Peripheral Metabolism of Thyroxine in Thyroidectomized Rats Treated with Cortisone[1] (35778)

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In man, following the administration of ACTH or cortisone, there is a diminished thyroidal uptake of radioiodine (1). Several mechanisms may be postulated to account for this phenomena. The cortisone effect may be a direct alteration of the thyroid's ability to respond to thyrotropic hormone (TSH) as suggested by enhanced uptake of radioiodine by thyroid glands of hypophysectomized, pituitary replaced, nephrectomized rats, treated with cortisone (2). In addition, cortisone may act directly at either the level of the hypothalamus or the pituitary gland by influencing TSH release. However, the effect of cortisone on the thyroid gland may be indirect, since in the rat it transiently augments renal clearance of radioiodide (3). Furthermore, cortisone might be altering peripheral metabolism of thyroxine as is indicated by the observation that cortisone in man reduces the peripheral disappearance of thyroxine (4). Decreased peripheral disappearance of thyroxine is observed in patients subjected to surgical stress despite a concomitant decrease in plasma thyroxine-binding protein (5). Reduced hepatic content of thyroxine has been noted in man and in rats treated with corticoids (6); whereas cortisol reportedly inhibits the enzymatic deiodination of thyroxine by rat liver slices (7).

In order to gain further insight into the mechanism(s) by which adrenal corticoids influence thyroid gland function the following experiments were performed to study the *in vivo* effect of cortisone on the peripheral metabolism of thyroxine using the hormonal equilibrium method of Escobar del Ray *et al.* (8). This approach was chosen since the studies of Escobar del Ray *et al.* (8) indicate that peripheral metabolism of thyroxine can affect thyroid gland function by acting at either the pituitary or hypothalamic level to influence TSH release.

Methods. In two separate experiments male albino rats of the Badger strain, weighing approximately 120 g were surgically thyroidectomized under ether anesthesia. Throughout the experiment they were fed a Remington diet *ad libitum* which contained 6 μg of iodine/100 g of diet. On the day after surgery, L-thyroxine replacement therapy was begun. Therapy consisted of daily subcutaneous doses 2.5 μg of L-thyroxine dissolved in a 0.2 ml of 0.9% NaCl (containing 2% rat serum to stabilize the iodinated compounds) per rat. The L-thyroxine labeled with ^{125}I was obtained from Abbott Laboratories. An aliquot of the stock solution was subjected to paper chromatography in the system *t*-amyl alcohol: 2 *N* NH_4OH. Carrier iodide, L-thyroxine, and propylthiouracil were added prior to chromatography to enhance the stability of the iodinated compounds. After chromatography the paper strip was stained for iodide with palladium chloride and for thyroxine with diazotized sulfanilic acid. The paper was cut into 1-cm strips; and the strips were counted in a well-type gamma scintillation counter. Initially less than 3% of the radioactivity was present as iodide and periodic reexamination of the stock solution throughout the 2 months of the experiment revealed no increase in the iodide content.

To obtain a specific activity of 0.2 μCi/μg, ^{125}I-thyroxine was added to the stock thyroxine solution. The addition of radioactive thyroxine did not cause an appreciable increase in L-thyroxine content. Eight days af-

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ter surgery injection of the radioactive solution was begun and continued throughout the experiment. At the onset of the radiothyroxine treatment, the animals were housed in individual steel metabolism cages which permitted separate collection of urine and feces. Samples of excreta were counted in a well-type gamma scintillation counter as was a sample of the injected solution. From the radioactivity in the injected solution and the radioactivity recovered in the excreta, the percentage of the daily administered dose excreted for each 2-day period of radiothyroxine treatment was calculated.

