine is present in the body in three different "pools" - i.e., the intrathyroidal iodine pool, the extrathyroidal organic iodine pool, and the extrathyroidal inorganic iodine pool. The accompanying diagram will serve as an aid to the subsequent discussion of this section (50).



2. Intrathyroidal Iodine Metabolism.

Iodide, derived from intake and from the degradation of organic iodine in the tissues, is made available to the thyroid gland or to the kidneys for excretion. The iodide entering the thyroid gland is rapidly bound to complex organic molecules. One can study the rate of iodide entry into the thyroid gland by use of the accumulation gradient, concentration gradient, or the thyroid uptake at defined intervals, such as six hours or twenty-four hours after administration of radioactive iodide. The rate of such accumulation of radioiodide is in inverse proportion to the amount of iodide in the serum and thyroid gland and the functional activity of the parenchymal elements of the gland, (as influenced by the thyrotrophic hormone) (51).

Briefly, the subsequent steps involve the oxidation of iodide to iodine, the formation of diiodotyrosine and oxidation and condensation of the latter compound to form thyroxine. Thyroxine then becomes peptide-linked after its formation and is secreted into the lumen of the thyroid follicle to be stored as thyroglobulin, or is split from the peptide and released into the blood as thyroxine. The latter process is also under the influence of thyrotrophic hormone (51).

3. Extrathyroidal Iodine Metabolism.

Upon release from the thyroid gland, thyroid hormone - thyroxine and triiodothyronine - becomes associated with a specific protein(s) of plasma. This organically bound iodine in the blood is known as protein-bound iodine. The rapidity with which organic iodine leaves the thyroid gland can be roughly gauged by the conversion ratio, calculated 24 hours after administration of radioiodide by the use of the formula (52):

$$\frac{\text{Plasma protein-bound } I^{131}}{\text{Total plasma } I^{131}} \times 100$$

In 1952, it was demonstrated (53--55) that protein-bound iodine of serum was associated with a globulin having an electrophoretic mobility at pH 8.6 between those of $alpha_1$- and $alpha_2$-globulins. It was further shown that this thyroxine-binding protein had a binding capacity towards thyroxine of 0.5 μg./ml. (56) and that in the euthyroid state, only about one-third of the binding sites were occupied.

The thyroid hormone released into the blood becomes distributed in tissue spaces; the hormone so distributed in the body is referred to as the extrathyroidal organic iodine pool. The amount of iodine in this pool is governed by the balance between the amount released

by the thyroid gland per unit time and the amount removed by the tissues. The proportional rate of turnover of extrathyroidal organic iodine can be estimated by determining the biological half-life in the plasma of intravenously administered I^{131}-labelled thyroxine. Most of the organic iodine is degraded to inorganic iodine which then becomes part of the extrathyroidal iodide pool; some, however, is excreted, undegraded, in the feces while excretion in the urine is questionable.

Iodide, in the extrathyroidal inorganic iodine pool, has the same fate as newly ingested iodide - excretion by the kidney or accumulation by the thyroid gland (57).

B. Effect of Thyroid Hormone on Protein Metabolism:

Various reports (58) have suggested that protein metabolism is affected by thyroid hormone. Lukens (58) concluded that the increased protein catabolism of hyperthyroidism depends on the presence of hormones of the pituitary-adrenocortical system and probably on their increased activity. It has been shown that there is a tendency to nitrogen loss in hyperthyroidism but nitrogen retention can be attained if the caloric intake exceeds the basal requirement by a large amount (59).

In hypothyroidism, the overall rate of protein metabolism has been found by many authors to be diminished. "The effects (on protein metabolism) may be seen as a reduction in either the rate of protein synthesis or the rate of protein breakdown, depending on the experimental conditions under which the study is made (58)."

C. Effect of Thyroid Hormone on Lipid Metabolism:

Disturbances in lipid metabolism are found in clinical and experimental situations in which the thyroid function is altered. In hyperthyroidism, the plasma concentration of cholesterol is commonly decreased. In hypothyroidism, the plasma cholesterol, phospholipid, and

beta-lipoprotein are increased (60).

There are certain experimental findings which relate the hyperlipemic states of hypothyroidism and the nephrotic syndrome. It has been shown that an increased concentration of cholesterol is confined to the plasma in the hypothyroid animal (61). Marsh and Drabkin's data support a similar viewpoint with regard to experimental nephrosis (62). In both experimental states there is found to be a diminution in the rate of disappearance from plasma of cholesterol (63, 64, 66). In both experimental states of hyperlipemia, it has been possible to lower plasma cholesterol concentration with preparations of desiccated thyroid (67, 68).

The mechanisms by which thyroid hormone controls lipid metabolism are not completely understood. It is established, however, that the rate of biosynthesis of cholesterol is in direct proportion to the basal metabolic rate (69), an indirect measure of thyroid function. Furthermore, in hypothyroid animals there is a decrease in the rate of biliary secretion of cholesterol (66). These findings indicate that the thyroid hormone accelerates the synthesis of cholesterol and also its excretion and degradation (70).

As there are certain similarities found in hyperlipemic plasma of hypothyroidism and nephrosis, there are differences as well. The neutral fat fraction of lipids is increased to a greater extent in nephrotic plasma (71). The free cholesterol:phospholipid ratio is not altered in the hypothyroid state; it is in nephrosis (71). Peters and Man (72) have emphasized the importance of the free cholesterol: phospholipid ratio. They stated that "the finding, in a patient's serum, of a disturbed relationship of free cholesterol to phospholipid suggests that the patient is not suffering from hypothyroidism or that the hypothyroidism is complicated by some other factor."

D. Basal Metabolism:

Basal metabolism is defined as the rate of heat production in a recumbent subject who is awake, mentally and physically relaxed and who has not eaten food for twelve hours (73). The determination of basal metabolism (BMR) is generally considered to be an indirect means of appraising the secretory activity of the thyroid gland.

Many factors, in addition to the activity of the thyroid gland, influence basal metabolism. Such variables as age, sex, and the amounts and relative proportions of fat, muscle, bone and water in the body affect the basal metabolic rate. The BMR is also influenced by the activity of smooth and skeletal muscle, various organs, and other glands, especially the adrenal cortex (73).

Additional variables affect its determination. It has been felt that the largest single contribution to the errors of BMR determinations is the estimation of surface area. Other sources of error involve technical difficulties, instrumentation, and the presence of other disease states (73).

In spite of the factors which influence the basal metabolic rate and the variables involved in its determination, there is found to be a correlation with the circulating level of thyroid hormone. Thus Salter found a logarithmic relationship between the BMR and the serum level of protein-bound iodine (74).

Day, on the other hand, felt that the BMR offers no better than a 50% correlation with the final clinical appraisal of the thyroid secretory activity. He did feel, however, that an optimum value of 80% correlation would be found if special care were taken and repetition of the test practised (73).

IV. THE EFFECT OF THE NEPHROTIC SYNDROME ON THYROID FUNCTION.

A. Introduction:

The measurement of the serum protein-bound iodine (PBI) serves as a quantitative method for estimating the concentration of circulating thyroid hormone. In 1948, Peters and Man (75) noted that the serum PBI level was reduced in two nephrotic patients. These low concentrations of serum protein-bound iodine were associated with significant urinary losses of organically bound iodine in both cases.

At this time, the serum PBI was considered to be the most direct approach to estimating the secretory activity of the thyroid gland (76). These considerations led Recant and Riggs (44) to study certain aspects of thyroid function employing radioiodine and labelled thyroxine. Subsequent reports of a similar nature have appeared in the literature (29 , 45, 46, 77, 78).

B. Serum Protein-Bound Iodine:

Recant and Riggs (44) found subnormal values for serum PBI in 13 of 17 cases (mean concentration 2.2 μg.%). Fiaschi et al. (46), on the other hand, noted subnormal values in only seven of 21 nephrotic subjects. The mean value was 4.39 (± 0.27) μg.% for adult subjects, and 4.82 (± 0.63) μg.% in children. Both mean values were within the limits of normal. The other studies revealed mean values below the normal range (29, 45, 77). In compiling all values from these studies, the author has found that the serum PBI was reduced in 60% of the nephrotic subjects studied.

C. Iodine Metabolism:

1. Introduction.

Four groups of investigators have studied the dynamics of iodine metabolism employing radioiodine (29, 44, 46, 77, 78). All patients in these investigations were clinically representative of the nephrotic syndrome, were adults (except for certain subjects in Recant and Riggs'

study (44), and had low serum protein-bound iodine levels (except for two of four subjects in Fiaschi's studies of the turnover of labelled thyroxine (46)).

The results of these investigations will be considered in regard to the phase of iodine metabolism to which they refer.

2. Intrathyroidal Iodine Metabolism.

(a) I^{131} Uptake:

The 24-hour radioiodine uptake was found to be in the upper limit of normal or increased in nephrotic subjects (44, 46, 77). In one study the six-hour uptake was found to be markedly increased (46). The accumulation gradient was normal or increased (44, 78).

(b) Storage of Organic Iodine:

Recant and Riggs (44) used 1-methyl 2-mercapto-imidazole for seven days to induce a complete block in hormone synthesis (indicated by a greatly diminished uptake of radioiodide) in one nephrotic and one control subject. With intermittent stimulation of the thyroid gland, with thyrotrophic hormone, they found that the serum PBI failed to rise in the nephrotic subject. This finding led them to conclude that the hormonal stores in the thyroid gland may be diminished in the nephrotic syndrome.

(c) The Release of Organic Iodine:

Fiaschi et al. (46) found that not only was there an increase in the rate at which radioiodide enters the thyroid gland, but there was also an increase in the rate of release of thyroidal radioiodine. These investigators measured thyroidal radioactivity at six and twenty-four hours in 21 nephrotic subjects. They found

that the thyroidal radioiodine content at these periods of measurement were closely similar or that the 24-hour measurement was apparently lower than the six-hour measurement. The latter circumstance prevailed in children. Similarly, Gruchand et al. (77), measuring the conversion ratio, found an increase in the rate at which radioiodine eventually left the thyroid gland in one of two nephrotic subjects. These results can be explained on the basis of an increase in the rate of turnover of iodine in the thyroid gland. An alternate explanation is that thyroidal organic iodine was diminished in quantity. The actual rate of secretion of thyroidal iodine was not measured.

There is available data on only three nephrotic subjects regarding the actual rate of release of organic iodine. In these patients, Rasmussen (29) found that the daily release was 35-44 μg. of iodine per day (normal values 38-100). Rasmussen's data suggest that either the mechanism of release of hormonal iodine in nephrotic subjects was impaired in some manner or that the stores of thyroidal iodine were diminished.

3. Extrathyroidal Organic Iodine Metabolism.

(a) Organic Iodine Pool:

Rasmussen (29) found that the apparent space of distribution of thyroxine in the body was 10.6-13.2 litres (normal values = 8-11 litres) in his three nephrotic subjects under investigation. The amount of thyroxine in this pool was calculated to be 191-224 μg. iodine (normal = 350-500 μg. iodine).

(b) Biological Half-life of Thyroxine:

The biological half-life of circulating thyroid hormone has been measured following endogenous or exogenous labelling of

thyroxine in patients with low serum PBI values. The biological half-life of thyroxine was shortened in eight subjects (29, 46, 78), and normal in two others (44).

(c) Loss of Protein-bound Iodine:

Rasmussen (29) demonstrated that significant organic iodine losses occur in the urine of nephrotic subjects. The amounts lost represented 25-50% of the daily release from the thyroid gland in his subjects. In addition, this investigator found 12-26% of released organic iodine in the feces whereas in the normal situation only 10-12% was lost by this route. Recant and Riggs (44) found urinary losses of 6.0-36.1 μg. of iodine per twenty-four hours in a study of four nephrotic patients. These authors demonstrated also, in two patients studied, that only 6-7% of an administered dose of d,l-thyroxine was recovered in the urine. The dose administered to these subjects was, however, 5-10 times the normal amount secreted by the thyroid gland, and it is probable that considerable quantities of hormone were lost by biliary excretion (117).

(d) Peripheral Utilization of Organic Iodine:

Rasmussen (29) found that the amount of hormonal iodine degraded per day was 19-28 μg. (normal = 35-90 μg.). The proportional degradation of extrathyroidal organic iodine per day, however, was found to be in the normal range (8.5-9.9% per day).

Fiaschi et al. (46) found that the total turnover rate of extra-thyroidal organic iodine (i.e. the total amount of iodine lost in the urine plus the amount degraded each day) in four patients so studied was 22.4% per day (normal = 10.72-12.22%).

(e) Additional Considerations:

(i) Thyroxine Binding in Nephrotic Sera. The thyroxine binding protein in the plasma of nephrotic patients has been found to be qualitatively similar to that of euthyroid subjects (79). In addition, the thyroxine binding capacity, although somewhat reduced, was sufficient to bind normal amounts of circulating thyroxine (78, 79).

(ii) Organic Iodine in Nephrotic Urine. Rasmussen (29) demonstrated that the organic iodine in the urine was in the form of thyroxine although a small percentage (6-8%) was in the form of diiodotyrosine. By chromatography, Cruchand et al. (77) showed the identity of protein-bound thyroxine in the urine and serum.

4. Discussion.

The alterations in parameters of thyroid function noted in the above studies have led to certain conflicting views on the state of thyroid function in nephrosis.

Recant and Riggs (44) concluded that even though the hormonal stores of the thyroid gland might be diminished in certain cases of nephrosis, the thyroid function and the supply of hormone to the tissues was normal. They suggested that the low concentration of PBI in plasma was due to the change in either the concentration or binding capacity of the plasma proteins. The latter possibility was not confirmed by Robbins et al. (79). Though there is a reduction in several plasma proteins, there is as yet no evidence of a decrease in the concentration of the specific thyroxine-binding globulin. These authors also suggested that since plasma proteins

were unable to bind thyroid hormone in a normal manner, the ability of the tissues to clear hormone would be increased. In addition, it was suggested that thyroxine was present in the circulation in an unbound state thus permitting the tissues to obtain a normal supply of hormone. Although 1-2% of circulating thyroxine is unbound (80) in the normal state, there is no direct evidence that the ability of the peripheral tissues to clear circulating hormone is increased in human nephrosis.

