Thyroid Function in a Uremic Rat Model

EVIDENCE SUGGESTING TISSUE HYPOTHYROIDISM

VICTORIA S. LIM, CARLOS HENRIQUEZ, HISAO SEO, SAMUEL REFETOFF, and ENIO MARTINO, *Renal Division, Department of Medicine, Michael Reese Hospital and Medical Center, and Thyroid Study Unit, Department of Medicine, University of Chicago, Chicago, Illinois 60616*

ABSTRACT The main objective of this study was to determine whether the principal abnormality of thyroid function observed in patients with chronic renal failure, low serum triiodothyronine (T_3) concentration, causes hypothyroidism at the tissue level. A partially nephrectomized (Nx) uremic rat model was developed and the following parameters of thyroid function were assessed: serum total thyroxine (TT_4), total T_3 (TT_3), and thyrotropin and liver T_3 content, and activity of two thyroid hormone-dependent enzymes, mitochondrial α-glycerophosphate dehydrogenase (αGPD) and cytosol malate dehydrogenase (MDH). The results were compared to those of intact control (C), thyroidectomized (Tx), and nephrectomized-thyroidectomized (NxTx) littermates.

Results expressed as mean±SEM showed that Nx rats had a fivefold increase in blood urea nitrogen, (112±20 mg/dl in Nx, and 22±1 mg/dl in C) and manifested all the changes of of thyroid function observed in uremic men, including a low serum TT_3 level (30±7 ng/dl in Nx and 50±6 ng/dl in C). In the liver, T_3 was significantly reduced (18±2 ng/total liver in Nx and 35±3 ng/total liver in C) as well as the activities of αGPD (8.8±1.0 and 16.1±1.5 ΔOD/min per total liver in Nx and C, respectively) and MDH (6.3±1.6 and 12.6±2.2 U/total liver in Nx and C, respectively). The reduction in liver enzyme activities correlated significantly with the decrease in T_3 content.

The changes in Tx rats were as expected, showing a profound reduction in serum hormone levels, liver T_3 content, and liver enzyme activities. Serum thyrotropin was markedly elevated to 2,390±212 ng/ml as compared to 703±61 in C and 441±87 ng/ml in Nx rats. The NxTx rats showed the combined effects of nephrectomy and thyroidectomy; blood urea nitrogen was elevated to 203, and serum levels of TT_4, TT_3, and thyrotropin were 0.4, <10, and 2,525, respectively. Total liver T_3 and αGPD and MDH were strikingly low; the corresponding values were 3.5, 2.4, and 2.5.

L-triiodothyronine replacement (0.4 μg/100 g body wt/d) for 4 wk in the Nx rats resulted in significant increases in liver enzyme activities, αGPD and MDH rose by 70 and 60% over their respective basal values without alteration in the severity of azotemia.

From these data, we conclude that the reduction of liver T_3 content in the uremic rats, accompanied by a decrease in αGPD and MDH activity, indicates the presence of hypothyroidism at the tissue level. Restoration of enzyme activities toward normal levels after T_3 administration provided further supporting evidence that the diminution in liver enzyme activity was causally related to tissue T_3 deficiency.

INTRODUCTION

Patients with chronic renal failure have low serum levels of total triiodothyronine (TT_3)[1] due, in part, to a defect in the peripheral conversion of thyroxine (T_4) to triiodothyronine (T_3) (1–11). However, the physiologic significance of the low serum T_3 concentration in a variety of conditions (4–6), including uremia, has not been clearly defined. Thus, the question of whether uremic patients are hypothyroid remains unanswered. Investigators who propose that uremic patients are euthyroid base their conclusion on the observation that serum thyrotropin (TSH) is not elevated and thyroid

This work was presented in part at the 12th Annual Meeting of the American Society of Nephrology, Boston, 18–20 November 1979, and the 8th International Thyroid Conference, Sydney, Australia, 3–8 February 1980.

Received for publication 7 February 1980 and in revised form 20 June 1980.

[1] *Abbreviations used in this paper*: BUN, blood urea nitrogen; C, control; FT_4I_x, free thyroxine index; GPD, glycerophosphate dehydrogenase; MDH, malate dehydrogenase; Nx, nephrecotomized; NxTx, nephrectomized-thyroidectomized; T_3, triiodothyronine; T_4, thyroxine; TSH, thyrotropin; TT_3, total T_3; TT_4, total T_4; Tx, thyroidectomized.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/11/0946/09* $1.00
Volume 66 November 1980 946–954

function tests, independent of hormone measurements such as the basal metabolic rate, the systolic time interval, and the deep tendon reflex relaxation time, are normal (7). The argument for hypothyroidism is purely theoretical and is based on the concept that T_3 is the hormone through which the major metabolic effects of T_4 are exerted (12, 13). The absence of TSH elevation should not be regarded as unequivocal evidence of euthyroidism because disturbances in the hypothalamic-pituitary axis are known to occur in uremia (14–17). The controversy regarding thyroid status in uremia is partly because most studies have concentrated on the measurement of thyroid hormone in serum. There is no information available on the content of T_3 in tissues. This is important because the bulk of extrathyroidal T_3 is generated in the peripheral tissues, at the sites of action (12, 13).

We studied thyroid function in a uremic rat model. T_3 concentrations in serum as well as in liver were measured. In addition, the activities of two liver enzymes regulated by thyroid hormone (18–21), mitochondrial α-glycerophosphate dehydrogenase (αGPD) and cytosol malate dehydrogenase (MDH), were determined. The results showed an early reduction in liver T_3 content accompanied by a significant diminution in both enzyme activities. These data, coupled with the correction of the observed enzyme deficiencies after T_3 treatment, suggest that hypothyroidism at the tissue level accompanies the uremic state.