When the excreted radioactivity became constant and approximately equal to the injected dose, the animals were randomly divided into control and experimental sets. Each animal in the experiment received 5 mg of cortisone subcutaneously daily for 6 days at which time both control and experimental sets were killed by exsanguination under ether anesthesia. Protein-bound radioactivity was determined on serum samples precipitated and washed with cold 5% trichloroacetic acid and dissolved in concentrated sodium hydroxide. Samples of urine were analyzed by paper chromatography in the system *t*-amyl alcohol: 2 *N* NH_4OH, stained for iodide and thyroxine, cut into strips 1 cm wide, and counted in a well-type gamma scintillation counter. Completeness of thyroidectomy was demonstrated at autopsy by comparison of trachea/muscle ^{125}I ratio. Data were analyzed by Student's *t* test.

Results. All the radioactivity present in the urine consisted of radioiodide. The percentage of the daily administered radioactivity recovered in the urine and feces during each 48-hr period of radiothyroxine treatment is shown in Fig. 1. Equilibrium was reached on approximately the sixth day of radiothyroxine treatment. Cortisone treatment was begun on the 15th day and did not significantly alter the distribution or quality of radioactivity excretion. Table I contains the average 2-day urinary, fecal, and total excreted radioactivity as a percentage (± SE) of the daily injected radioactivity of both groups for the period of the cortisone treatment. The protein-bound radioactivity at the time of sacrifice can be presented as the percentage of the daily injected dose per milliliter of serum. For the first experiment this protein-bound radioiodide activity (% of daily injected dose/ml of plasma) was 2.29 ± 0.43 (n = 4) in the control group and 2.66 ± 0.76 (n = 5) in the cortisone treated. For the second experiment the data are 1.37 ± 0.14 (n = 5) for the control group and 1.21 ± 0.19 (n = 4) for the cortisone treated. Both experiments demonstrate no effect of cortisone on the level of protein-bound iodine. In addition, there is no significant difference between control and cortisone-treated animals when comparing urinary, fecal, and total excreted radioactivity in either of the replicate experiments (Table I).

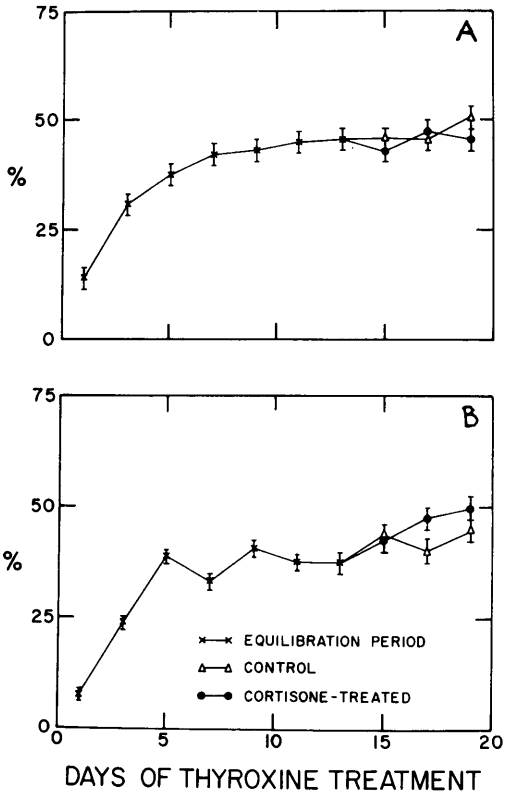

FIG. 1. Percentage (± standard error of the mean) of daily administered ^{125}I radioactivity recovered in urine (A); and feces (B) of thyroxine maintained, thyroidectomized rats treated with cortisone after a 14-day equilibration period.

Discussion. Isotopic equilibration of thy-

TABLE I. Injected Radioactivity Excreted (%) in each 48-hr Period.