Rasmussen (29) drew attention to the apparent thyroxine deficiency and its attendant sequellae in his nephrotic subjects. The deficiency was believed to be the result of the losses of organic iodine in the urine, the proportionately greater losses of iodine in the feces, and dilution of released organic iodine in an expanded extracellular volume. These alterations might be expected to induce a compensatory increase in anterior pituitary release of thyrotrophic hormone. Thyroidal compensation was not found in his patients, however, as evidenced by the normal I^{131} uptake and the reduction in release of hormonal iodine. In addition, it was observed that the actual amounts of hormone undergoing peripheral degradation were in the range of those observed in hypopituitarism. Although the reason for the apparent failure to augment the pituitary release of thyrotrophic hormone was unknown, it was suggested that it may be due to the depletion of body protein in the nephrotic state. The suggested pituitary failure in nephrosis was therefore regarded as similar to that seen in chronic malnutrition.

Accepting these considerations, it would appear that thyroid function in the nephrotic syndrome was dependent on the ability of

the pituitary gland to compensate for circumstances of the nephrotic state per se (severity of edema as determined by degree and duration, the quantity of protein-bound iodine lost by urinary excretion, protein depletion as determined by the severity of proteinuria and nitrogen balance). This concept would also imply that thyroid function might undergo sequential changes during the course of nephrosis.

These variables in the nephrotic subject and the sequential changes of the syndrome could explain the results of other workers. Although the increase in turnover time of iodine within the thyroid gland and the depletion of thyroidal iodine (46, 77) could be explained on the basis of a state of iodine deprivation, this viewpoint lacks the support of experimental evidence.

It has been suggested that the nature of the alteration in thyroidal function, unknown at the present time, might become evident if several aspects of thyroxine metabolism were studied in various stages of the disease (80). This approach would appear necessary to more adequately interpret the experimental findings presently available.

V. EXPERIMENTAL NEPHROSIS.

A. Introduction:

Experimental nephrosis has been induced in rats by the administration of either antikidney serum (81) or the aminonucleoside of puromycin (82). The former method is the longer established and most frequently used method of induction, and since it was the method used in the author's experiments, it will be given sole consideration in this section.

The use of antikidney serum to induce renal disease in animals has been studied by many investigators, the pioneers in this field being Masugi (83) and Smadel (84). The principle involved is the following: nephrotoxic serum is prepared by injecting homogenized kidney tissue of one animal species (Species I) into another animal of a different species (Species II) whose serum becomes antigenic to the injected foreign protein. If the serum of Species II is then injected into animals of Species I, the latter develop a characteristic renal disease.

Heymann and Lund (81) modified the method of preparation of homogenized kidney tissue as well as the dosage in administering nephrotoxic serum. By the use of rabbit anti-rat kidney serum they were able to induce in rats a renal disease which resembled clinical nephrosis in many ways. The subsequent remarks refer to the disease state produced by this method.

B. Histology:

Administration of antikidney serum to rats resulted in degenerative changes in the renal glomeruli and tubules. Glomerular changes have been observed one-half hour after antikidney serum administration

while the tubular changes occurred 4-6 hours later (85). Initially, there was noted to be basement membrane thickening, cytoplasmic swelling of the endothelium without proliferative changes, and fatty infiltration of the glomerular tuft.

Subsequently, these degenerative changes of the glomeruli were found to persist or were replaced by fibrosis or cellular proliferation of the glomerulus and its capsule. With persistence of the degenerative glomerular changes, the clinical course was noted to follow a pattern similar to that of "pure" or lipoid nephrosis of humans. The proliferative phase was associated with mild degrees of hypertension and uremia and was felt to be representative of "mixed" nephrosis of humans (81).

The degenerative changes in the renal tubules consisted of swelling, granularity, and fatty changes of the cytoplasm - histologic changes similar to the tubular changes in human nephrosis (81).

With the light microscope, the histologic features of glomerular changes were seen to resemble closely the glomerular changes of lipoid nephrosis of children (81). Ehrich et al. (86) supported this viewpoint but indicated that the morphological changes were also representative of the basement membrane changes in the nephrotic syndrome of diffuse glomerulonephritis, diffuse amyloidosis, intercapillary glomerulosclerosis, and lupus erythematosus disseminatus.

Piel et al. (87) studied the histologic changes of the glomerulus with the electron microscope. It was observed that obliteration of the spaces between the foot processes and thickening of the basement membrane were persistent changes. The former change occurred one hour after administration of antikidney sera. The latter change

occurred at six hours and corresponded with the onset of proteinuria.

C. Biochemical Features:

Proteinuria, hypoproteinuria and hyperlipemia were observed within eight hours after antikidney serum administration (85). These changes were most severe during the initial period of the renal disease. It has been found in the early stages that daily albumin losses amount to more than twice the total circulating albumin of normal rats (88). The total lipids of plasma rose to three or more times normal values (81).

The plasma protein concentration was found to diminish to half that of control animals, in the early stage of the disease. The diminution was found to correspond to degree of edema. Edema appeared from three days to two weeks following nephro-toxin serum administration and was frequently associated with ascites. The edematous phase remained from one to three weeks (81).

In the subsequent course of the experimental disease, there was a slow subsidence in the changes in plasma lipids and total protein observed during the initial phase. However, hyperlipemia and hypoproteinemia were evident as long as the disease persisted. Proteinuria, although less severe than during the initial phase of illness, was always a feature of the disease state (81).

During the clinical course of the disease, over two thirds of the rats remained normotensive while the remainder revealed a slight to moderate degree of hypertension. Such hypertension was found to be fluctuating and not fixed. Although the blood non-protein nitrogen values were elevated initially, they returned to normal within four

weeks while the remainder revealed slight elevations of non-protein nitrogen during the subsequent course of the disease state. Hyaline and granular casts were observed in the urinary sediment. Gross hematuria rarely occurred (81).

D. Natural History:

Nephrotoxic disease in rats was found to take one of three courses (81). In 30% of animals, spontaneous cure of the disease occurred within one to eight months following antikidney serum administration. An additional 30% developed a fulminating renal disease, characterized by massive ascites, edema, proteinuria, and edema resulting in death within the first two weeks. The remaining animals developed a chronic renal disease of an uninterrupted nature or marked by remissions and exaccerbations.

There was believed to be no clinical counterpart to the fulminating renal disease occurring in the first two weeks. Of those animals that survived, it was found that the renal disease was self limited or was chronic in type. In the chronic form, there were inconstant elevations of blood pressure and mild elevation of the non-protein nitrogen in only some of the animals. Such findings are well in keeping with the natural history of the nephrotic syndrome in the human.

E. Metabolic Considerations:

There has been found no basic differences in the mechanism of protein loss between the human and experimental varieties of nephrosis. The urinary protein of the nephrotic animals has been found to be derived from the plasma (88). In addition, albumin and globulins of urinary protein have been found to have no qualitative differences from normal plasma proteins by electrophoretic analysis (89). Subsequent to this protein loss there has been demonstrated an increase in synthesis of

plasma albumin (62, 88), a finding similar to that observed in the nephrotic syndrome in humans (19, 20). From these considerations there appears to be little basic difference in protein metabolism between experimental and clinical nephrosis.

The hyperlipemia of experimental nephrosis is associated with an increase in beta-lipoproteins (91). It has been demonstrated that there is no increase in the rate of absorption of lipids from the intestine (92), nor a decrease in the rate of excretion of lipids (93) in nephrotic animals. The half-life of labelled chylomicra and cholesterol has been found to be prolonged (65, 94). Hyperlipemia has been claimed to be due, at least in part, to a retention of lipid in the serum (65, 95) together with an increased synthesis and discharge of lipid (65, 96). These features of hyperlipemia in experimental nephrotic animals are consistent with observations in human nephrosis.

F. Conclusion:

In the preceding discussion there was shown to be a close similarity between experimental nephrosis and the nephrotic syndrome in humans. This resemblance was found to exist in the basic morphological change of the renal glomerulus, biochemical and clinical features, natural history, and in the metabolic disturbances of protein and lipid metabolism. In total, it is apparent that the experimental nephrosis is in many ways representative of the nephrotic syndrome in humans, and therefore appears to be a valid experimental tool to use in studying the changes in thyroid function occurring in the nephrotic state.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. EXPERIMENTAL ANIMALS.

Experimental procedures were performed using male adult Sprague-Dawley rats. The animals were kept in a room of constant temperature (78° ± 1° F.) and constant humidity (40 ± 2%) with the lights adjusted to go on at 7:00 a.m. and off at 7:00 p.m.

The animals were housed in individual cages in experiments involving measurement of food consumption; otherwise, stock cages were employed. When excreta were to be measured, individual metabolism cages were employed; these permitted the collection of urine while the feces were retained on a wire mesh screen below the floor of the cage.

Three dietary preparations were utilized. (a) The standard laboratory diet had the following composition:

protein	20.4%
fat	13.9%
carbohydrate	49.1%

The ingredients are:

	Grams per Dry Weight
Starch	350
Sucrose	100
Dextrose	81
Casein	220
Vegetable Oil	150
Brewer's Yeast	37
*Salt Mixture	40
**Vitamin Mixture	22
Powdered Cellulose	75
Sodium Chloride	6
Total	1,081 Grams

* Jones-Foster salt mixture (97).

** Contains Vitamin A, Vitamin D, alpha tocopherol, ascorbic acid, inositol, choline chloride, menadione, p-amino benzoic acid, niacin, riboflavin, pyridoxine hydrochloride, thiamine hydrochloride, calcium pantothenate, biotin, folic acid, Vitamin B_{12}.

(b) The low iodine diet consisted of the standard diet with potassium iodide omitted from the salt mixture; it had an iodine content of 142 µg./kg. This diet was used in all experiments involving thyroidal uptake and release of radioiodine. (c) The third preparation used was Purina Fox Chow which contained no more than 20% protein by weight and approximately 1,500 µg. of iodine per kg. (98). To all of the above diets tetracycline (750 mg./kg. dry weight) was added as prophylaxis against infection.

The animals were allowed free access to food or were fed by gavage. When free feeding was permitted but it was desired to know the food consumption, specially constructed food boxes were employed. Gavage was used in some experiments to ensure a constant daily food intake. For this purpose 1140 cc. of water were added to 1,081 g. of diet (either standard or low iodine) and mixed in a Waring Blender. This gave a smooth suspension which was administered intragastrically by polyethylene catheter. Feedings of 11 cc. were given at 10:00 a.m. and 6:00 p.m.; this volume provided 55 calories daily. The iodine intake with the low iodine diet fed in this manner was 1.85 µg., an amount sufficient to prevent goitre formation (99).

II. IODINATED COMPOUNDS EMPLOYED.

Radioiodide (NaI^{131}) was obtained from the Charles E. Frosst Company, Montreal and was carrier free.

Sodium L-triiodothyronine was obtained as highly purified preparations from the Research Laboratories of the Smith, Kline and French Corporation, Philadelphia, Pennsylvania. For administration it was dissolved in dilute sodium hydroxide solution adjusted to a final pH of 9.0.

Radioactive L-thyroxine was obtained from the Abbott Laboratories, Oak Ridge, Tennessee. This chromatographically pure preparation had a specific activity of 36 mc./mg.

III. METHODS.

A. Production of Experimental Nephrosis:

The method used was that established by Heymann and Lund (81). It consists essentially of the following steps: (1) immunization of rabbits to normal rat kidney tissue by the repeated injection of kidney homogenate, and (2) removal of rabbit serum containing antibodies to rat kidney, and injection of the serum into normal rats. The rabbit antibodies react with the kidney tissue resulting in local damage which leads to development of the nephrotic syndrome.

B. Collection of Biological Materials for Analysis:

Urine was collected under toluol in amber bottles. Glacial acetic acid or acetate buffer at pH 5.0 was added to inhibit bacterial growth. A collection period was 24 hours in length, unless otherwise stated, and was terminated by facilitating urination with an ether soaked swab of cotton, held close to the animal's nose. The cages were rinsed with a spray of distilled water, which was then added to the urine bottle, thus providing a complete collection of urinary solutes. The urine was filtered to remove sediment before analysis.

Small quantities of serum were obtained by collecting blood from the cut end of the tail and larger quantities of serum by exsanguination from the abdominal aorta.

C. Chemical Analysis:

Urine and serum proteins were determined quantitatively by a microtechnique using a biuret reagent (100). Urine proteins were prepared for analysis by initial protein precipitation with 10% trichloroacetic acid.

Total urinary nitrogen was determined by a micro-Kjeldahl procedure.

Thyroid and serum specimens were analyzed for total and protein-bound iodine at the Boston Medical Laboratories, 19 Bay State Road, Boston, Massachusetts.

D. Radioactive Assay:

1. Thyroidal Radioactivity.

Thyroid radioactivity was measured in vivo with a scintillation detector using a collimator of 1.6 cm. in diameter. The procedure was as follows: The animals were anaesthetized with ether and placed in a supine position on a mounting board. The fore and hind legs were attached by rubber bands and the neck extended by traction with a rubber band attached to the upper incisor teeth. The neck was shaved and the position of the thyroid cartilage marked. The animal was then placed under the counter with the thyroid cartilage in the center of the area exposed to the collimator. The height of the collimator above the animal board was fixed to 3.8 cm.

An error was involved in repositioning the animal on the mounting board and was estimated in the following manner: An initial count was obtained over the thyroid cartilage. The animal was then removed, placed anew on the board, repositioned under the counting aperture, and a second count determined. A series of four determinations was made on each of five animals. The results are listed in the accompanying table.