METHODS

Experimental design

Four groups of rats, control (C), thyroidectomized (Tx), nephrectomized (Nx), and nephrectomized-thyroidectomized (NxTx), were examined. The Nx rats were studied 4, 5, and 6 wk after nephrectomy. Male Sprague Dawley rats, weighing 120–150 g at the start of each experiment, were obtained from Animal Resource Laboratory, Madison, Wis. Uremic rats were prepared by a two-stage 5/6 nephrectomy. The upper and lower poles of the left kidney were removed by ligation and the raw surfaces were cauterized to prevent bleeding and minimize compensatory hypertrophy. The entire right kidney was removed 5–7 d later. Only 50–60% of the Nx rats were used; 30–40% died within 72 h of the second stage of nephrectomy and another 10% were discarded because blood urea nitrogen (BUN) was insufficiently elevated (<50 mg/dl). Thyroidectomy was performed by a standard surgical technique. Mortality in the Tx group was negligible and hypothyroidism always ensued. In the NxTx rats, thyroidectomy was done 5 wk after nephrectomy. As these rats appeared very ill, studies were performed 3–5 d after thyroidectomy. The mortality in this group was ~75%. About 70% survived the second stage of nephrectomy but only 25% survived the subsequent thyroidectomy. Unless otherwise specified, rats were fed regular chow and given access to tap water ad lib. Tx and NxTx rats were given 0.9% calcium chloride as the drinking water. In addition, NxTx rats received 10 mg of calcium gluconate, by intraperitoneal injection, every other day after thyroidectomy.

In another experiment, only C and 6-wk Nx rats were used. Each group was further divided in half; one-half received no treatment whereas the other received exogenous L-triidothyronine (Sigma Chemical Co., St. Louis, Mo.) supplement at 0.4 μg/100 g of body wt/d by intraperitoneal injections for 4 consecutive wk starting 2 wk after the second stage of nephrectomy. The dose was considered to be optimal for T_3 replacement based on the studies by Garcia et al. (22) on the effect of T_3 on TSH response to TSH-releasing hormone in Tx rats.

Rats were killed by cardiac exsanguination under light ether anesthesia. The liver was perfused *in situ* with normal saline solution until light tan. The entire liver was removed and weighed. 1 g was frozen on dry ice immediately and kept at −30°C until extraction was done on another day for measurement of T_3 content. Additionally, 1 gram of liver was processed freshly for quantitation of mitochondrial αGPD activity and another 2 g for determination of MDH activity. The two kidneys of C and Tx rats and the kidney remnants of Nx and NxTx rats were removed and weighed. Serum was saved for measurements of BUN, total thyroxine (TT_4), free thyroxine index (FT_4I_x), T_4 binding capacity, TT_3, and thyroid stimulating hormone (TSH). In the rats that received exogenous T_3 treatment, additional serum samples were obtained 2 h after the last T_3 injection for determination of TT_3 levels.

As serum thyroid hormone concentrations (23) and αGPD and MDH activities (24) vary with age, littermates were used for each experiment. Execution of surgical procedures was staggered so that rats in each experimental group were of the same age at the time of study.

Analytical procedures

Determination of T_3 content. Liver T_3 was extracted using the method of Nejad et al. (25). Briefly, 1 g of frozen liver was homogenized in 2 ml of chilled water containing 50 μM propylthiouracil. 10 μl of the homogenate was saved for protein determination and the remainder was extracted twice with 6 ml of 95% ethanol. An equal volume of chloroform (12 ml) was added to the pooled ethanol extract placed in a separatory funnel. T_3 was extracted from the ethanol-chloroform mixture using 1/3 vol of 2 N NH_4OH three times. The pooled aqueous phase was concentrated in a flash evaporator and subsequently lyophilized. The residue was reconstituted in 0.5 M phosphate buffer containing 1% bovine serum albumin, pH 7.5, and was stored at −20°C until analyzed for T_3 content.

Recovery was monitored in every batch of T_3 extraction by addition of sufficient [^{125}I]T_3 to a second 1 g piece of liver before extraction and corrections for recovery were carried out for each sample.

Assessment of enzyme activity. Mitochondrial αGPD (EC 1.1.1.8) activity was measured in the mitochondrial fraction prepared from 1 g of freshly perfused liver. Homogenization was done in 9 vol of 0.25 M sucrose, 50 mM Tris-HCl, pH 7.5. After centrifugation at 1,000 *g*, 4°C for 10 min, the supernate was again centrifuged at 12,000 *g*, 4°C for 10 min, and the resultant pellet containing mitochondria was resuspended in 1 ml of 0.125 M phosphate buffer, pH 7.5. αGPD activity was analyzed in triplicate (three sets of blanks and samples) according to the method of Lee and Lardy (19). Reactions were carried out for 15 min. The difference in the mean optical density in the assay tubes and in the blanks read at 500 nm (ΔOD) and the protein concentration, measured in an aliquot of the mitochondrial preparation, was used for the calculation of the enzyme activity expressed as ΔOD per minute per total liver and per mg of protein.

Cytosol MDH (EC 1.1.1.40) activity was measured in 2 g of fresh liver. Homogenization was done in 3 vol of chilled 0.25 M sucrose. The homogenate was centrifuged at 9,000 *g* 4°C for 10 min, and the supernate was recentrifuged at 100,000 *g*,

4°C for 60 min. This supernate, containing the cytosol fraction, was passed through a 0.22 μMillipore filter (Millipore Corp., Bedford, Mass.) and diluted 5- or 10-fold with a solution containing 10 mM EDTA, 50 mM Tris HCl, 200 mM magnesium acetate, and 2 mM 2-mercaptoethanol, pH 7.4. MDH activity was then measured using the method of Ochoa (26) as modified by Hsu and Lardy (27), by monitoring the reduction of NADP to NADPH, producing changes in the optical density at 340 nm. Appropriate blanks, without added substrate, and a standard preparation of purified chicken liver enzyme (Sigma Chemical Co., St. Louis, Mo.) were used in each assay. Enzyme activity was expressed as units of MDH per milligram of cytosol protein and per total liver.