	Excretion					
	Total		Urinary		Fecal	
Days	Control	Cortisone	Control	Cortisone	Control	Cortisone
Expt. I[a]						
15, 16	91.73 ± 2.82[b]	86.74 ± 5.13	50.94 ± 2.50	48.77 ± 4.44	42.47 ± 4.82	39.97 ± 1.31
17, 18	87.04 ± 2.42	96.43 ± 9.06	48.37 ± 3.32	51.17 ± 2.99	38.09 ± 2.31	45.07 ± 6.38
19, 20	96.10 ± 3.61	101.16 ± 6.86	55.41 ± 2.68	51.04 ± 1.96	40.66 ± 3.85	50.11 ± 5.81
Expt. II[a]						
15, 16	86.35 ± 7.46	83.45 ± 3.82	42.13 ± 2.40	38.00 ± 1.52	44.2 ± 5.32	45.56 ± 4.20
17, 18	87.36 ± 6.04	92.98 ± 3.13	43.96 ± 0.87	43.62 ± 2.59	43.45 ± 6.77	49.36 ± 5.21
19, 20	96.19 ± 2.49[a]	96.22 ± 6.46	46.05 ± 2.41	41.06 ± 4.16	50.13 ± 0.51	49.15 ± 8.42

[a] In experiment I the control group consisted of 4 animals and the cortisone-treated group had 5 animals. In experiment II there were 5 controls and 4 cortisone-treated animals.

[b] Standard error of the mean.

roidectomized rats with radioactive L-thyroxine was first described by Escobar del Ray *et al.* (9). They used 120–150-g animals and physiological doses of L-thyroxine, and achieved isotopic equilibrium in 5–6 days. In our experiments using similar size animals and dosages, the time required for isotopic equilibration was essentially the same. In thyroidectomized, thyroxine-maintained, isotopically equilibrated rats the urinary iodide excretion can be taken as a measure of the peripheral deiodination of the hormone provided the renal iodide excretion remains unaltered.

Cortisone has been shown to influence several parameters of thyroid function by causing a reduction of iodide uptake, thyroid weight, and synthesis of thyroidal protein in intact cortisone-treated rats (11, 12). Cortisone transiently augments renal iodide clearance and this property was at first thought to explain reduced thyroidal iodide uptake (2). However renal iodide clearance is markedly dependent on the clearance of water and electrolytes and is augmented by cortisone in adrenalectomized and hypophysectomized animals, but not in intact animals (12). Furthermore, in man, the thyroidal suppressive effect can be differentiated from the renal effect (13).

In the present experiments, cortisone treatment did not alter the urinary excretion of radiothyroxine-derived radioiodide. In view of the fact that the corticoid effect on renal iodide excretion is transient and manifested only in adrenalectomized and hypophysectomized animals, it is possible to state that *in vivo* cortisone does not alter the peripheral deiodination in thyroidectomized, thyroxine-maintained animal.

Alternative explanations for the thyroidal suppressive effect of cortisone have involved either a direct suppression of thyroidal response to TSH or an alteration of TSH release. We have previously investigated the possibility of a cortisone-induced suppression of thyroidal response to TSH by the use of nephrectomized, hypophysectomized, pituitary replaced rats. In such animals, cortisone augments the ability of the gland to take up iodide (3).

Several investigators have suggested that cortisone may suppress release of TSH from the pituitary. Brown-Grant *et al.* (14) found that cortisone treatment reversibly slowed the release of radioiodine from the thyroids of treated rats. If cortisone suppresses the peripheral metabolism of thyroxine as is suggested from reduced peripheral disappearance rates in treated animals and by the reduced deiodination of radiothyroxine by rat liver slices *in vitro* (7), it is conceivable that a negative feedback mechanism could bring about reduced TSH release. Our data show that *in vivo* cortisone treatment does not induce significant alterations in the peripheral deiodination of thyroxine as reflected by urinary excretion of radioiodide. Therefore, it is

reasonable to conclude that if cortisone does exert a thyroid suppressive effect by inhibition of TSH release, the inhibition does not depend on alteration of thyroxine peripheral deiodination.

Summary. The present study supports the conclusion that cortisone, administered to thyroidectomized, thyroxine-maintained rats does not alter their ability to deiodinate peripheral thyroxine. This is based on the observation that in two separate experiments there was no demonstrable cortisone effect on the level of serum protein-bound protein iodine. In addition, there was no significant difference between control and cortisone-treated animals in regard to either their urinary or fecal ^{125}I excretion as well as no difference in total excretion of radioactivity.

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