TABLE I

Animal Number	Counts per Minute (Mean + Standard Deviation)	Coefficient of Variation
1	3130 ± 104.8	3.35%
2	1746 ± 54.6	3.14%
3	1590 ± 49.7	3.12%
4	1440 ± 19.2	1.33%
5	853 ± 6.9	0.81%
Average		2.35%

From the above table, an average value of 2.35% represents the coefficient of variation for this method of placing the thyroid gland in the position for counting. The error involved may not be as great as the average value would indicate, since the counts from animals 4 and 5 were performed at a later time when there was more experience with the technique.

Extrathyroidal radioactivity was determined by counting over the right anterior chest. After correction for background the chest count was multiplied by the factor 0.65 (101), representing the ratio of neck:chest circumference. The thyroidal radioactivity was calculated after corrections for room background, extrathyroidal radioactivity and for physical decay of radioiodine.

To calculate the percentage of a dose of radioiodine present in the thyroid gland at the various times of in vivo measurement, the following procedure was employed: Following total thyroidectomy (102), the glands were placed in test tubes containing 10% trichloroacetic acid and a measurement of radioactivity was obtained. By comparison with the count given by an aliquot of the administered

dose assayed at the same time, it was possible to calculate the percent of the administered dose present in the thyroid gland at the time of sacrifice. An estimate of the in vivo count at this time was made by extrapolation of the thyroid radioactivity decay curve. The relationship between in vivo and in vitro assays was then established and it was then possible to calculate the activity measured in vivo as a percent of the administered dose measured in vitro.

A scintillation well counter assembly was used for all in vitro determinations.

2. Accumulation Gradient.

The accumulation gradient is defined as the slope of the straight line obtained when the radioactivity in the thyroid gland, expressed as counts per second, is plotted on the ordinate against the square root of time in minutes on the abscissa. In humans, Stanley and Astwood (103) found that the linear relationship existed over an eight-hour interval after radioiodide administration. Data obtained during a comparable five-hour interval were used to calculate the gradient in the present study.

3. I^{131} Release Rate.

The I^{131} release rate is a reflection of the fractional rate of turnover of hormone in the thyroid gland. Since virtually all the thyroidal radioiodine is bound at 40 hours after NaI^{131} administration (104, 105), daily interval determinations after this time were used to calculate the I^{131} release rate. The interval determinations of thyroidal radioactivity were converted to logarithmic function and plotted against time in days. A regression line for

each animal was obtained by the method of least squares and its slope taken as the rate constant of I^{131} release from the thyroid gland.

4. Urinary Radioactivity.

When urinary radioactivity was to be measured, 1.0-5.0 mg. of sodium iodide were added to the collection bottles to minimize the loss of radioactive iodine by sublimation. A 3.0 ml. aliquot was used in determining total urinary radioactivity. The procedure for the determination of protein-bound radioactivity was as follows:

(a) To 3.0 ml. of urine were added 3.0 ml. of 20% trichloroacetic acid (TCA) to precipitate protein. The precipitate was stirred with a glass stirring rod, which was then removed and used in subsequent steps involving agitation of the precipitate.

(b) The sample was centrifuged at 3,000 r.p.m. for 10 minutes. The resulting supernatant was decanted and discarded.

(c) The precipitate was washed three times with 3.0 ml. aliquots of 10% TCA.

(d) The precipitate was dissolved in 3.0 ml. of 10% NaOH, and the solution assayed for radioactivity.

The possibility that urinary protein might bind with radioiodide in the collection bottles was examined. To test this hypothesis, 5 μc. of NaI^{131} were added to three collections of nephrotic urine containing 300-450 mg. of protein. The urines were incubated at room temperature for 24 hours. The percent of the administered dose recovered as protein-bound I^{131} was 0.500, 0.915 and 0.780. It was considered that these values probably represented contamination of the TCA precipitate with inorganic I^{131} rather than true protein binding, and was not an unduly great error for the technique.

The feces were stored in the cold in bottles containing water and excess of sodium iodide. A fecal homogenate was made by adding distilled water and mixing with a glass stirring rod, and a 2.0 ml. aliquot used for radioactivity estimation.

E. Determination of Oxygen Consumption:

Oxygen consumption was determined with a closed circuit respirometer using 95% oxygen and involving the direct measurement of the oxygen consumed (106). Quartz Mason jars, kept submerged in a constant temperature bath of 20° C., served as animal chambers. The individual animal chambers were connected to spirometers contained in a separate water tank. The animal chambers had a wire screen serving as an animal stand under which was placed an adequate amount of soda lime used as a carbon dioxide adsorbent. The spirometers were equipped with an indicator from which the volume of oxygen consumed could be readily assessed.

Calibration of the individual spirometer volume was necessary. Thus a graduated pipette was connected by means of rubber tubing to a reservoir of water contained in a 50 ml. sealed Erlenmeyer flask. A second opening in the rubber stopper of this flask served as an air passage to communicate with the bellows. The volume of each division of the spirometer scale was determined by filling and emptying of the volumetric pipette. Three individual determinations were made and the mean values taken. The volume was corrected to 760 mm. H_2O pressure and the volume of the divisions of the scale calculated.

The procedure for measuring oxygen consumption was as follows: The spirometers were tested for the patency of inlet and outlet tubes, and oxygen was flushed through the spirometers. The animals were placed in the chambers allowing for a 15-20 minute period before taking

the initial determination of oxygen consumption. Three 15-minute periods were used to calculate the oxygen consumption results, with no daily consumption so calculated unless the individual periods were within a 30% agreement of one another. The animals were placed in a different chamber for each daily determination to minimize differences within the apparatus.

Oxygen consumption was expressed as liters per square meter per hour and was calculated in the following fashion:

$$\text{L./sq. M./hr.} = \frac{V}{t} \times \frac{P}{760} \times \frac{273}{T} \times \frac{10{,}000}{(\text{weight})^{2/3} \times 10} \times \frac{1}{1{,}000}$$

L./sq. M./hr. = volume of oxygen consumed per square meter surface area per hour corrected for standard temperature and pressure.

V = mean observed volume (ml.) of oxygen consumed for three intervals.

t = time (hours) = 0.25 hr.

P = barometric pressure (ml. of water).

T = absolute temperature at time of test = 301° A.

Wt. = weight of the animal.

$\frac{10{,}000}{(\text{weight})^{2/3} \times 10}$ = formula for converting body weight (in grams) to surface area (in square meters).

$\frac{1}{1{,}000}$ = factor converting cubic centimeters to liters.

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

EXPERIMENT I.

Purpose:

This experiment was carried out to determine the metabolic fate of administered radioiodide in experimental nephrosis by measuring the thyroidal uptake and release of radioiodine and the excretion pattern of radioactivity in the urine and feces.

Method:

Five nephrotic animals weighing from 152 to 260 g. and six control animals weighing from 210 to 246 g. were used. Nephrosis had been induced in the first group five weeks previously. In the nephrotic animals there was no evidence of edema during the experiment and the mean daily urinary losses of protein were 104 to 414 mg. Throughout the experiment the low iodine diet was administered by gavage in order to control the iodine intake and to insure that the caloric intake would be adequate to maintain initial body weight. Distilled water was available ad lib.

On the eleventh day, 10 μc. of NaI^{131} without added carrier were given intravenously in 0.5 ml. of saline. The radioactivity of the thyroid gland was then measured four times at accurately timed intervals of approximately one hour to permit calculation of the accumulation gradient. Further measurements of thyroidal radioactivity were made at 18 hours after NaI^{131} injection and subsequently at 24-hour intervals for six days. From the latter series of measurements was calculated the specific rate constant for the release of I^{131} from the thyroid gland.

The urine was collected quantitatively following administration of radioiodide, and 24-hour collections were analyzed for total and protein-bound radioactivity as well as for the total protein content. The total fecal excretion of radioactivity during the experiment was determined from the pooled daily collections.

After the final count of thyroidal radioactivity the animals were sacrificed, a total thyroidectomy was performed and the glands analyzed for free and protein-bound iodine content. Aliquots of serum were analyzed for total and protein-bound iodine and for total and protein-bound radioactivity.

Results:

A. Accumulation Gradient.

The accumulation gradient was higher in the nephrotic animals. The mean values obtained (Table I) were 1.50 $\pm$ 0.11 for the control animals and 3.27 $\pm$ 0.65 for the nephrotic animals. The difference between the groups was significant at the 5% level.

B. 18-Hour Uptakes.

The values are shown in Table I. It is seen that the nephrotic animals had considerably higher thyroidal radioactivity at 18 hours than did the controls. Statistical analysis of the mean values for each group revealed that this difference in uptake was significant at the 1% level.

C. Radioiodine Release Rate.

The mean rate of I^{131} release in the nephrotic animals was greater than for the controls (Table I). This difference was significant at better than the 5% level.

D. Urinary Excretion of Radioactivity.

The mean daily urinary excretion of total and protein-bound radioactivity is found in Table II. Three significant differences were noted:

1. The total I^{131} excretion was greater in the control group during the 20-hour interval after administration of NaI^{131} ($P < .05$).

2. The excretion of inorganic radioiodine was greater in the nephrotic group during the latter periods of measurement ($P < .01$).

3. The excretion of protein-bound radioactivity was much greater in the nephrotic animals during all periods of measurement ($P < .01$).

E. Fecal Excretion of Radioactivity.

There was no significant difference between the two groups of animals in the fecal excretion of radioactivity (Table III).

F. Analyses of Serum.

No significant differences between the two groups of animals existed in the serum content of free and protein-bound iodine (Table IV) or in the total and protein-bound radioactivity (Table V). There was a statistically significant difference between the specific activities of protein-bound iodine in the two groups of animals ($P < .05$).

G. Thyroid Glands.

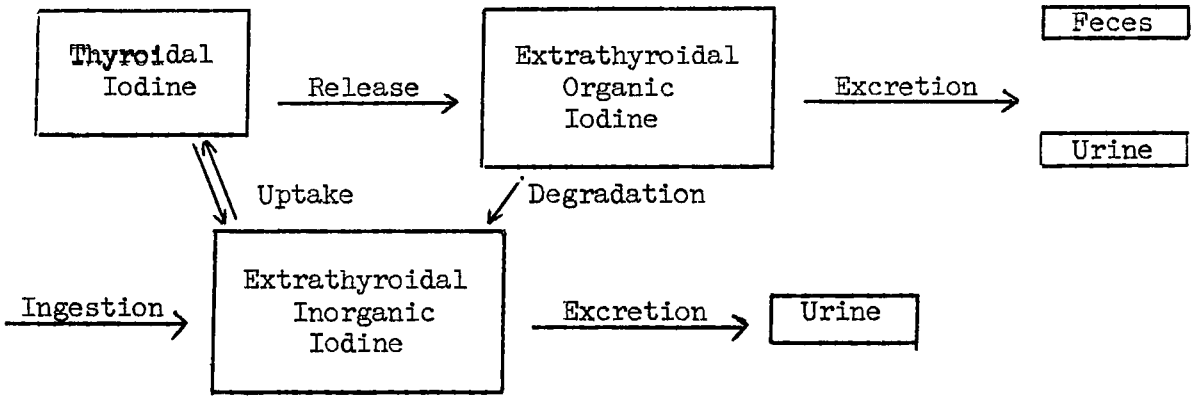
There was a significant difference between the two groups in protein-bound iodine content of the glands (Table IV). The mean value was 6.0 µg. in the nephrotic animals and 8.6 µg. in the control animals. There was no significant difference in free iodine content of the thyroid gland in the two groups of animals.

H. Urinary Losses of Protein and PBI^{131}.

The daily individual urinary losses of protein and protein-bound radioactivity in the nephrotic animals are found in Table VI.

Discussion:

An assumed model (modified from Rasmussen (29)) will serve as the basis for the discussion of this experiment:



The experimental data revealed an increase in the accumulation gradient and an increased 18-hour uptake of radioiodide by the thyroid glands in nephrotic animals. Since the accumulation of radioiodide is inversely proportional to the serum iodide concentration, it might be thought that the above parameters of thyroidal uptake were appreciably affected by this factor in the present experiment. This is unlikely for the experiments of Childs et al. (107) suggest that the difference in the serum iodide concentration would not have appreciably affected thyroidal uptake in the present experiment. Although the magnitude of the difference between the two groups of animals in the accumulation of radioiodide may have been influenced by the serum iodide concentration, it is concluded that the nephrotic animals exhibited an increased thyroidal avidity for inorganic iodide.

Subsequently there was an increased fractional rate of release of I^{131} from the thyroid glands in the nephrotic group. If the total amount of hormone (represented by organically bound iodine) in the gland is known, then the rate of discharge of I^{131} can be used to determine the actual rate of secretion of hormone. In this experiment, the release of organic iodine from the thyroid gland was greater in the nephrotic animals by approximately 31% (See Appendix I for calculation).

The loss of PBI^{131} in the urine for the interval 20-140 hours was approximately 30 times greater in the nephrotic than in control animals. Three factors may have contributed to this: an increased release of PBI^{131} from the thyroid, increased specific activity of serum PBI, and association of PBI^{131} with the increased amount of proteins lost in the urine. Since the release of radioactivity from the thyroid glands and the relative specific activity of serum PBI were each approximately 2.6 times greater in nephrotic animals than in control animals (See Appendix II and Table V), it is obvious that these factors cannot account for the urinary PBI^{131} losses. The urinary protein-bound radioactivity represents, therefore, losses due to the proteinuria of the nephrotic state.

The urinary loss of PBI^{131} represents $19.31 \pm 6.65\%$ of the net amount of I^{131} released from the thyroid (Table VII). This is equivalent to 25% of the net I^{131} release in control animals. This must be considered in conjunction with the calculation, noted above, indicating that the rate of hormone release was 31% greater in the nephrotic animals. The results are therefore compatible with the concept that the increase in thyroid function, as manifested by increased I^{131} uptake and release, is a compensation for the urinary loss of hormone.