Other measurements. Serum TT_4 was measured by a competitive binding assay and the results of a resin T_4 uptake test were used to calculate FT_4I_x as described (28).

Serum and tissue T_3 were measured by a radioimmunoassay (29) using a specific antiserum, a gift from Dr. G. Burke (Cook County Hospital, Chicago, Ill.). Determinations in serum were carried out in the presence of 8-anilino-1-naphthalene sulfonic acid and those in tissues were done in the absence of this substance. Corresponding standard curves contained hypothyroid rat serum or buffer only. The sensitivity of the assay was 10 ng/dl and the intra- and interassay coefficients of variation were 7 and 15%, respectively.

Serum T_4 binding capacity was measured by saturation (30) and expressed as percent of control.

Serum TSH was measured by a radioimmunoassay (31) using materials from two kits (Nos. 3 and 4) obtained from National Institute of Arthritis Metabolism and Digestive Diseases. The assay sensitivity was 100 ng/ml. The intra- and interassay coefficients of variation were 3.1 and 16.4%, respectively.

The BUN was determined by the standard microcolorimetric technique with diacetyl monoxime and ferric nitrate reagents.

Protein concentration was measured using the method of Lowry (32).

Statistical analyses, including Student's *t* test, analysis of variance, and linear regression analysis, were done by using the Biomedical Computer Program package (Health Science Computer Facility, University of California, Los Angeles, Calif.). Group data are presented as mean±SEM.

RESULTS

Kidney and thyroid function of all groups of rats are compared in Table I. After nephrectomy, mean BUN in the Nx rats increased fivefold and the mean weight

TABLE I
Thyroid and Renal Function in C, Tx, Nx, and NxTx Rats

	Body weight	Kidney weight	Liver weight	BUN	Serum			
					TT_4	FT_4I_x	TT_3	TSH
	g	g	g	mg/dl	μg/dl		ng/dl	ng/ml
C (11)	373	2.8	14.5	22	4.7	6.3	52	703
	±12	±0.1	±0.9	±1	±0.3	±0.4	±6	±61
Tx (8)	349	2.1	11.6	28	0.4	0.4	18	2249
	±8	±0.1	±0.6	±2	±0.1	±0.1	±2	±136
P	NS	<0.001	<0.02	<0.001	<0.001	<0.001	<0.001	<0.001
Nx4 (14)	283	1.1	12.6	112	3.5	4.7	43	510
	±13	±0.1	±0.6	±11	±0.3	±0.4	±4	±58
P	<0.001	<0.001	NS	<0.001	<0.01	<0.02	NS	<0.05
Nx5 (12)	276	1.0	12.1	113	3.3	4.6	44	383
	±13	±0.1	±0.8	±10	±0.3	±0.5	±5	±56
P	<0.001	<0.001	NS	<0.001	<0.01	<0.02	NS	<0.001
Nx6 (11)	256	1.1	12.6	112	3.3	4.6	30	441
	±25	±0.1	±0.8	±20	±0.4	±0.6	±7	±87
P	<0.001	<0.001	NS	<0.001	<0.01	<0.02	<0.02	<0.02
NxTx (6)	173	0.8	8.0	203	0.4	0.5	<10	2525
	±8	±0.1	±0.2	±24	±0.2	±0.2	±0	±292
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	—	<0.001
DF*	5/56	5/56	5/56	5/56	5/56	5/56	5/56	5/56
F ratio	17.909	63.226	6.37	22.171	31.334	28.535	9.747	83.906
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

All values are given as mean±SEM. (Nx), 4, 5, and 6 indicate weeks postnephrectomy. The number of rats per group is in parenthesis. The significance of the difference between the means of each experimental group and the control was assessed by the Student's *t* test.

* Individual values from all six groups were tested by the analysis of variance; DF, the degree of freedom, F ratio, and *P* values are listed.

of the kidney remnant was <40% of the combined kidney weights of the control littermates. Mean body weight was also decreased by ~30%. The postnephrectomy hypertrophy of the kidney appeared to have been completed during the first 4 wk after the second stage operation as there was no difference in either the mean kidney weights or the mean BUN levels among the 4-, 5-, and 6-wk Nx rats. Thyroid function also differed significantly in the Nx rats. Mean serum TT_4 and FT_4I_x were decreased by ~30% 4 wk postnephrectomy and remained stable during the subsequent 2 wk of observation. Serum T_4 binding capacity was not affected by nephrectomy, being 98±6% in the 6-wk Nx rats as compared to 100±4% in the C group. The mean serum TT_3 showed a declining trend during the 4th and 5th wk, but the level became significantly reduced to 30 ng/dl at 6 wk postnephrectomy. Mean serum TSH concentrations were significantly lower in all three Nx rat groups. The Tx rats showed changes typical of severe hypothyroidism: mean serum TT_4 and FT_4I_x were reduced to <10%, mean serum TT_3 to about 35%, and mean serum TSH increased by 300% of control littermates. The slight but significantly elevated BUN in the Tx rats is consistent with the known effect of thyroid hormone in augmenting glomerular filtration rate (33). The NxTx rats showed the combined effects of nephrectomy and thyroidectomy. Mean serum TT_4 and FT_4I_x were reduced to the same extent as the Tx littermates, but the reduction in serum TT_3 to <10 ng/dl was more severe than could be accounted for by thyroidectomy alone. Mean serum TSH was elevated to a comparable degree as in the Tx rats. When compared to Nx rats, NxTx rats had a mean BUN level twofold higher, and the kidney remnant weighed an average of 20% less. These rats generally appeared very ill and their mean body weight was 40% less than the nonthyroidectomized uremic rats.