For the interval of 68-140 hours the urinary loss of inorganic radioactivity was greater in the nephrotic animals by a factor of 1.68. If it is assumed that the ratio $\frac{\text{thyroid uptake of } I^{131}}{\text{urinary excretion of inorganic } I^{131}}$ was the same during this interval of time as on the first day, then the ratio of values for I^{131} produced by degradation of PBI^{131} is 1 (control animals):2 (nephrotic animals). (See Appendix III for calculations). When the urinary loss of protein-bound radioactivity and the fecal excretion were then taken into account it was found that the radioiodine losses from the extrathyroidal

organic iodine pool were greater in the nephrotic animals by a factor of 1.88 compared to a factor of 2.6 for the net I^{131} release from the thyroid gland determined from measurements of thyroidal radioactivity. This discrepancy is probably due to a summation of errors involved in the calculations.

Since it has been calculated that the release of thyroid hormone in the nephrotic animals was 31% greater than in the controls, and since urinary excretion of PBI accounted for most of the increase of the thyroid hormone released, the amount of PBI remaining for tissue utilization and fecal excretion was the same in the two groups. The increase in urinary inorganic I^{131} in the nephrotic animals is thus due to the increase in specific activity of serum protein-bound iodine in the nephrotic animals.

From a comparison of the daily urinary losses in nephrotic animals of protein and protein-bound radioactivity, it appeared that PBI^{131} losses were greater in animals in which proteinuria was more severe. Because of the small amount of data it was decided to re-examine the above parameters in an experiment of a similar design.

TABLE I

ACCUMULATION GRADIENT, 18-HOUR UPTAKE, AND I^{131} RELEASE RATE

Control Animals	Accumulation Gradient	18-Hour Uptake (Counts per Minute)	I^{131} Release Rate †
#2	0.67	1350	-.0151
#3	1.11	2283	-.0277
#4	1.12	1918	-.0539
#5	2.69	2384	-.0533
#6	1.56	2629	-.0184
#7	1.85	3806	-.0338
Mean ± Standard Error	1.50 ± 0.11	2395 ± 334*	-.0337 ± .007
Nephrotic Animals			
#2	3.91	5470	-.0721
#3	2.37	5110	-.0431
#4	1.89	3338	-.0466
#6	2.65	5066	-.0482
#7	5.50	6681	-.0797
Mean ± Standard Error	3.27 ± 0.65	5133 ± 536*	-.0579 ± .002†
Statistical Significance	P<.05	P < .01	P < .05

† Fraction/24 hours.

* 181.8 ± 4.74 counts per minute = 1% uptake.

TABLE II

DAILY URINARY RADIOACTIVITY EXCRETION
AS PERCENT OF ADMINISTERED DOSE

(VALUES EXPRESSED AS MEAN ± STANDARD ERROR)

Hours after Administration of Radioiodide	Control Animals		Nephrotic Animals	
	Total I^{131}	PBI^{131}*	Total I^{131}	PBI^{131}*
0--20	57.66 ± 2.40**	.203 ± .01	46.54 ± 4.35**	0.83 ± 0.21
20--44	10.77 ± 1.20	.031 ± .003	9.59 ± 1.43	0.58 ± 0.19
44--68	2.75 ± 0.41	.014 ± .002	4.47 ± 0.65	0.73 ± 0.30
68--92	1.34 ± 0.11	.018 ± .002	2.96 ± 0.56†	0.66 ± 0.21
92--116	0.91 ± 0.06	.012 ± .002	1.97 ± 0.23†	0.35 ± 0.22
116--140	0.79 ± 0.04	.017 ± .006	1.55 ± 0.18†	0.34 ± 0.11
Total (0--140)	74.27 ± 3.01	.296 ± .013	67.22 ± 4.99†	3.47 ± 0.11

* PBI^{131} excretion was greater in nephrotic animals during all periods of measurement ($P < .01$).

** Urinary excretion of total radioactivity was greater in control animals ($P < .05$).

† Inorganic I^{131} excretion was greater in nephrotic animals during these periods of measurement ($P < .01$).

TABLE III

FECAL EXCRETION OF RADIOACTIVITY FROM 0--162 HOURS AFTER RADIOIODIDE ADMINISTRATION (VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Control Animals		Nephrotic Animals	
#2	2.68	#2	5.36
#3	4.05	#3	5.70
#4	3.20	#4	4.23
#5	11.50	#6	6.90
#6	5.08	#7	11.20
#7	5.10		
Mean ± S. E.	5.27 ± 1.31%		6.05 ± 1.43%

TABLE IV

IODINE VALUES OF SERUM AND THYROID GLAND

	Serum (μg./100 ml.)			Thyroid Gland (μg.)	
Control Animals	Protein Bound Iodine	Total Iodine	Free Iodine	Protein Bound Iodine	Free Iodine
#2	3.6	4.8	0.8	11.0	0.75
#3	3.4	5.0	1.6	9.6	1.00
#4	2.8	6.0	3.2	7.2	0.75
#6	2.0	2.8	0.8	9.2	1.45
#7	3.4	4.4	1.0	8.8	0.75
#5				5.2	1.45
Mean ± Standard Error	3.04 ± 0.567	4.60 ± 0.52	1.48 ± 0.46	8.50 ± 0.83	1.025 ± 0.17
Nephrotic Animals					
#2	1.2	2.4	1.2	6.0	1.45
#3	2.8	3.2	0.4	6.6	0.80
#4	3.8	3.8	0	6.4	1.20
#6	3.0	3.0	0	6.1	1.40
#7	0.8	2.6	1.8	5.0	1.15
Mean ± Standard Error	2.32 ± 0.292	3.00 ± 0.11	0.68 ± 0.35	6.02 ± 0.45	1.20 ± 0.11
Statistical Significance	$P > .05$	$P < .05$	$P > .05$	$P > .05$	$P > .05$

TABLE V

RADIOACTIVITY ASSAY OF A 1.0 ML. ALIQUOT OF SERUM

SEVEN DAYS AFTER RADIOIODIDE ADMINISTRATION

Control Animals	Total Radioactivity	Protein Bound Radioactivity	
	Percent of Administered Dose	Percent of Administered Dose	Relative Specific Activity*
#2	3.08×10^{-2}	2.67×10^{-2}	0.74
#3	4.45×10^{-2}	3.90×10^{-2}	1.5
#4	3.45×10^{-2}	2.75×10^{-2}	0.98
#5	4.01×10^{-2}	3.39×10^{-2}	1.69
#7	6.36×10^{-2}	5.47×10^{-2}	1.61
Mean ± Standard Error	$(4.27 \pm 0.8) \times 10^{-2}$	$(3.64 \pm 0.9) \times 10^{-2}$	1.23 ±0.18
Nephrotic Animals			
#2	5.62×10^{-2}	4.88×10^{-2}	4.07
#3	8.73×10^{-2}	7.76×10^{-2}	2.77
#4	6.94×10^{-2}	6.16×10^{-2}	1.62
#6	6.74×10^{-2}	5.07×10^{-2}	1.69
#7	3.56×10^{-2}	3.16×10^{-2}	3.95
Mean ± Standard Error	$(6.12 \pm 0.8) \times 10^{-2}$	$(5.41 \pm 0.8) \times 10^{-2}$	2.82 ± 0.53
Statistical Significance	$P > .05$	$P > .05$	$P < .01$

* $\frac{PBI^{131}}{PBI^{127}(\mu g./ml.)}$

TABLE VI

URINARY EXCRETION OF PROTEIN AND PBI^{131}
IN NEPHROTIC ANIMALS

PBI^{131} (% of Administered Dose)

Animal	Hours after I^{131} Administration					
	0--20	20--44	44--68	68--92	92--116	116--140
#2	0.97	0.62	0.80	0.50	0.37	0.37
#3	1.56	0.73	0.86	0.90	0.55	0.54
#4	0.48	0.18	0.07	0.11	0.04	0.04
#6	0.41	0.18	0.17	0.20	0.10	0.10
#7	0.72	1.19	1.77	1.31	0.71	0.72

Protein (Milligrams)

Animal	Hours after I^{131} Administration						
	0--20	20--44	44--68	68--92	92--116	116--140	Mean ± S. E.
#2	310	364	390	322	300	312	333 ± 14.6
#3	277	380	352	394	343	386	355.2 ± 18.8
#4	110	110	114	111	70	108	103.8 ± 10.2
#6	168	209	199	204	176	230	194.7 ± 13.9
#7	290	426	471	430	505	360	413.7 ± 20.3

TABLE VII

URINARY PBI^{131} EXCRETION AND THYROIDAL I^{131} RELEASE

BY THE NEPHROTIC ANIMALS FROM 42--138 HOURS

(VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Nephrotic Animals	Urinary PBI^{131} Excretion	Thyroidal I^{131} Release*	$\frac{\text{Urinary PBI}^{131}\text{ Excretion}}{\text{Thyroidal I}^{131}\text{ Release}} \times 100$
#2	2.036	13.18	15.45
#3	2.910	8.85	32.88
#4	.287	5.87	4.89
#6	.602	9.50	6.34
#7	4.425	11.97	36.97
Mean ± Standard Error	2.05 ± 0.76	9.87 ± 2.26	19.31 ± 6.65

* The percent of the administered dose released from the thyroid gland was determined from the activity at 42 and 138 hours as calculated from the iodine release rate constants. 181.8 ± 4.74 counts per minute = 1% release.

EXPERIMENT II.

Purpose:

Since there were found significant urinary losses of protein-bound iodine in nephrotic animals in Experiment I, it was considered necessary to obtain additional information on such losses in order to determine whether there was a relationship between daily urinary losses of protein and hormonal iodine.

Method:

Seven nephrotic animals weighing from 240 to 354 g. and six control animals weighing from 306 to 366 g. were used. Nephrosis had been present in the first group for at least four weeks and at the beginning of the experiment there was no evidence of edema. The mean daily loss of protein ranged from 242 to 598 mg. in the nephrotic animals. The animals were maintained on a low iodine diet administered by gavage, with distilled water being available ad lib.

After 12 days on this diet, 10 μc. of NaI^{131} without added carrier were given intraperitoneally. Complete daily urine collections were made beginning 24 hours after the administration of radioiodide and were analyzed for the protein-bound radioactivity and total protein content.

Results:

The urinary losses of protein-bound radioactivity were much greater in the nephrotic animals during all periods of measurement (Table I). The daily losses of protein in the nephrotic animals are found in Table II.

Discussion:

In Experiment I it was demonstrated that the urinary losses of protein-bound iodine were characteristic of the nephrotic state and appeared to vary with the severity of proteinuria. In the present experiment a similar

relationship was observed. To attempt to elucidate the relationship between these two parameters, the combined data from the two experiments are presented in graphic fashion (Figure I).

The figure demonstrates that there exists an abrupt change in urinary loss of protein-bound radioiodine when the daily proteinuria was 250-300 mg. When the daily protein loss was greater than this amount, the organic iodine loss increased rapidly in a roughly linear relationship to daily proteinuria. The abrupt change in hormonal iodine loss when daily protein losses were 250-300 mg. or more may be explained in two ways. The change may be explained by a distinct renal threshold for thyroxine-binding globulin or by the assumption that the thyroxine-binding globulin is a large or asymmetrical molecule which is not excreted until the glomerular "filter" is severely damaged as reflected in proteinuria of 250-300 mg. daily. Regardless of the mechanism involved, it is apparent that when the loss of protein is severe the loss of hormonal iodine may generally be expected to be more marked than would be found with protein losses of lesser severity.

TABLE I

DAILY URINARY LOSSES OF PROTEIN-BOUND RADIOIODINE
(VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Control Animals	Hours after I^{131} Administration			
	24--48	48--72	72--96	96--120
#1	.066	.005	.008	.007
#2	.015	.010	.008	.030
#3	.006	.004	.004	.060
#4	.012	.012	.012	.039
#5	.005	.012	.008	.014
#6	.008	.012	.006	.011
Mean ± S. E.	.026 ± .016	.010 ± .002	.008 ± .001	.028 ± .008
Nephrotic Animals				
#4	.878	.950	.902	.650
#5	.421	.451	.430	.336
#6	.828	.888	.455	.452
#7	.468	.585	.340	.384
#8	1.22	.950	.735	.496
#9	.83	1.23	.867	.480
#15	1.77	1.34	.885	.60
Mean ± S. E.	.916 ± .18	.913 ± .12	.659 ± .09	.487 ± .04

TABLE II

DAILY PROTEINURIA IN NEPHROTIC ANIMALS

(EXPRESSED AS MILLIGRAMS)

Animal	Hours after I^{131} Administration				Mean ± Standard Error
	24--48	48--72	72--96	96--120	
#4	380	401	374	357	379 ± 9.0
#5	324	394	334	368	355 ± 16.1
#6	242	278	231	263	253 ± 10.5
#7	284	275	280	304	285.5 ± 9.4
#8	598	381	272	462	428.2 ± 68.8
#9	471	397	448	453	442.2 ± 23.6
#15	505	588	507	547	536.7 ± 20.1

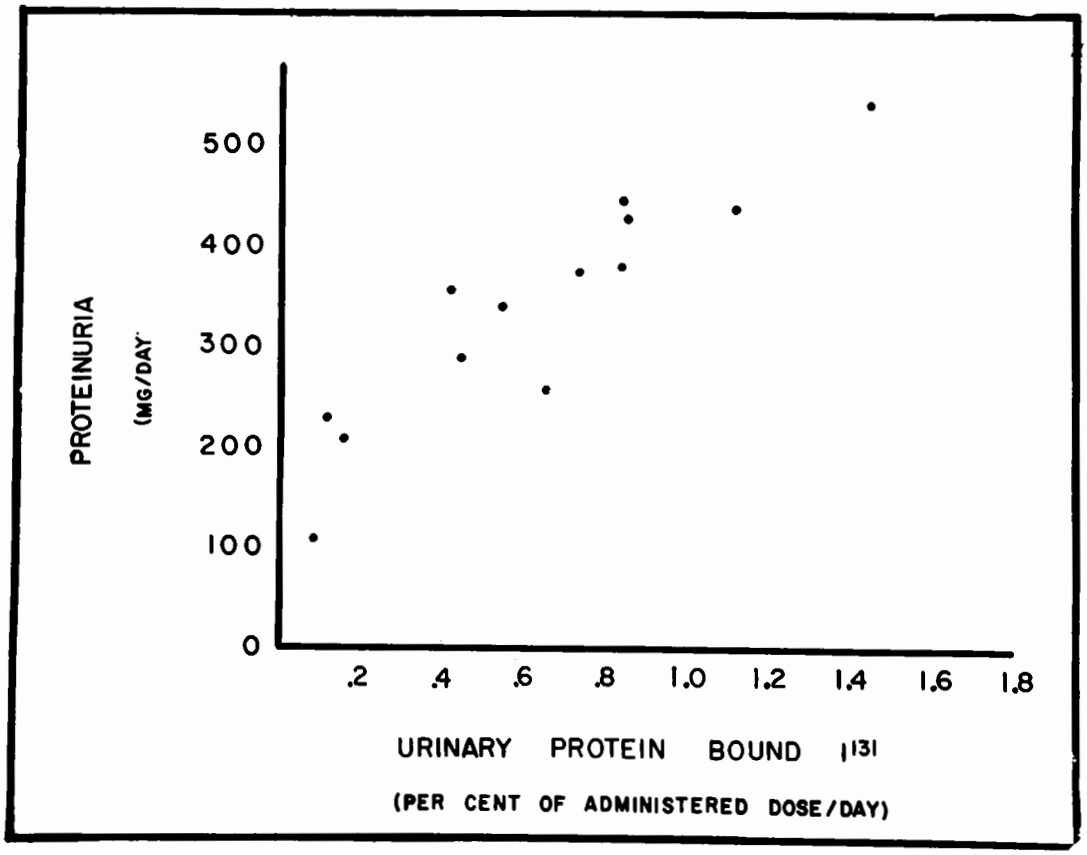


FIGURE I - RELATIONSHIP BETWEEN URINARY PROTEIN AND URINARY PBI^{131}. VALUES ARE THE MEANS FOR THE INTERVAL 48-120 HOURS AFTER ADMINISTRATION OF INORGANIC I^{131}.

EXPERIMENT III.

Purpose:

The purpose of this experiment was to investigate the thyroidal accumulation of radioiodide and the radioiodine release rate in nephrotic animals placed on a freely accessible low iodine diet.

In Experiment I in which a gavage regimen was used thyroidal I^{131} uptake and the fractional rate of release of thyroidal radioactivity in the nephrotic animals were increased in comparison to control animals. The 18-hour uptake in the control animals, however, was only 14% of the administered dose, considerably lower than anticipated. In a subsequent experiment it was found that when six normal animals were placed on a freely accessible low iodine diet for 10 days, a mean of 45% of the administered radioiodide appeared in the thyroid glands.

Since stresses of various types are known to inhibit both accumulation and release of iodine by the thyroid (109), it was considered that the procedure of tube feeding and the involved distortion of food intake rhythm may have constituted a stressful situation. Thus it was felt that these parameters of thyroid function might be more adequately measured by allowing the animals free access to a low iodine diet.

Method:

Seven nephrotic and seven control animals were used. As the weights of the animals ranged from 198 to 372 g. at the beginning of the experiment, each control animal was paired with a nephrotic animal on the basis of weight. This was done to reduce the following possible variables in the method of determining *in vivo* activity: firstly, since the distance of the thyroid gland to the radioactivity detector is inversely proportional to the circumference of the rat's neck, it was thought that variations in the

animal's weight would alter this distance; secondly, it was considered that the ease of placing the animal in a position for counting might be influenced by the animal's weight. At the time of radioiodide injection, the mean difference in weight between the paired animals was 10 ± 4 g.

The animals were placed in individual cages and the diet consumption measured daily to determine whether there was any significant difference in the iodine intake in the two groups. Such a difference might account for variations in the dilution of the total iodine pool of the animals within the groups and would make it difficult to evaluate the initial uptake and the release rate constant.

At the end of five days of low iodine feeding, 10 μc. of NaI^{131} without added carrier were injected intraperitoneally. The uptake was measured at 16 hours and the disappearance of thyroidal radioiodine determined at daily intervals from 38-158 hours after administration.

Results:

A. Dietary Intake of Iodine.

The mean daily dietary intake in the five-day period prior to administration of NaI^{131} was 18.03 ± 0.14 g. for the nephrotic animals and 15.94 ± 0.82 g. for the control animals. During the seven days of *in vivo* measurement, the nephrotic animals had a mean daily intake of 12.81 ± 0.76 g. compared to a value of 12.90 ± 0.69 g. for the control animals. There was found to be no statistically significant difference in food intake between the two groups of animals paired as to their weights.

B. 16-hour Uptake.

The mean value for the 16-hour uptake of the tracer dose of radioiodide in the nephrotic group was 3665 ± 299 counts per minute and in the control group it was 3278 ± 426 counts per minute (Table I). There was no significant

difference between these mean values or between the radioiodide uptake of the two groups of animals paired as to their weight (Table II).

C. Radioiodine Release Rate Constant.

When the release rate constants in the paired animals were compared, it was found that the release rate constant was more rapid in the nephrotic animals. This finding was statistically significant at the 1% level (Table III). From these constants the half-life of thyroidal radioiodine was found to be approximately five days in control animals and four days in nephrotic animals.

Discussion:

A. The fractional release rate of thyroidal radioiodine in the control animals was more rapid in this experiment than in Experiment I. Both of these rates are somewhat lower, however, than those found by Wolff (110). With rats on a freely accessible low iodine diet since weaning he measured thyroidal radioactivity 40 hours following radioiodide administration and at successive daily intervals. From these determinations, he found that the half-life of thyroidal I^{131} was 3.3 days. In this experiment, the mean half-life of thyroidal I^{131} in the control animals was approximately five days. Since the low iodine diet was administered for a shorter period of time in this experiment, it is possible that a lesser depletion of thyroidal iodine content occurred in this experiment. This could account for the longer half-life measured in this experiment.

In the first experiment, the mean half-life of thyroidal I^{131} was not reached in the experimental period but was estimated to be nine days in the control animals. In addition, the thyroidal I^{131} uptake and I^{131} release rate in the control animals were lower than observed in the present experiment. The difference in the two experiments may be explained on the basis of the method of feeding. Feeding by gavage introduces a certain degree of stress

in experimental animals. This has been demonstrated by Cohn et al. (109) who found a depression in I^{131} uptake and release of radioiodine in tube-fed animals. The prolonged biological half-life of thyroidal iodine in the control animals of Experiment I is an apparent addition to Cohn's data on the effect of gavage feeding in studies of thyroid function.

B. In the present experiment the 18-hour uptake was found to be similar in the two groups of animals. Since the serum iodide concentration was not determined, it is unknown whether the actual iodine uptake is similar in magnitude in the two groups of animals. If five days of a low iodine diet did not deplete the inorganic iodine pool, it could be concluded that the thyroid gland accumulates a normal quantity of iodide for hormonal synthesis.

It has been demonstrated (111) that the thyroidal iodine content is similar in nephrotic and control animals after 10 days of a freely fed low iodine diet. The thyroidal iodine content was found to be 10.0 and 9.5 µg. in nephrotic and normal rats, respectively. In the present experiment, the radioiodine release rate was 30% greater in the nephrotic animals. It seems warranted to conclude, therefore, that an actual increase in the release of thyroidal iodine occurred in the nephrotic animals.

TABLE I

IN VIVO ACTIVITY OF THYROID GLANDS

AS MEAN COUNTS PER MINUTE ± STANDARD ERROR

Hours after NaI^{131} Administration	Control (7 Animals)	Nephrotic (7 Animals)
16	3278 ± 426	3665 ± 299
38	2848 ± 344	3224 ± 378
62	2518 ± 316	2634 ± 351
86	2048 ± 252	2133 ± 304
110	1590 ± 180 *	1691 ± 256
134	1630 ± 212	1522 ± 225 **
158	1377 ± 184	1233 ± 136 **

* Mean of six determinations (data not obtained in one animal).
** Mean of five determinations (death of two animals).

TABLE II

16-HOUR UPTAKE BY CONTROL AND NEPHROTIC ANIMALS, PAIRED AS TO THEIR WEIGHT

(VALUES EXPRESSED AS COUNTS PER MINUTE)

Control	Nephrotic
2919	4473
2169	3485
3574	3188
2316	3429
2972	5047
3453	2906
5544	3128
Mean ± S. E. 3278 ± 426	3665 ± 299

TABLE III

IODINE RELEASE RATE CONSTANT FOR CONTROL AND NEPHROTIC ANIMALS, PAIRED AS TO THEIR WEIGHT

(VALUES EXPRESSED AS FRACTION OF DOSE/24 HOURS)

Control	Nephrotic
-.0663	-.0849
-.0464	-.0472
-.0752	-.1035
-.0687	-.0875
-.0818	-.1050
-.0509	-.0924
-.0724	-.0769
Mean ± S. E. -.0659 ± .006	-.0863 ± .005

EXPERIMENT IV

Purpose:

The purpose of this experiment was to study the metabolic fate of thyroxine in control and nephrotic rats by measuring the urinary excretion of inorganic and protein-bound radioactivity following the administration of radioactive l-thyroxine.

Method:

Seven nephrotic animals and six control animals were used. The range of animal weights was between 265 and 436 g. The animals were allowed free access to a diet of Purina Fox Chow and tap water. Radioactive l-thyroxine (0.417 mg.) labelled with 15 μc. of I^{131} was administered intraperitoneally in 0.5 ml. of saline to each animal. The urine was collected over 24-hour intervals following radioiodide administration and was assayed for total and protein-bound radioactivity as well as for protein content.

Results:

A. Urinary Excretion of Protein-bound Radioiodine.

The nephrotic animals excreted 0.8-26.5% of the administered radioactivity in protein-bound form during the 96-hour period of measurement (Table I). The excretion of protein-bound radioiodine by the control animals was negligible. Hence the total urinary radioactivity in this group was considered to be in an inorganic form.

B. Urinary Excretion of Inorganic Radioiodide.

The rate of inorganic radioiodide excretion was similar in the two groups of animals (Tables II and III). During the 96 hours of measurement, the mean total excretion of inorganic radioiodide was 57.62 ± 1.85% of the administered dose in the control animals and 44.66 ± 7.54% of the administered dose in the nephrotic animals. The difference between these results was not statistically significant.

Discussion:

A. Peripheral Utilization of Thyroxine.

The inorganic radioiodine resulting from peripheral utilization of organic radioiodine has two metabolic fates, namely excretion or accumulation by the thyroid gland. Although no determinations on *in vivo* activity on the latter were performed, it can be assumed that the radioiodide uptake would be negligible. (Gross and Leblond (112) found that I^{131} accumulation from peripheral degradation of radiothyroxine was less than 3% at 72 hours when animals received a diet similar to that of the present experiment.) Because of the high iodine diet, the serum concentration of inorganic iodide in the two groups of animals should be similar. Since the nephrotic animals were not edematous and since the mean weights of the control and nephrotic animals were similar (327.5 and 317.7 g., respectively), it was assumed that the size of the extrathyroidal iodine pool was not markedly different in the two groups of animals.

If the renal clearance of iodide was unimpaired, the proportion of the radiothyroxine which underwent peripheral utilization would be indicated by the daily urinary losses of inorganic radioiodide, which were similar in the two groups of animals.

It will be noted that the excretion of radioactivity in two of the nephrotic animals (#12 and #16) was much smaller than the excretion in any of the other animals within the group. These animals received the same quantity of radioactive thyroxine as the other animals and excreted significant amounts of protein in the urine. Complete collections of urine were obtained. If these two nephrotic animals whose excretion patterns of radioactivity defied explanation are not considered for comparative purposes, it is seen that the urinary excretion of radioactivity in the two groups of animals in the period of study was strikingly similar (Table III).

B. Peripheral Utilization of Thyroxine.

If the urinary excretion of inorganic radioiodide reflects the actual rate of peripheral utilization of circulating hormonal iodine, it is necessary to consider certain factors which were not measured in the present experiment. These are the volume of distribution of radiothyroxine, the concentration of serum protein-bound iodine, and the reaccumulation of radioiodide by the thyroid gland.

It was considered that the mean volume of distribution of radiothyroxine was similar in the two animal groups (Section A). It was also assumed that there was no difference in the mean serum PBI values between the groups. (From independent work (111), it was found that serum PBI values, determined 10 days after low iodine feeding ad lib., were 4.9 ± 0.3 and 4.5 ± 0.4 µg./100 ml. in control and nephrotic animals, respectively).

It can be safely assumed that the accumulation of radioiodide by the thyroid gland was negligible in the present experiment (Section A).

Accepting these considerations, the renal excretion of radioiodide permits an interpretation of peripheral hormonal degradation. Since there was no significant difference in the urinary radioiodide excretion observed in the two groups, it is concluded that the peripheral utilization of hormonal iodine was unaltered from normal levels in the nephrotic animals.

Table IV reveals that the urinary PBI^{131} loss in the nephrotic animals averaged 25% of the amount of organic radioiodine which underwent peripheral degradation. In Experiment I, it was demonstrated that the renal loss of organic iodine represented 20% of the amount released from the thyroid gland - or approximately 25% of released organic iodine whose fate was peripheral degradation. It is apparent, therefore, that the urinary loss of protein-bound radioactivity from exogenous labelling is in agreement with the corresponding loss after endogenous labelling of thyroxine.