T_3 content and the activity of the two thyroid-hormone dependent enzymes in the liver are summarized in Table II. Nephrectomy resulted in a significant reduction in liver T_3 content as well as the activities of the thyroid hormone-dependent enzymes. Mean total

TABLE II

Liver T_3 Content, Mitochondrial αGPD, and Cytosol MDH Activities in C, Tx, Nx, and NxTx Rats

	T_3			αGPD		MDH	
	ng/total liver	*ng/g*	*pg/mg protein*	*ΔOD/min/ total liver*	*ΔOD/min/ mg protein*	*units/total liver*	*units/mg protein*
C (11)	35.4 ±3.1	2.47 ±0.21	19 ±1	16.13 ±1.54	1.700 ±0.139	12.64 ±2.21	0.037 ±0.003
Tx (8)	5.3 ±0.9	0.45 ±0.05	4 ±0.4	5.07 ±0.62	0.595 ±0.055	5.76 ±1.15	0.019 ±0.002
P	<0.001	<0.001	<0.001	<0.01	<0.001	<0.05	<0.001
Nx4 (14)	28.8 ±3.1	2.32 ±0.20	20 ±0.2	10.70 ±0.86	1.351 ±0.107	—	—
P	NS	NS	NS	<0.01	NS		
Nx5 (12)	20.5 ±3.5	1.62 ±0.19	13 ±2	10.29 ±1.03	1.268 ±0.092	—	—
P	<0.01	<0.01	<0.01	<0.01	<0.02		
Nx6 (11)	18.5 ±2.4	1.45 ±0.14	12 ±1	8.77 ±1.00	1.061 ±0.101	6.26 ±1.59	0.024 ±0.003
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.02
NxTx (6)	3.5 ±0.3	0.44 ±0.04	5 ±0.4	2.36 ±0.27	0.646 ±0.123	2.48 ±0.15	0.020 ±0.001
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005	<0.001
DF*	5/56	5/56	5/56	5/56	5/56	3/22	3/22
F ratio	16.641	18.109	15.84	17.854	14.05	9.148	11.837
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0005	<0.0005

All values are represented as means±SEM. (Nx)4, 5, and 6 refer to weeks postnephrectomy. The number of rats per group is in parenthesis. The significance of the difference between the means of each experimental group and the control was assessed by the Student *t* test.

* Individual values from all six groups were tested by the analysis of variance; DF, the degree of freedom, F ratio, and *P* values are summarized.

liver T_3 content and total liver mitochondrial αGPD activity were reduced on the fourth week; the decrease became statistically significant on the 5th wk and further declined on the 6th wk postnephrectomy. Results are similar when expressed in terms of total tissue or mitochondrial protein concentration. Cytosol MDH activity was examined only in the 6-wk Nx rats; both total liver MDH and MDH/milligrams of cytosol protein were significantly reduced. The changes after Tx were as expected: liver T_3 and αGPD and MDH activities were all markedly reduced. In the NxTx rats, mean liver weight was ~50% that of control rats so that total liver T_3 content and total liver enzyme activities were further decreased to levels lower than either Tx or Nx alone. As the protein concentration in the liver and the subcellular fractions were also greatly reduced, T_3 content and enzyme activities in this group of rats when corrected for protein concentration were reduced to the same level as in the Tx littermates.

It appeared that the reduction in αGPD and MDH activities in the Nx rats correlated best with the reduction in liver T_3 content (Figs. 1 and 2). Significant positive correlations were found when the Nx rats were examined independently of the C and Tx rats. There was no significant difference when the two regression lines were compared. Control rats having higher T_3 content and greater enzyme activities were grouped in the upper section whereas the Tx rats with the least amount

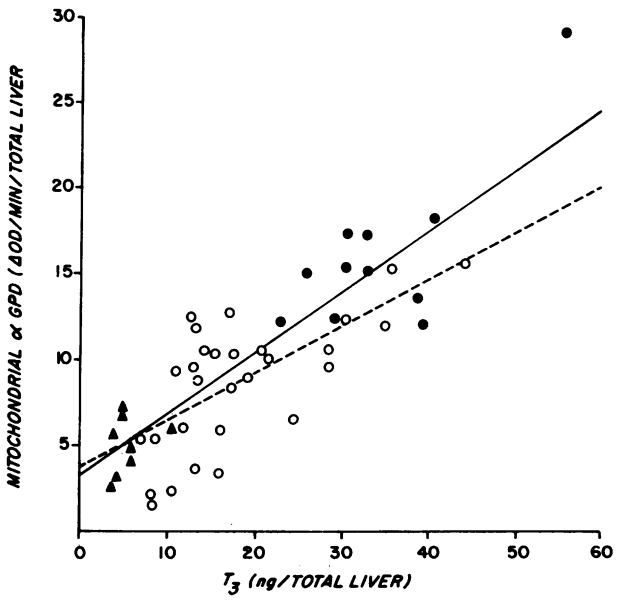

FIGURE 1 Correlation between total liver T_3 and total liver mitochondrial αGPD. Two linear regression lines are presented. The solid line was derived from C (●) and Tx (▲) rats (n = 19, r = 0.896, and $P < 0.01$). The dotted line was constructed from 5- and 6-wk Nx (○) rats (n = 29, r = 0.666, and $P < 0.01$). There is no statistical difference between the two lines.

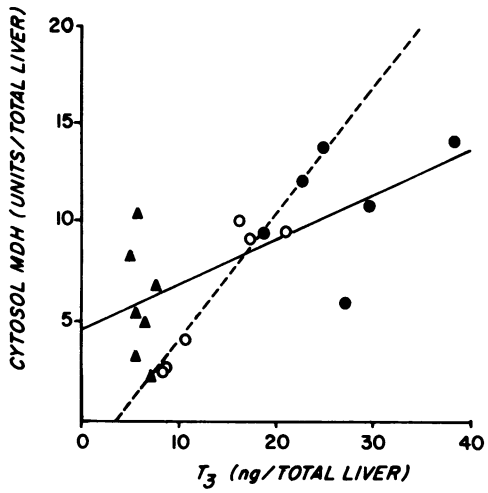

FIGURE 2 Total liver cytosol MDH was plotted as a function of total liver T_3 showing excellent correlations between these two parameters. The solid line (n = 13, r = 0.688, and $P < 0.01$) was derived from C (●) and Tx (▲) rats, whereas the dotted line (n = 6, r = 0.946, and $P < 0.01$) was based on the data of 6-wk Nx (○) rats. The two regression lines are not different statistically.

of T_3 and the lowest enzyme activities were tightly packed in the left lower corner. The Nx rats having intermediary values were scattered in between.

The effects of T_3 treatment in the Nx rats are depicted in Figs. 3 and 4. Both C and 6-wk Nx rats were either not treated or given 0.4 μg T_3/100 g of body wt by daily intraperitoneal injections for 4 consecutive wk starting 2 wk after nephrectomy. In both groups of rats, mean serum TT_3 concentration increased to 185 and 180 ng/dl, respectively, 2 h after T_3 injection and serum TSH was suppressed to levels <100 ng/ml. Mean concentrations of BUN were not changed by T_3 treatment. At the tissue level, T_3 treatment did not significantly alter the mean T_3 content, αGPD, or MDH activities of the C rats. The only change was an increase in the specific activity of αGPD. In the Nx rats, changes were more apparent. Total liver αGPD and MDH rose by 70 and 60% over their respective base-line values. These increases became more significant when corrected for protein concentration. In fact, the reduced enzyme activities of Nx rats were normalized to values comparable to untreated controls. These changes in enzyme activities were in agreement with the corresponding normalization of the liver T_3 content, which rose from 12 to 17 pg/mg protein. Other important information not shown in the figures is that of body weights and serum TT_4. Despite improvement in thyroid function, T_3 treatment had no beneficial effect on body weight, which was 340±8 g in C and 251±13 g in Nx rats ($P < 0.001$). Mean serum TT_4 was decreased in both groups of T_3 treated rats, being 2.5±0.2 μg/dl in the C and 1.5±0.2 μg/dl in the Nx rats ($P < 0.001$).

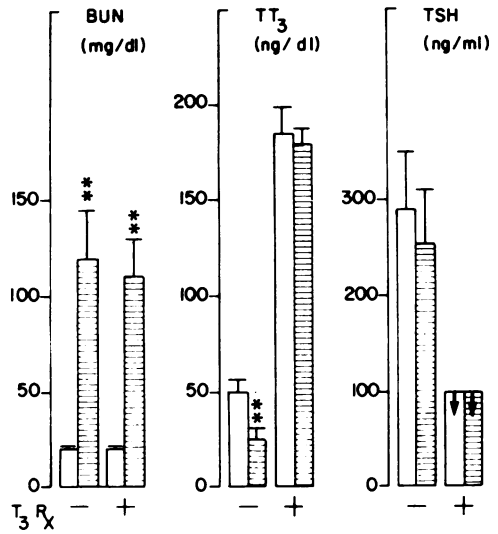

FIGURE 3 Effect of T_3 treatment on renal and thyroid function in C (□) and Nx (▤) rats. Values, representing mean±SEM, are depicted for nontreated (−) and treated (+) rats. Treatment consisted of 0.4 μg T_3/100 g body wt per day given by intraperitoneal injection for 4 wk. Statistically significant differences between control and nephrectomized rats were indicated by **, $P < 0.005$ to <0.0005 using one-tailed t test. Mean serum TT_4 (μg/dl) levels were 2.5±0.2 and 1.5±0.2, respectively, for the treated C and treated Nx rats. Data for untreated groups are in Table I.

DISCUSSION

The main objective of this study was to examine the physiologic significance of the principal abnormality associated with chronic renal failure, low serum T_3 concentration (1, 4–9), by searching for evidence of hypothyroidism at the tissue level. We attempted to answer the question by quantitation of T_3 content in the liver and by measurement of the activities of two thyroid hormone-dependent hepatic enzymes. A uremic rat model produced by a two-stage 5/6 nephrectomy was developed. After an initial 30% loss of body weight, a stable and reproducible form of chronic azotemia was achieved with a five- to sixfold elevation of BUN during the entire period of observation. More importantly, the uremic rat exhibited changes of thyroid function typical of man with chronic renal failure (1–11) and thus constituted a suitable experimental model. There was a slight decrease in serum TT_4, FT_4I_x, and a marked diminution in serum TT_3 concentration; serum TSH level was normal or slightly reduced (Table I). In the NxTx rats, serum TT_4 and FT_4I_x were decreased to the same extent as in the Tx rats, but serum TT_3 was reduced to a greater degree. This observation indicated that the low serum TT_3 in NxTx rats is not only due to lack of T_4 but also to an impairment in T_4 to T_3 conversion caused by uremia.