C. Urinary Excretion of Protein-bound Radioactivity.

Appreciable amounts of protein-bound radioactivity were lost in the urine of nephrotic animals. This is seen from the fact that as much as 26.54% of the administered dose of radiothyroxine was excreted in the urine as protein-bound radioiodine.

The rate of thyroxine-I^{131} excretion (Table V) was determined for each animal by extrapolating to zero time a semilogarithmic plot of urinary PBI^{131} (as percentage of dose) on time (days after thyroxine administration). The mean value obtained was 16.31% of the dose per day which was in close agreement with the comparable estimates made for endogenous hormonal iodine in Experiment I.

By labelling of thyroxine in an endogenous manner, it was demonstrated that the urinary excretion of protein-bound iodine varied directly with the severity of proteinuria in nephrotic animals when the urinary loss of protein was above 250-300 mg. per day. Similarly, by exogenous labelling of thyroxine, the present results indicate that the hormonal iodine losses were greater with increasing proteinuria and especially above a daily protein loss of 300 mg. (Figure II).

TABLE I

URINARY EXCRETION OF PROTEIN BOUND RADIOIODINE

(VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Hours after Administration of Radiothyroxine	Control Animals						Mean ± Standard Error
	#1	#5	#6	#8	#14	#15	
0--24	.065	.190	.145	.177	.123	.100	.133 ± .019
24--48	.028	.030	.048	.041	.035	.028	.035 ± .003
48--72	0	0	0	0	0	0	
72--96	0	0	0	0	0	0	
Total (0--96)	.093	.220	.193	.218	.158	.128	.168 ± .022

Hours after Administration of Radiothyroxine	Nephrotic Animals							Mean ± Standard Error
	#3	#4	#8	#12	#16	#22	#25	
0--24	15.29	5.40	21.72	.49	.92	7.16	13.00	9.14 ± 2.97
24--48	4.92	1.71	4.16	.22	.28	4.41	2.13	2.24 ± .74
48--72	.82	.35	.47	.08	.11	.94	.32	.44 ± .12
72--96	.22	.09	.19	.04	.05	.27	.03	.13 ± .04
Total (0--96)	21.25	7.55	26.54	.83	1.36	12.78	15.48	11.95 ± 3.87

TABLE II

URINARY EXCRETION OF TOTAL RADIOACTIVITY

(VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Hours after Administration of Radiothyroxine	Control Animals *						Mean ± Standard Error
	#1	#5	#6	#8	#14	#15	
0--24	42.65	34.30	39.62	39.20	33.55	31.98	36.88 ± 1.72
24--48	11.37	13.60	15.47	14.06	14.57	12.50	13.59 ± 0.6
48--72	4.29	4.60	5.31	5.99	5.78	4.40	5.06 ± 0.3
72--96	1.58	1.00	2.07	2.51	3.05	2.25	2.08 ± 0.3
Total (0--96)	59.89	53.50	62.47	61.76	56.95	51.13	57.61 ± 2.92

Hours after Administration of Radiothyroxine	Nephrotic Animals							Mean ± Standard Error
	#3	#4	#8	#12	#16	#22	#25	
0--24	53.73	39.69	57.29	16.34	8.64	25.24	45.98	35.27 ± 7.07
24--48	20.34	12.53	14.29	3.68	2.76	34.63	10.65	14.13 ± 4.12
48--72	9.47	5.13	3.53	1.10	0.97	11.28	3.83	5.04 ± 1.50
72--96	1.35	1.25	1.38	0.39	0.45	2.99	4.28	1.73 ± 0.53
Total (0--96)	84.89	58.60	76.49	21.51	12.82	74.14	64.74	56.17 ± 13.22

* Since the urinary excretion of protein-bound radioactivity for the 96-hour period of measurement was .168 ± .021 percent of the administered dose in the control animals, the values represented in this table are indicative of urinary excretion of inorganic I^{131}.

TABLE III

URINARY INORGANIC I^{131} EXCRETION BY NEPHROTIC ANIMALS

(VALUES EXPRESSED AS PERCENT OF ADMINISTERED DOSE)

Hours	#3	#4	#8	#12	#16	#22	#25	Mean ± Standard Error
0--24	38.44	34.29	35.57	15.85	7.72	18.08	32.98	26.13 ± 4.53
24--48	15.42	10.82	10.13	3.46	2.48	30.22	8.52	11.58 ± 3.95
48--72	8.65	4.78	3.06	1.02	0.86	10.34	3.51	4.60 ± 1.37
72--96	1.13	1.16	1.19	0.35	0.40	2.72	4.25	1.74 ± 0.50
Total (0--96)	63.64	51.05	49.95	20.68	11.46	61.36	49.26	44.05 ± 10.35

CONTROL ANIMALS*

	#1	#5	#6	#8	#14	#15	Mean ± Standard Error
Total (0--96)	59.89	53.50	62.47	61.76	56.95	51.13	57.61 ± 1.92

* Values obtained from Table II.

TABLE IV

FRACTIONAL PERCENTAGE OF URINARY PBI^{131} EXCRETION RELATED TO URINARY INORGANIC I^{131} EXCRETION IN NEPHROTIC ANIMALS (96 HOURS)

Animal	Urinary Inorganic I^{131} % of Administered Dose	Urinary PBI^{131}	$\frac{\text{Urinary PBI}^{131}}{\text{Urinary Inorganic I}^{131}} \times 100$
#3	63.6	21.25	33.6
#4	51.05	7.55	14.8
#8	49.95	26.54	53.0
#12	20.68	.83	4.0
#16	11.46	1.36	11.9
#22	61.36	12.78	20.8
#25	49.26	15.48	31.5
Mean ± S. E.	43.85 ± 8.98	12.25 ± 3.67	24.22 ± 8.88

TABLE V

URINARY LOSS OF PBI^{131} AND PROTEIN AFTER ADMINISTRATION OF L-THYROXINE-I^{131}

Animal	PBI^{131} % Administered Dose Per Day	Mean Proteinuria Milligrams Per Day
#3	25	507
#4	10	358
#8	30	597
#12	0.8	266
#16	1.4	472
#22	18	619
#25	29	556
Mean ± S. E.	16.31 ± 4.73	

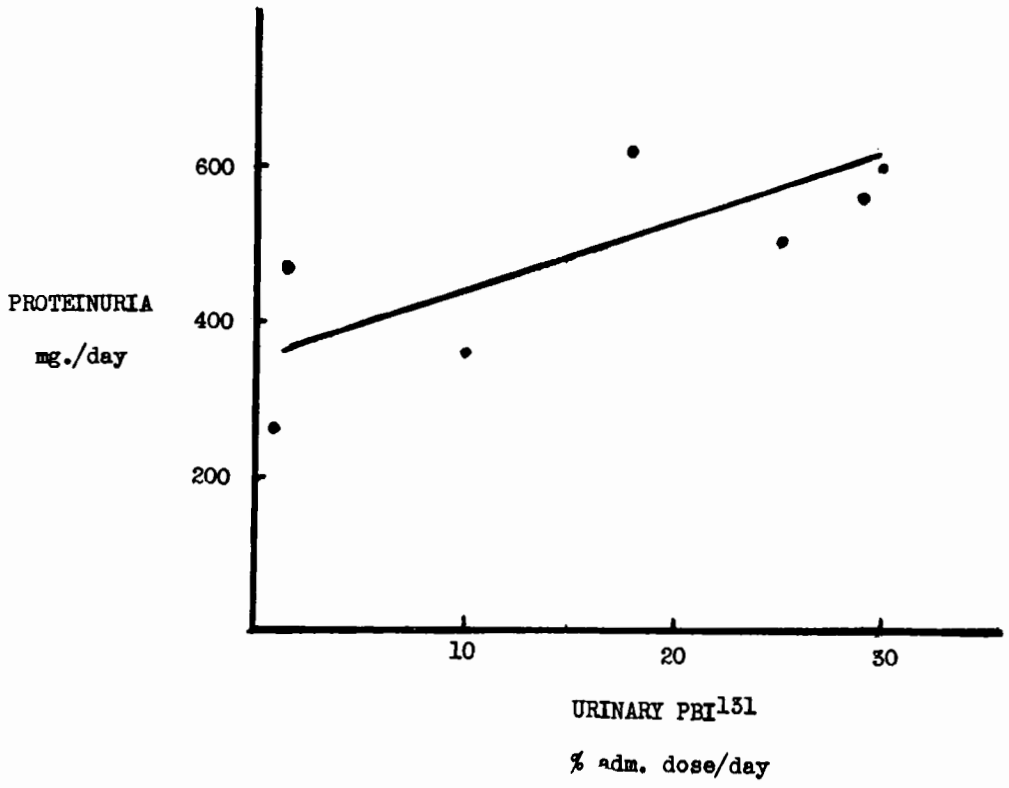


FIGURE II - RELATIONSHIP BETWEEN URINARY PROTEIN AND URINARY PROTEIN-BOUND RADIOIODINE FOLLOWING ADMINISTRATION OF THYROXINE-I^{131} TO NEPHROTIC RATS.

EXPERIMENT V

Purpose:

The purpose of this experiment was (1) to determine if the rate of oxygen consumption is altered in experimental nephrotic animals, (2) to study the changes in oxygen consumption in these animals with increasing doses of triiodothyronine, and (3) to study the effect of triiodothyronine on urinary non-protein nitrogen in control and nephrotic animals and on the proteinuria of nephrotic animals.

Method:

Seven nephrotic and five control animals were used in this experiment. All determinations of oxygen consumption were made in the morning following 15-17 hours of fasting, according to the method described previously (See Materials and Methods). Three consecutive daily measurements of oxygen consumption were obtained during the control period of the experiment. From these measurements a mean value for each animal was obtained and subsequent measurements were expressed as percent increase or decrease from these values. Urine was then collected on two successive days for determination of nitrogen content in both groups of animals and for protein content in the nephrotic animals. These measurements served as a control for further determinations during the experiment.

The dosage of triiodothyronine during the experiment was calculated for each animal on the basis of body weight during the control period. This weight in grams was converted to weight to the three-quarter power; the value obtained is referred to as the metabolic mass, and has been recommended by Brody (113) as a sound basis for comparing dosages of biotics in animals of different body size. This was considered advisable because of the wide range of body weights of the animals in this experiment, i.e. 183 to 342 g.

The effect of triiodothyronine on oxygen consumption was studied at three dosage levels, namely, 4, 8, and 16 µg./100 g. metabolic mass per day. (For a 250 g. rat, this corresponded to a daily dose of 2.7, 5.4 and 10.8 µg. of triiodothyronine per day). Triiodothyronine was injected subcutaneously in the morning at the lower dosage level, and in the morning and afternoon at the other levels.

When the dose of triiodothyronine was 4 and 8 µg./100 g. metabolic mass, the urine was collected for quantitative urine determinations (referred to above) on the days when the oxygen consumption was not measured.

The following protocol was used in studying the effect of triiodothyronine on oxygen consumption and urinary excretion of nitrogen and protein:

Day	Measurement of Oxygen Consumption	Determination of Urinary Nitrogen and Protein	Triiodothyronine Administration*
1	+		
2	+		
3	+		
4		+	
5		+	
6		+	4
7		+	4
8	+		4
9	+		4
10	+		8
11		+	8
12	+		8
13		+	8
14	+		8
15	+		8
16	+		8
17		+	8
18			8
19			16
20	+		16
21	+		16
22	+		16

* Dose of triiodothyronine expressed as ug./100 g. metabolic mass.

+ Indicates days in which measurement was made.

Results:

A. Oxygen Consumption Rates in Control and Nephrotic Animals During the Control Period.

The oxygen consumption rates of control and nephrotic animals during the control period are shown in Table I. The mean rate of oxygen consumption in the nephrotic animals was 7.16 $\pm$ 0.09 and in the control animals was 7.0 $\pm$ 0.09. There was no significant difference between these values.

B. Effect of Triiodothyronine on the Oxygen Consumption Rate.

The rate of oxygen consumption in the experimental period, expressed as a percentage increase or decrease of the oxygen consumption during the control period for the two groups of animals is found in Table II. Figure III shows the rate of increase of oxygen consumption with increasing doses of triiodothyronine in the two groups of animals.

At a dosage level of 4 µg./100 g. metabolic mass, an increase in oxygen consumption of approximately ten percent was found in both groups of animals (Table II). When this dose was doubled, there was a progressive increase in the mean rate of oxygen consumption in the two groups until the fourteenth day of the experiment, at which time an increase of approximately 30% in oxygen consumption in the two groups was noted. This dose was given for two more days and the mean rate in the nephrotic animals remained at approximately the same level as on day 14, whereas the mean rate in the control animals rose to 40% above basal levels.

When the daily dose was increased to 16 µg./100 g. metabolic mass, there was an increase in the oxygen consumption in all animals. The most marked increases were found in the control animals; however, the mean increase was not found to be significantly greater than that of the nephrotic animals.

C. Effect of Triiodothyronine on Urinary Excretion of Nitrogen and Protein.

There was an increase in nitrogen excretion in both groups of animals with triiodothyronine administration (Table III). This increase was noted after the initial injection of triiodothyronine in the control group, and was maintained at approximately the same level after further injections. The increase in nitrogen excretion in the control group was not significantly different from that in the nephrotic animals ($P > .05$).

The effect of triiodothyronine on protein excretion in the nephrotic animals is shown in Table IV. There was no consistent change in proteinuria with the doses of triiodothyronine used.

Discussion:

A. Rate of Oxygen Consumption in Experimental Nephrotic Animals.

During the control period of the experiment, it was found that the control and nephrotic animals had similar rates of oxygen consumption. In clinical nephrosis, a low basal metabolic rate is frequently found. Although the reason for this is unknown, it has been proposed that these low values are due to such causes as a protein deficiency state, under-nutrition, edema and relative hypothyroidism.