An important finding in this study was a reduction in liver T_3 content in the Nx rats. The decrease was most marked when expressed as T_3 content per total liver, an average of 50% of the control values 6 wk after nephrectomy (Table II). The reduction in liver T_3 was also significant when expressed as nanograms per milligram protein. It is unlikely that the decrease in liver T_3 is due to a lack of T_4 as serum TT_4 was only slightly

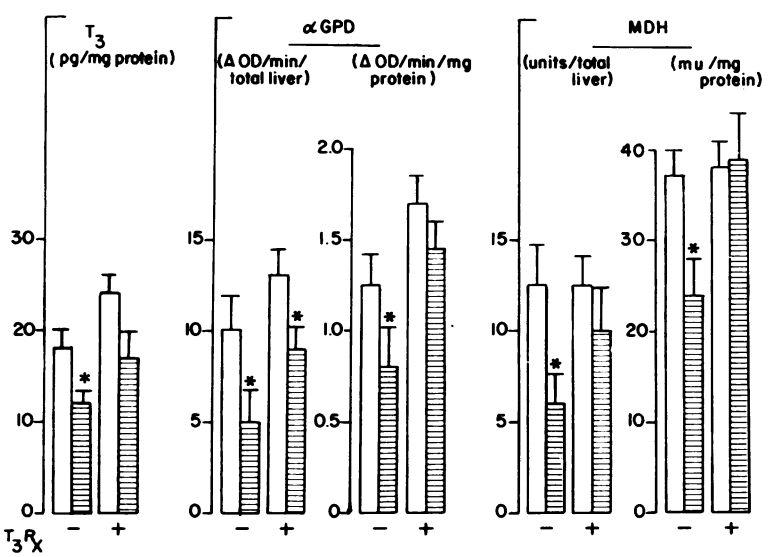

FIGURE 4 Effect of T_3 treatment on liver T_3 and αGPD and MDH activities in C (□) and Nx (▤) rats. Values, representing mean±SEM, are depicted for nontreated (−) and treated (+) rats. The treated rats were given 0.4 μg T_3/100 g body wt per day by intraperitoneal injection for 4 wk. αGPD and MDH are presented both as total activity for liver and as specific activity (corrected for protein concentration). Statistically significant differences between C and Nx rats are indicated by *, $P < 0.05$ to <0.025 using one-tailed t test.

reduced. Moreover, while the reduction in serum TT_4 occurred early and remained stable, a significant decrease in liver T_3 was not noted until 5 wk after nephrectomy with further diminution at 6 wk. Using isotopically labeled T_4 and T_3 we have previously shown that uremic patients have impaired ability in T_4 to T_3 conversion (9), a mechanism most likely responsible for the low liver T_3 content in the present experimental model. Reduction in tissue T_3 content is not unique to uremia; starvation and partial hepatectomy in the rat have been shown to produce a decresae in nuclear T_3 binding capacity and T_3 content (34, 35). In humans, Reichlin et al. (36) found decreased T_3 concentration in liver and kidney obtained at necropsy from patients who died from a variety of chronic illnesses.

T_3 concentration in the liver was several times higher than that in the serum and reduction in liver T_3 content preceded the appearance of significant diminution in serum T_3 concentration. Whereas the finding of low tissue T_3 content is not necessarily indicative of hypothyroidism, the concomitant reduction of mitochondrial αGPD and cytosol MDH in the Nx rats suggested the presence of metabolic consequences of T_3 deficiency. As shown in Fig. 1, changes in αGPD paralleled those of T_3 content. A similar correlation was found between the MDH activity and liver T_3 content in the 6-wk Nx rats (Fig. 2). The linear regressions correlating liver enzyme activities and T_3 content were not different in azotemic rats when compared to Tx and C rats with intact renal function.

It is unlikely that starvation was responsible for the reduction in enzyme activities in the uremic rat as enzymes were restored to normal despite failure of these animals to achieve a higher body weight during the 4-wk period of T_3 replacement. The possibility that metabolic consequences directly related to uremia, rather than reduced T_3 supply, were responsible for the observed enzymatic changes, was considered. In fact, although thyroid hormone replacement in the Tx rats is known to correct the activity of these two liver enzymes (20), it has been shown that the apparently T_3-deficient severely starved and partially hepatectomized rats are resistant to the induction of liver MDH by thyroid hormone (35). Relative resistance of the starving rat to a single dose of T_3 by augmentation in resting oxygen consumption has also been demonstrated (37). Nevertheless, chronic administration of physiologic doses of T_3 prevented the diminution of αGPD and MDH activities seen in the uremic rat. Such an observation underscores the causal relationship between tissue T_3 and enzyme induction in this animal model, especially when the increase in enzyme activities was associated with a repletion of T_3 content (Fig. 4). The slightly higher liver T_3 content in the C rats given T_3 is either due to a more efficient endogenous generation of T_3 from the residual nonsuppressible T_4 in these rats or, alternatively, to abnormal catabolism of T_3 in the Nx rats. It is important to note that although T_3 treatment prevented the decline in liver enzyme activity in the uremic rat, it had no effect on the magnitude of weight loss or the degree of uremia associated with nephrectomy.

Although these data clearly indicate that the reduction of liver αGPD and MDH activities in uremia is causally related to tissue T_3 deficiency, they are in apparent contradiction with results reported in the starving and partially hepatectomized rats, models bearing similarities with the uremic rat. The resistance to T_3 in the starving and partially hepatectomized rats was manifested by the failure to observe an increase in MDH but not αGPD activity after the administration of a single pharmacologic dose of T_3 (35). Unlike uremic and hypothyroid rats (20), basal enzyme activity in these acute experimental models was not decreased (35). No resistance to T_3 was observed in fasting human subjects with low serum TT_3 when the hormone was given chronically and in physiologic doses (38).