It is difficult to evaluate the above possibilities in causation in clinical nephrosis in the light of the observed normal rate of oxygen consumption in experimental nephrotic animals. However, certain conditions of the experiment are worthy of mention. The investigation was performed with non-edematous nephrotic rats which were receiving sufficient calories in their diet to maintain their body weight. Although no values of serum protein concentration were obtained in this experiment, the urinary excretion of protein in the nephrotic animals would indicate that a state of hypoalbuminemia was present (i.e. there existed a degree of protein deficiency). In

previous experiments, it was demonstrated that there probably was no decrease in the peripheral degradation of circulating hormonal iodine in experimental nephrosis. This would indicate that there was not a state of relative hypothyroidism or tissue hypometabolism in the nephrotic animals presently under investigation. The results of the present experiment may therefore be confirmatory evidence to this previous experimental consideration.

It is, however, possible that the observed finding of a normal basal metabolic rate in the nephrotic animal may constitute a difference between experimental and clinical nephrosis. Although the nature of the difference can only be speculated on, the observed difference in the present study was that the basal metabolic rate was measured in a non-edematous state. In the edematous state, the metabolic mass would be a smaller fraction of total body weight, as a consequence of the addition of metabolically inert fluid to the subject's weight. In such a state, a decrease in the basal metabolic rate would be expected if there exists no appreciable metabolic variation between experimental and human nephrosis.

The present evidence does indicate that normal rates of oxygen consumption were found in all nephrotic animals and in instances where proteinuria was marked. It would be most improbable, therefore, that abnormal BMR values would be found by studying a larger group of animals under similar conditions. The presence of a low basal metabolism would therefore seem to be an unusual occurrence in experimental nephrosis.

B. <u>The Effect of Triiodothyronine on Oxygen Consumption</u>.

In human nephrosis, an increased tolerance to thyroid preparation has been reported. These reports (7, 28) appeared in the early literature of nephrosis. The above view, however, lacks the experimental verification with preparations of thyroxine (or its analogues) of unquestioned potency. It is, therefore, unknown whether there exists a tolerance to thyroid preparations

in human nephrosis. In experimental nephrosis, there was no marked tolerance to a thyroid preparation (i.e. triiodothyronine) in the nephrotic animal as observed from the rate of increase of oxygen consumption.

The observed difference in oxygen consumption as effected by triiodothyronine in the two groups of animals may have been due to a difference in metabolic channelling of triiodothyronine in the nephrotic animal. It is unlikely that this difference can be accounted for on the basis of renal excretion of triiodothyronine since only 1% of an administered dose of l-triiodothyronine-I^{131} has been found to be lost by this route (111). The results in the present experiment indicate that there is an approximate 30% difference in oxygen consumption with triiodothyronine administration. These results are in good agreement with the changes in oxygen consumption with thyroxine administration in nephrotic animals (111). In this situation, the urinary loss of thyroxine in nephrotic animals was considered to be at least partly responsible for the observed difference.

C. The Effect of Triiodothyronine on Nitrogen Excretion:

There exists recent evidence that in certain cases of human nephrosis the amount of circulating hormone undergoing peripheral degradation is markedly diminished from normal values, as in hypothyroidism (29). Such a situation would imply that an observed catabolism of protein would be explained, at least in part, by a degree of hypothyroidism and that the administration of thyroxine should result in nitrogen retention as observed in clinical hypothyroidism (114). Although such studies have not been done in human nephrosis, the excretion of nitrogen has been measured in the nephrotic animal to detect any change in the catabolism of protein as effected by triiodothyronine.

In experimental hypothyroidism in rats, 5-10 µg. of thyroxine resulted in a decrease in nitrogen excretion (114). Such a dose is equal to the daily

thyroidal release of thyroxine (105, 115) and equivalent in metabolic activity to the dose of 4 μg./100 mg. metabolic mass used in the present experiment (116). This dose of triiodothyronine did not essentially change urinary excretion of nitrogen in the nephrotic animal. Such a finding can be explained on the assumption that there was no degree of hypothyroidism in nephrotic animals as indicated by an alteration in nitrogen excretion. Such an explanation is consistent with the considerations of other experimental results in the study.

TABLE I

OXYGEN CONSUMPTION RATES FOR CONTROL AND NEPHROTIC ANIMALS DURING CONTROL PERIOD OF EXPERIMENT

(VALUES EXPRESSED AS LITERS PER SQUARE METER PER HOUR)

Control Animals

Day	#2	#3	#4	#5	#6	#7	#12
1	7.31	6.74	7.02	7.00	7.43	7.48	6.85
2	7.17	7.03	6.19	6.13	6.79	7.03	7.38
3	6.88	7.28	6.89	6.63	6.95	7.36	7.33
Mean	7.12	7.02	6.70	6.59	7.06	7.29	7.19

Group Mean ± Standard Error 7.0 ± 0.09

Nephrotic Animals

Day	#4	#5	#6	#9	#14	#15	#16	#22
1	7.64	7.35	7.34	6.61	7.00	7.34	6.65	7.19
2	7.96	7.23	6.95	7.06	7.15	7.51	7.07	7.06
3	7.68	6.84	6.69	6.95	7.33	6.86	6.96	7.42
Mean	7.76	7.14	6.99	6.87	7.16	7.24	6.89	7.22

Group Mean ± Standard Error 7.16 ± 0.09

TABLE II

EFFECT OF TRIIODOTHYRONINE ON OXYGEN CONSUMPTION RATES OF CONTROL AND NEPHROTIC ANIMALS (VALUES EXPRESSED AS PERCENT INCREASE OR DECREASE IN OXYGEN CONSUMPTION RATE FROM MEAN VALUES OBTAINED DURING THE CONTROL PERIOD OF MEASUREMENT)

Control Animals

Dose*	Day	#2	#3	#6	#7	#12	Mean
4	8	9.7	8.1	13.5	8.1	18.5	11.6
	9	-2.2	-3.1	5.4	2.5	8.2	2.2
	10	9.8	7.7	9.1	8.2	18.1	10.6
8	12	17.1	27.1	22.1	22.6	20.2	21.8
	14	48.0	20.7	22.1	33.2	23.5	29.5
	15	40.3	51.7	24.5	20.2	57.9	38.9
	16	N.R.O.	30.6	N.R.O.	N.R.O.	52.0	41.3
16	20	81.5	N.R.O.	99.6	50.6	N.R.O.	77.3
	22	N.R.O.	N.R.O.	44.9	54.2	N.R.O.	49.5

Nephrotic Animals

Dose*	Day	#4	#5	#6	#14	#15	#16	#22	Mean
4	8	10.1	13.3	-.2	-1.3	17.3	12.0	3.8	7.9
	9	10.7	-.8	-9.6	-.4	15.9	5.4	7.9	4.0
	10	6.6	5.5	6.0	21.5	15.9	4.6	0	8.5
8	12	13.3	40.6	25.3	25.0	19.2	7.1	9.1	19.5
	14	N.R.O.	39.9	26.7	45.3	23.9	18.6	13.8	29.7
	15	22.0	40.1	24.6	23.9	23.9	18.0	11.8	23.4
	16	37.2	40.6	13.4	10.1	39.4	21.9	16.6	25.6
16	20	46.6	N.R.O.	N.R.O.	30.0	61.5	36.7	N.R.O.	43.7
	21	42.1	N.R.O.	N.R.O.	39.1	44.2	40.5	N.R.O.	41.5

N.R.O. = No results obtained.

* Dose of triiodothyronine in µg./100 g. metabolic mass.

TABLE III

DAILY URINARY NITROGEN (IN MILLIGRAMS) OF CONTROL AND NEPHROTIC ANIMALS

	Control Animals						Nephrotic Animals							
Day	#2	#3	#4	#6	#12	Mean ± Standard Error	#4	#5	#6	#14	#15	#16	#22	Mean ± Standard Error
4	269	298	320	316	256	291.8 ± 11.7	296	319	331	331	282	350	326	319.3 ± 8.7
5	290	309	334	291	278	300.4 ± 9.7	319	328	343	326	304	329	337	326.6 ± 4.1
* 6	302	301	354	287	371	323 ± 16.7	327	243	322	326	296	350	379	320.4 ± 16.2
* 7	345	SL	302	356	275	319 ± 16.3	SL	327	319	323	314	330	339	325 ± 4.1
* 8														
* 9														
**10														
**11	328	342	332	320	304	325 ± 6.4	386	344	346	336	366	369	376	360.4 ± 7.3
**12														
**13	332	329	347	382	301	338 ± 13.2	290	346	380	333	319	329	390	341 ± 13.1
**14														
**15														
**16														
**17	322	339	309	302	362	327 ± 10.8	350	313	343	352	351	365	396	353.7 ± 9.5

* Administration of tri-iodo-thyronine in a dosage of 4 γg. per 100 grams metabolic mass, administered once daily.

** Administration of tri-iodo-thyronine in a dosage of 4 γg. per 100 grams metabolic mass, administered twice daily.

SL = specimen lost

TABLE IV

DAILY URINARY PROTEIN EXCRETION IN MILLIGRAMS

IN THE NEPHROTIC ANIMALS

Day	#4	#5	#6	#14	#15	#16	#22
4	254	511	575	601	259	580	532
5	258	586	682	575	209	502	620
* 6	237	513	642	664	200	508	607
* 7	SL	461	396	600	185	523	544
* 8							
* 9							
** 10							
** 11	216	625	741	576	228	568	744
** 12							
** 13	205	740	755	841	194	498	730
** 14							
** 15							
** 16							
** 17	175	589	708	605	137	545	757

* Administration of tri-iodo-thyronine in a dosage of 4 γ g. per 100 grams metabolic mass, administered once daily

** Administration of tri-iodo-thyronine in a dosage of 4 γ g. per 100 grams metabolic mass, administered twice daily

SL specimen lost

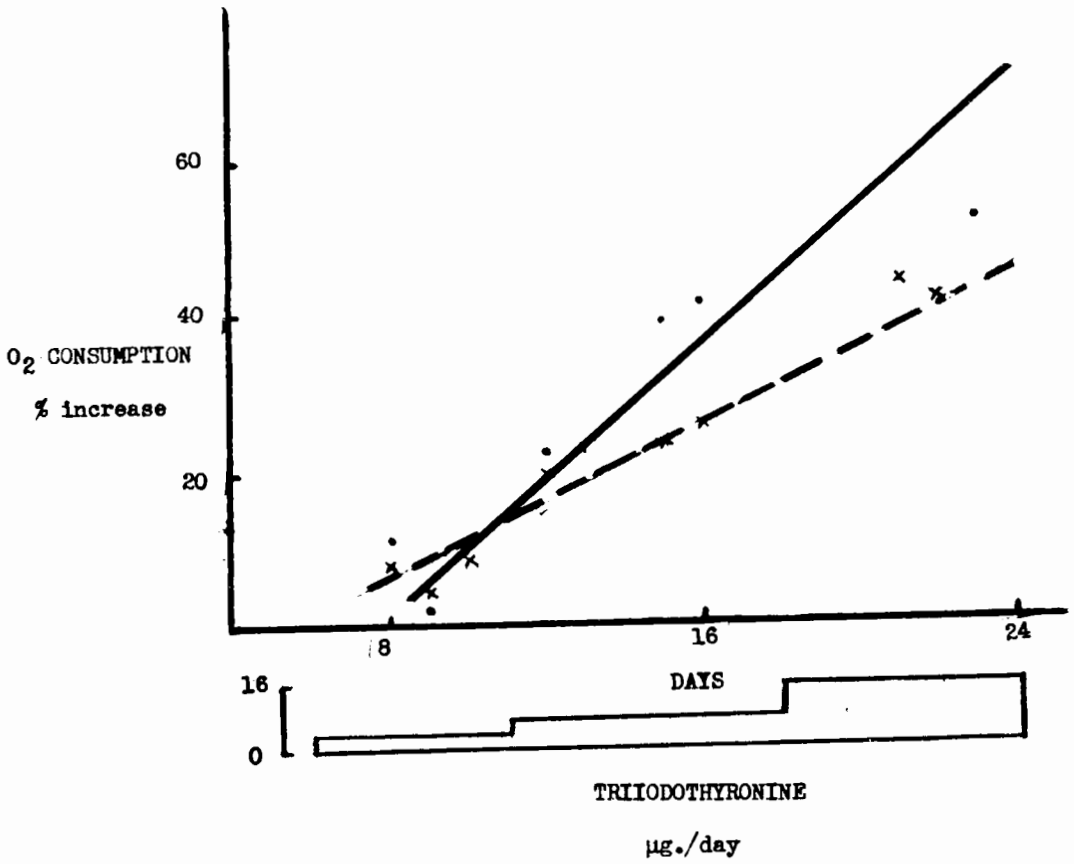


FIGURE III - RATE OF INCREASE OF OXYGEN CONSUMPTION WITH INCREASING DOSES OF TRIIODOTHYRONINE. (SOLID LINE INDICATES CONTROL ANIMALS AND BROKEN LINE INDICATES NEPHROTIC ANIMALS.)

DISCUSSION

DISCUSSION

I. Thyroid Function in Experimental Nephrosis.

The following observations have been made in this study of the thyroid function in experimental nephrotic animals: (a) appreciable urinary losses of protein-bound iodine, (b) an alteration in intrathyroidal iodine metabolism, (c) approximately equal urinary losses of radioiodide in nephrotic and normal animals following the administration of radiothyroxine, (d) the presence of a normal basal metabolic rate, and (e) absence of a marked difference in the increase of oxygen consumption or nitrogen excretion with triiodothyronine administration.

A. Urinary losses of protein-bound iodine occurred in nephrotic animals. The loss of this fraction was found to be dependent on the proteinuria in this disease state since the losses could not be accounted for by the increased release of radioiodine from the thyroid glands or by the specific activity of the serum PBI. Moreover, these losses were dependent on the quantitative loss of protein. There was observed an abrupt change in urinary loss of protein-bound iodine when the daily proteinuria was 250-300 mg.; above this daily protein loss the organic iodine losses were roughly linear to proteinuria. This indicates that the renal losses of protein-bound iodine were determined either by the existence of a threshold for thyroxine binding globulin or by the severity of damage to the "glomerular filter". In the latter instance, the implication is that the thyroxine binding globulin will not be present in the urine until the glomerular damage is severe enough to allow passage of molecules of such size and pattern as this globulin represents. Regardless as to which mechanism is operative or whether they exist in combination, the observed losses of protein-bound iodine indicate that there exists an impairment in the renal handling of thyroxine binding globulin in nephrotic animals and that the loss of this protein fraction can be roughly estimated from the daily protein excretion.

B. There was an alteration in intrathyroidal iodine metabolism in nephrotic animals. In Experiments I and III an increased I^{131} release rate was found and was representative of an actual increase in the release of thyroidal iodine. In a steady state of intrathyroidal metabolism, a commensurate increase in uptake of inorganic iodide would be expected. In Experiment I, there was an increased I^{131} uptake in nephrotic animals. However, the differences between control and nephrotic animals could be explained at least in part by the dilution of serum inorganic iodine in the nephrotic animals. In Experiment III there was no significant difference in I^{131} uptake in a situation where the radioiodide uptake was not felt to be influenced by relative differences in the concentration of serum iodide. However, no difference has been found in the thyroidal content of organic iodine under the conditions of Experiment III (111). This additional information suggests that the thyroid gland in the nephrotic animals compensated for the increased release of hormonal iodine by an increase in iodine uptake even though this was not detected in this experiment. A possible explanation for the difference between the organic iodine content of the thyroid gland in the two groups of animals in Experiment I may have been the stressful situation of tube feeding and the entailed distortion of food intake rhythm.

The fate of organically bound iodine released from the thyroid was altered in the nephrotic animal in that a significant fraction of the circulating hormonal iodine was lost by renal excretion. Thus, 15-20% (Experiments I and III) of the amount released from the thyroid gland in the nephrotic animal was lost as urinary PBI^{131}. The observed increase in the rate of release of thyroidal radioactivity may have been a secondary phenomenon to compensate for the urinary losses of hormonal iodine. The adequacy of this compensation is seen from the fact that the increase in release was

approximately equal to the renal losses of protein-bound radioiodine. The results of radiothyroxine administration appeared to confirm this concept.

C. The urinary losses of inorganic iodine were similar in nephrotic and control animals following the administration of radiothyroxine. From the design of this experiment, it was considered probable that the relationship of radioiodide EXCRETION to total hormonal iodine utilized was the same in the two groups of animals. Admittedly this assumption is only valid if the renal clearance of iodide is unchanged in the nephrotic animal. However, if the assumptions of this experiment were correct, then the supply of the hormone to the tissues was not altered in the nephrotic state.

It is probable that the sole duty of the thyroid gland is to maintain an optimal concentration of thyroid hormone in the tissues (76). Physiological variations may be expected to occur when the supply of hormone is inadequate or when the loss of hormone from the extrathyroidal iodine pool demands a compensatory increase in the rate of secretion. It is highly unlikely that the former consideration is applicable to the nephrotic animal since the experimental results revealed an increase in hormone secretion. The presence of a normal rate of peripheral utilization implies that the thyroid supplied a normal amount of hormone to the peripheral tissues, but to do so released hormonal iodine at an increased rate in order to compensate for renal loss of circulating hormone.

D. A normal basal metabolic rate was found in experimentally nephrotic animals. This finding may well represent an essential difference between human and experimental nephrosis even though the measurements in the latter instance were made in the non-edematous stage of the disease. The method of measurement in the experimental animal has been demonstrated to be a sensitive index of thyroid function in induced states of hypothyroidism and hyperthyroidism (106). Therefore, if the measurement of oxygen consumption

in the nephrotic animal reflected the activity of the thyroid gland, the observations of the present study are consistent with those of the peripheral utilization of thyroxine in the nephrotic animal and with the suggestion that the peripheral tissues received an adequate amount of thyroid hormone.

E. Oxygen consumption and nitrogen excretion were measured in nephrotic and control animals receiving triiodothyronine. An increase in the rate of oxygen consumption occurred in nephrotic and control animals with increasing doses of triiodothyronine. The rate of increase was less in nephrotic animals than in normal animals, results similar to those later obtained with thyroxine administration (111). With these two thyroid preparations, the observed rate of increase in oxygen consumption was 30% less in nephrotic animals as compared to controls. Renal loss of organic iodine may explain part of this difference in the case of thyroxine, but not in the case of triiodothyronine.

The insignificant renal loss of triiodothyronine in the nephrotic animal (111) constitutes a difference from the metabolic fate of thyroxine as found in the present experiments. This difference is probably not characteristic of the nephrotic animal but probably characteristic of the metabolism of triiodothyronine **per se**. This viewpoint is supported by the fact that the binding of triiodothyronine is weaker than that of thyroxine (116) and that the rate of biliary excretion of triiodothyronine is greater than that of thyroxine (117).

In the hypothyroid animal a significant nitrogen retention would be expected with administration of physiologic amounts of triiodothyronine (114). In the nephrotic animal nitrogen retention was not observed with this dose. This observation suggests that hypothyroidism, as indicated by changes in nitrogen excretion, was not evident in nephrotic animals.

II. Comparison of Thyroid Function in Clinical and Experimental Nephrosis.

In experimental nephrosis, urinary excretion accounts for a significant fraction of the thyroxine entering the circulation. This is compatible with the findings of Rasmussen (29) and Peters and Man (75) in human nephrosis but at variance with those of Recant and Riggs (44). It is possible that the disagreement among studies of human subjects may be related to only small differences in the severity of proteinuria in the subjects studied. In nephrotic animals, there was noted to be a marked increase in the excretion of protein-bound iodine when protein losses were greater than 250-300 mg. If a similar situation is found in the human, then it is probable that only certain cases of the nephrotic syndrome would reveal significant urinary losses of hormonal iodine - a situation in which the urinary losses of protein-bound iodine are determined by the severity of damage to the glomerular membrane or due to the existence of a renal threshold to thyroxine binding globulin.

The results in experimental nephrosis indicated that the thyroid gland probably fully compensated for the urinary loss of hormonal iodine. Such compensation is not always found in the human as evidenced by the frequent occurrence of low serum protein-bound iodine values. The degree of failure in nephrotic subjects is shown in Rasmussen's patients where thyroidal release of organic iodine was impaired to such an extent that peripheral degradation of released hormone was well below normal levels. He postulated that the failure of the thyroid gland to compensate was due to a degree of hypopituitarism secondary to protein deficiency. That this apparently did not obtain in the nephrotic rat may be a manifestation of differences in the metabolic channeling of available protein rather than of a fundamental difference in thyroid function and iodine metabolism.

It is also possible, as suggested by Rasmussen, that the nephrotic syndrome in humans undergoes sequential changes and that the changes in thyroid function are dependent on these changes. At the present time, the sequential changes of both disease states remain to be elucidated. The differences encountered in thyroid function in the experimental and human situation may, however, not be due to an essential difference between the two disease states but due to the stage at which thyroid function has been measured.

CONCLUSIONS

CONCLUSIONS

The results of this study of the thyroid function in animals with experimental nephrosis have led to the following conclusions:

1. Significant losses of protein-bound iodine occurred in the urine. This loss was approximately 15-20% of the thyroid hormone secreted by the thyroid.

2. The urinary losses of protein-bound iodine were dependent on the severity of proteinuria.

3. The increase in the rate of release of thyroidal iodine is apparently compensatory to the urinary losses of protein-bound iodine.

4. The thyroid gland probably fully compensated for the metabolic disturbance in iodine metabolism as evidenced by the normal rate of peripheral degradation of radiothyroxine.

5. The basal metabolic rate was normal.

6. The changes in oxygen consumption on administration of increasing doses of triiodothyronine suggested but did not conclusively demonstrate a decrease in response to this hormone.

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APPENDIX I

The release of organic iodine from the thyroid gland (μg./day) = thyroidal content organic iodine (μg.) x fractional I^{131} release rate (fraction/day). The daily release of organic iodine for the two groups of animals is listed below:

NEPHROTIC ANIMALS				CONTROL ANIMALS			
No.	PBI* μg.	I^{131} Release Rate** Fraction/Day	μg./Day	No.	PBI* μg.	I^{131} Release Rate** Fraction/Day	μg./Day
2	6.0	.0721	.43	2	11	.0151	.166
3	6.6	.0431	.28	3	9.6	.0277	.258
4	6.4	.0466	.30	4	7.2	.0539	.388
6	6.1	.0482	.29	5	5.2	.0533	.277
7	5.0	.0797	.40	6	9.2	.0184	.169
				7	8.8	.0338	.297
Mean ± S.E.	6.0 ± 0.45	.0579 ± .002	.34		8.50 ± 0.8	.0337 ± .007	.26

* The above calculations assume that there was no depletion of thyroidal stores of organic iodine during the experimental period.

** Since reaccumulation of radioiodide occurred continually during the experiment, the true rate of I^{131} release from the thyroid is equal to the measured rate plus the rate of I^{131} reaccumulation. Although the latter figure is unknown, it was considered to be minimal since Gross and Leblond (112) have found that thyroidal reaccumulation of radioiodide from peripheral degradation of radiothyroxine was less than 3% of the administered dose at 72 hours.

The mean thyroidal iodine release is therefore approximately 31% greater in the nephrotic animals:

$$\frac{\text{Nephrotic}}{\text{Control}} = \frac{.34}{.26} = \frac{1.31}{1}$$

APPENDIX II

The percent of the administered dose of radioiodide released from the thyroid gland as organic radioiodine has been established from the 42 and 162-hour counts as calculated from the iodine release rate constant of each animal. The difference in these values was converted to percent of the administered dose released by reference to the *in vitro* measurement of thyroidal radioactivity.

Thyroidal Radioactivity

	No.	42 Hours cts./min.	162 Hours cts./min.	1% Thyroidal Radioactivity as cts./min.	% Dose Released
CONTROL	2	1610	1350	214	1.22
	3	2500	1800	183	3.82
	5	2740	1430	166	7.89
	6	2650	2130	175	2.97
	7	3940	2560	186	7.42
	Mean ± S.E.				4.66 ± 1.29
NEPHROTIC	2	5010	2210	183	15.30
	3	5150	3200	162	12.04
	4	3050	1780	195	6.51
	6	4800	2750	161	12.73
	7	4890	2340	185	13.78
	Mean ± S.E.				12.07 ± 1.50

Since the mean values for release of thyroidal radioactivity as percent of the administered dose were 12.07 in the nephrotic animals and 4.66 in the control animals, this indicates that the relase of thyroidal radioactivity was 2.6 times greater in the nephrotic animals.

$$\frac{\text{Nephrotic}}{\text{Control}} = \frac{12.07}{4.66} = \frac{2.6}{1}$$

APPENDIX III

There are three routes of metabolism for hormonal radioiodine released from the thyroid gland: (a) degradation of hormonal radioiodine to non-precipitable components, i.e. inorganic iodide and iodinated tyrosine; (b) loss of protein-bound radioiodine in the urine; and (c) loss of radioiodine in the feces.

A. Degradation of Hormonal Radioiodine to Inorganic Iodine:

The ratio for the excretion of inorganic iodide in the initial 18 hours is: $\frac{\text{Control}}{\text{Nephrotic}} = \frac{57.46}{47.79} = \frac{1}{0.8}$.

During the latter three days of the experiment (68-140 hours) the ratio for the inorganic iodide excretion is: $\frac{\text{Control}}{\text{Nephrotic}} = \frac{3.0}{5.03} = \frac{1}{1.68}$. On the assumption that the excreted I^{131} was entirely derived from degradation of hormonal radioiodine, and that such radioiodide underwent the same metabolic fate as that originally administered, it may be calculated that the ratio of the amounts degraded was: $\frac{\text{Control}}{\text{Nephrotic}} = \frac{1}{1.68/0.8} = \frac{1}{2}$.

B. Loss of Protein-bound Radioactivity in the Urine from 68-140 Hours:

The ratio of urinary PBI^{131} loss to urinary inorganic radioiodine excretion is $\frac{.048}{3.00} = \frac{.016}{1}$ for control animals, and $\frac{1.15}{5.03} = \frac{0.23}{1}$ for nephrotic animals.

C. Loss of Radioactivity in the Feces:

Johnson and Albert (108) found that in the rat 3% of an administered dose of radioiodide appeared in the feces in 72 hours, the amount excreted having an approximate linear relationship with time. In the seven days of the present experiment, 5.27 $\pm$ 1.31% of the administered dose and 6.05 $\pm$ 1.43% of the administered dose were the amounts excreted in the feces by

the control and nephrotic animals, respectively. If a similar linear relationship with time exists in this experiment, approximately 0.8% of the administered dose is assumed to represent the mean daily fecal loss of radioactivity for the two groups of animals. Therefore, the ratio of fecal loss of radioactivity to the total inorganic iodine loss in the urine is $\frac{2.4}{3.0} = \frac{0.8}{1}$ for the control animals and $\frac{2.4}{5.03} = \frac{0.48}{1}$ for the nephrotic animals.

D. Conclusion:

Since the relative hormonal loss via urinary inorganic iodine is 1 for the control group and 2 for the nephrotic group, the amount of thyroidal I^{131} released from the thyroid gland represented by the urinary and fecal losses during the period of 68-140 hours after radioiodide administration is

$$\frac{\text{Control}}{\text{Nephrotic}} = \frac{1 + .016 + 0.8}{2 + 0.46 + 0.96} = \frac{1.82}{3.42} = \frac{1}{1.88} .$$