The lack of TSH elevation in the presence of tissue hypothyroidism and low serum levels of TT_3 and TT_4 in the uremic rats is puzzling. It certainly could not be attributable to low TSH reserve in the pituitary as serum TSH in the NxTx rats reached levels as high as in the nonuremic Tx rats (Table I). It is possible that intrapituitary T_3 content is normal in the uremic rats as monodeiodination of T_3 tends to be more efficient in the pituitary (39, 40). In such an instance, hypothyroidism would only be confined to the peripheral tissue, including the liver. Alternatively, there may be concomitant disturbances in the hypothalamic-pituitary axis with regard to TSH secretion associated with uremia. Impaired TSH response to TSH-releasing hormone is well documented in patients with chronic renal failure (8, 9). A resetting of the feedback regulation of TSH secretion at a lower level, proposed as the mechanism responsible for the failure to observe serum TSH elevation in response to starvation-induced decrease in T_3 generation in man (38), may also be operational in the uremic rat model.

In summary, the partially nephrectomized rat was an excellent experimental model to examine thyroid function in renal failure because uremia was chronic and stable and the changes in thyroid hormone profile in the circulation were surprisingly similar to that found in patients with chronic renal failure. In this model, T_3 content was markedly reduced and the αGPD and MDH activities were also significantly decreased. These data were interpreted as evidence consistent with hypothyroidism at the tissue level. Restoration of enzyme activity after T_3 treatment lent further support to this hypothesis. The physiologic significance of these findings with regard to total body economy and energy metabolism remains to be elucidated.

ACKNOWLEDGMENTS

We appreciate the advice and encouragement of Dr. Leslie DeGroot and Dr. Fredric L. Coe. We thank Mr. Swen R. Hagen for his technical assistance and Ms. Amy Strauss for her help in statistical analysis.

The work was supported by U. S. Public Health Service grants HD 10918 and AM 15070 and by the Michael Reese Research Institute.

REFERENCES

1. Silverberg, D. S., R. A. Ulan, D. M. Fawcett, J. B. Dorsetor, M. Grace, and K. Bettcher. 1973. Effects of chronic hemodialysis on thyroid function in chronic renal failure. *Can. Med. Assoc. J.* **109:** 282–286.
2. Ramirez, G., W. Jubiz, C. F. Gutch, H. A. Bloomer, R. Siegler, and W. J. Koff. Thyroid abnormalities in renal failure. 1973. *Ann. Intern. Med.* **79:** 500–504.
3. Joasoo, A., I. P. C. Murray, J. Parkin, M. R. Robertson, and D. Jeremy. 1974. Abnormalities of in vitro thyroid function tests in renal disease. *Q. J. Med.* **43:** 245–261.
4. Carter, J. N., C. J. Eastman, J. M. Corcoran, and L. Lazarus. 1974. Effect of severe chronic illness on thyroid function. *Lancet.* **II:** 971–974.
5. Chopra, I. J., U. Chopra, S. R. Smith, M. Reza, and D. H. Solomon. 1975. Reciprocal changes in serum concentration of 3,3′,5′-triiodothyronine (reverse T_3) and 3,3′,5-triiodothyronine (T_3) in systemic illnesses. *J. Clin. Endocrinol. Metab.* **41:** 1043–1049.
6. Bermudez, F., M. I. Surks, and J. H. Oppenheimer. 1975. High incidence of decreased serum triiodothyronine concentration in patients with nonthyroidal disease. *J. Clin. Endocrinol. Metab.* **41:** 27–40.
7. Spector, D. A., P. J. Davis, J. H. Helderman, B. Bell, and R. D. Utiger. 1976. Thyroid function and metabolic state in chronic renal failure. *Ann. Intern. Med.* **85:** 724–730.
8. Ramirez, G., W. O'Neil, Jr., W. Jubiz, and H. A. Bloomer. 1976. Thyroid dysfunction in uremia: Evidence for thyroid and hypophyseal abnormalities. *Ann. Intern. Med.* **84:** 672–676.
9. Lim, V. S., V. S. Fang, A. I. Katz, and S. Refetoff. 1977. Thyroid dysfunction in chronic renal failure. A study of the pituitary-thyroid axis and peripheral turnover kinetics of thyroxine and triiodothyronine. *J. Clin. Invest.* **60:** 522–534.
10. Dandona, P., D. Newton, and M. M. Platts. 1977. Long-term hemodialysis and thyroid function. *Br. Med. J.* **1:** 134–136.
11. Hershman, J. M., L. G. Krugman, J. D. Kopple, A. W. Reid, M. Azukizawa, and J. H. Schinaberger. 1978. Thyroid function in patients undergoing maintenance hemodialysis: unexplained low serum thyroxine. *Metab. Clin. Exp.* **27:** 755–759.
12. Braverman, L. E., S. H. Ingbar, and K. Sterling. 1970. Conversion of thyroxine (T_4) to triiodothyronine (T_3) in athyreotic human subjects. *J. Clin. Invest.* **49:** 855–864.
13. Surks, M. I., and J. H. Oppenheimer. 1977. Concentration of L-thyroxine and L-triiodothyronine specifically bound to nuclear receptors in rat liver and kidney. Quantitative evidence favoring a major role of T_3 in thyroid hormone action. *J. Clin. Invest.* **60:** 555–562.
14. Lim, V. S., and V. S. Fang. 1975. Gonadal dysfunction in uremic men: a study of the hypothalamo-pituitary axis before and after renal transplantation. *Am. J. Med.* **58:** 655–662.
15. Lim, V. S., S. C. Kathpalia, and L. A. Frohman. 1979. Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure: Reversal following transplantation. *J. Clin. Endocrinol. Metab.* **48:** 101–107.
16. Sievertsen, G., V. S. Lim, C. Nakawatase, and L. A. Frohman. 1980. Metabolic clearance and secretion rates of human prolactin in normal subjects and in patients with chronic renal failure. *J. Clin. Endocrinol. Metab.* **50:** 846–852.
17. Lim, V. S., C. Henriquez, G. Sievertsen, and L. A. Frohman. 1980. Ovarian function in chronic renal failure: evidence suggesting hypothalamic anovulation. *Ann. Intern. Med.* **93:** 21–27.
18. Ruegamer, W. R., W. W. Westerfeld, and D. A. Richert. 1964. α-Glycerophosphate dehydrogenase response to thyroxine in thyroidectomized, thiouracil-fed and temperature adapted rats. *Endocrinology.* **75:** 908–916.
19. Lee, Y. P., and H. A. Lardy. 1965. Influence of thyroid hormones on L-α-glycerophosphate dehydrogenase and other dehydrogenases in various organs of the rat. *J. Biol. Chem.* **240:** 1427–1436.
20. Oppenheimer, J. H., E. Silva, H. L. Schwartz, and M. I. Surks. 1977. Stimulation of hepatic mitochondrial α-glycerophosphate dehydrogenase and malic enzyme by L-triiodothyronine. *J. Clin. Invest.* **59:** 517–527.
21. Goodridge, A. G. 1976. Regulation of the synthesis of malic enzyme by thyroid hormone in liver cells in culture. *In* Thyroid Research. Proceedings of the VII International Thyroid Conference. J. Robbins and L. E. Braverman, editors. Excerpta Medica, Amsterdam. 355–358.
22. Garcia, M. D., F. Escobar del Rey, and G. Morreale de Escobar. 1976. Thyrotropin-releasing hormone and thyroid hormone interactions on thyrotropin secretion in the rat: lack of inhibiting effects of small doses of triiodo-L-thyronine in the hypothyroid rat. *Endocrinology.* **98:** 203–213.
23. Sewell, D. L., and B. S. Wostmann. 1975. Thyroid function and related hepatic enzymes in the germfree rat. *Metab. Clin. Exp.* **24:** 695–701.
24. Schwartz, H. L., M. A. Forciea, C. N. Mariash, and J. H. Oppenheimer. 1979. Age-related reduction in response of hepatic enzymes to 3,5,3′-triiodothyronine administration. *Endocrinology.* **105:** 41–46.
25. Nejad, I., J. Bollinger, M. A. Mitnick, P. Sullivan, and S. Reichlin. 1975. Measurement of plasma and tissue triiodothyronine concentration in the rat by radioimmunoassay. *Endocrinology.* **96:** 773–780.
26. Ochoa, S. 1955. Malic enzymes. *Methods Enzymol.* **1:** 739–753.
27. Hsu, R. Y., and H. A. Lardy. 1969. Malic enzymes. *Methods Enzymol.* **13:** 230–235.
28. Robin, N. I., S. R. Hagen, F. Collaco, S. Refetoff, and H. A. Selenkow. 1971. Serum tests for measurements of thyroid function. *Hormones (Basel).* **2:** 266–279.
29. Fang, V. S., and S. Refetoff. 1974. Radioimmunoassay for serum triiodothyronine (T_3): evaluation of simple techniques to control interference from binding proteins. *Clin. Chem.* **20:** 1150–1157.
30. Refetoff, S., S. R. Hagen, and H. A. Selenkow. 1972. Estimation of the T_4 binding capacity of serum TBG and TBPA by a single T_4 load ion exchange resin method. *J. Nucl. Med.* **13:** 2–12.
31. Reichlin, S., J. B. Martin, R. L. Boshaus, D. S. Schalch, J. G. Pierce, and J. Bollinger. 1971. Measurement of TSH in plasma and pituitary of the rat by a radioimmunoassay utilizing bovine TSH: effect of thyroidectomy or thyroxine administration on plasma TSH level. *Endocrinology.* **87:** 1022–1031.
32. Lowry, D. H., N. H. Rosebrough, H. L. Farr, and R. J.

Rondale. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

33. Michael, U. F., R. L. Barenberg, R. Chavez, C. A. Vaamonde, and S. Papper. 1972. Renal handling of sodium and water in the hypothyroid rat. *J. Clin. Invest.* **51:** 1405–1415.
34. DeGroot, L. J., A. H. Coleoni, P. A. Rue, H. Seo, E. Martino, and S. Refetoff. 1977. Reduced nuclear triiodothyronine receptors in starvation-induced hypothyroidism. *Biochem. Biophys. Res. Commun.* **79:** 173–178.
35. Dillmann, W. H., H. L. Schwartz, and J. H. Oppenheimer. 1978. Selective alterations in hepatic enzyme response after reduction of nuclear triiodothyronine receptor sites by partial hepatectomy and starvation. *Biochem. Biophys. Res. Commun.* **80:** 259–266.
36. Reichlin, S., J. Bollinger, I. Nejad, and P. Sullivan. 1973. Tissue thyroid hormone concentration of rat and man determined by radioimmunoassay: biologic significance. *Mt. Sinai J. Med.* **40:** 502–510.
37. Wimpfheimer, C., E. Saville, M. J. Voirol, E. Dauforth, Jr., and A. G. Burger. 1979. Starvation-induced decreased sensitivity of resting metabolic rate to triiodothyronine. *Science (Wash. D. C.).* **205:** 1272–1273.
38. Gardner, D. F., M. M. Kaplan, C. A. Stanley, and R. D. Utiger. 1979. Effect of triiodothyronine replacement on the metabolic and pituitary responses to starvation. *N. Engl. J. Med.* **300:** 579–584.
39. Silva, J. E., and P. R. Larsen. 1977. Pituitary nuclear 3,5,3′-triiodothyronine and thyrotropin secretion: an explanation for the effect of thyroxine. *Science (Wash. D. C.).* **198:** 617–618.
40. Silva, J. E., T. E. Dick, and P. R. Larsen. 1978. The contribution of local tissue thyroxine monodeiodination to the nuclear 3,5,3′-triiodothyronine in pituitary, liver, and kidney of euthyroid rats. *Endocrinology.* **103:** 1196–1207.