Effect of Stress and Cortisone on Plasma Protein-Bound Iodine and Thyroxine Metabolism in Rats. (19867)

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Cortisone and adrenocorticotrophic hormone suppress the uptake of I^{131} by the thyroid gland of experimental animals(1,2,17) and human beings(3-5). These substances also reduce the concentration of the plasma protein-bound iodine (PBI)(3,4,6,7). Various types of stress also depress I^{131} uptake by the thyroid of experimental animals(8-11) probably by increasing the secretory activity of the adrenal cortex. It therefore appeared probable that stress would reduce the plasma PBI by the same mechanism.

This line of reasoning ignores the importance of the rate at which the thyroid hormone is removed from the blood as a factor in determining the concentration of the hormone in the plasma. The following experiments were designed to investigate the effects of cortisone and stress upon the plasma PBI and on the peripheral utilization of thyroxine.

Methods. Two groups of male Sprague Dawley rats were used in these experiments. One group was thyroidectomized surgically and, after 6 months, treated with I^{131} (100 millicuries intraperitoneally to each animal) to assure complete destruction of the thyroid gland. The other group was of similar age, but was untreated. The thyroidectomized animals received 20 μg of racemic crystalline thyroxine subcutaneously each day starting seven days before the experiments were begun. At least one week was permitted to elapse between experiments. Protein-bound iodines were determined on 0.5 ml of plasma from heparinized blood obtained from the tail, using a modification of the alkaline ash method of Barker, Humphrey and Soley(12). Values obtained on 0.5 ml of plasma were not significantly different from the results obtained on 1.0 ml of plasma. In order to minimize the effects of repeated bleeding, not more than 2 samples were taken from any animal during a 24-hour period. The animals were subjected to the various stresses in sequence, with at least one week between experiments. Each animal served as his own control during the experiments. This method of calculation decreases the variation and increases the significance of the results statistically; however, the general conclusions obtained are not altered if the means of the entire group before and after treatment are compared. Three types of stress were studied. Animals exposed to low temperatures were kept in individual cages in a cold room at 4°C. Exercise stress was induced by placing the animals in a tank of water at 27°C and forcing them to swim for 2 hours. The thyroidectomized, thyroxine-maintained animals were so exhausted that the swim was terminated after only 105 minutes. The intact rats were also starved for periods up to 72 hours.

Results. In Table I, it can be seen that exposure to the cold produces a significant reduction in the PBI within 15 hours in intact, fasted rats. The PBI falls still further at 24 hours, but at 72 hours has returned to essentially normal levels. In contrast to this, prolonged fasting *per se* fails to alter the PBI during the first 15 hours, and produces a gradual continuing fall at 24 and 72 hours. The data presented in the second part of Table I show that when a constant source of thyroxine is maintained in thyroidectomized animals by injection, exposure to the cold depresses the PBI. The depression is even greater than the maximum observed in the intact rats ($p < .001$). These data indicate that the reduction in PBI as a result of exposure to the cold is not a result of decreased secretion of thyroid hormone; on the contrary, the fact that the fall of PBI is greater in the thyroidectomized rats indicates that an increase in thyroxine secretion probably occurs in the intact rats exposed to cold.

Violent exercise greatly reduces the PBI of intact rats (Table II, No. 1 and 2). The

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TABLE I. Effects of Cold Stress on Plasma PBI of Rats.

	Duration of exp., hr	Treatment	PBI*	Change of PBI, 4°C vs. control	P
		A. 6 normal rats			
1.	0	Fed control	4.4 ± .29		
2.	15	4°C (fasted)	3.9 ± .07	— .7 ± .03	<.001
		Control "	4.6 ± .15		
3.	24	4°C (fasted)	3.1 ± .16	— .9 ± .16	<.01
		Control "	4 ± .13		
4.	72	4°C (fasted)	4.6 ± .22	+1.5 ± .55	<.05
		Control "	3.1 ± .27		
B.	8 thyroidectomized rats maintained on 20 γ thyroxine subcut./day				
5.	0	Fed control	4.6 ± .30	—3.6 ± .52	<.001
6.	15	4°C (fed)	1 ± .55		

* Values throughout are expressed as mean ± stand. error of the mean $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

thyroidectomized rats received their daily injection of thyroxine between the first (preswim) sample (Table II, No. 5) and the second sample, taken after swimming (Table II, No. 6). Consequently, a rise of PBI occurred even after the swim. When this rise is compared with the rise occurring at a similar time interval after the injection of an identical amount of thyroxine without exercise stress (Table II, No. 3 vs No. 4), the degree of rise is found to be reduced by exercise. The effect of exercise is therefore to diminish the PBI even when it is maintained by an unvarying source of thyroxine.

The injection of a single dose of 12.5 mg of cortisone subcutaneously failed to alter the PBI of normal rats, but continued injection for three days greatly depressed the PBI (Table III, No. 1 and 2). In contrast to this, the injection of a similar dose of cortisone for three days greatly increased the PBI of thyroxine-maintained thyroidectomized rats (Table III, No. 3). Moreover, the administration of cortisone completely prevented the expected fall of PBI in thyroidectomized rats after a 15-hour exposure to the cold (Table III, No. 4).

Discussion. These data demonstrate that 3 types of stress (cold, exercise and starvation) reduce the plasma protein-bound iodine level in intact rats. This effect is not entirely a result of suppression of thyroid activity, since the PBI is depressed by cold and exercise in thyroidectomized rats maintained on injected thyroxine. Presumably, therefore, these stresses act, in part at least, by increasing the rate of metabolism and destruction of thyroxine. Indeed, in the case of cold stress the depression of the PBI is greater in thyroxine-maintained thyroidectomized animals than in intact rats, suggesting that the rate of secretion of thyroid hormone has been augmented by exposure of the animal to the cold. Moreover, after 72 hours in the cold, the animals have a normal PBI, suggesting that the accelerated activity of the thyroid gland has "caught up" with the tissue demands for thyroxine. This finding is in agreement with the evidence of numerous observers, that the activity of the thyroid is increased when the animal is in a cold environment(9,13,14). The apparent decrease of I^{131} uptake by the thyroid glands of animals exposed to a cold environment is probably an artefact, as pointed out by LeBlond *et al.*(9), resulting from an increased turnover rate of iodine in the hyperactive gland.

Cortisone, in contrast to stress, appears to reduce the rate of metabolism of thyroxine injected into thyroidectomized animals. The adrenal hormone, in fact, is capable of completely blocking the augmented rate of disappearance of thyroxine produced by cold stress. The mechanism of this effect is obscure but it may be related in some way to the ability of adrenal steroids to increase the metabolic rate under circumstances where alterations of thyroid function cannot have

TABLE II. Effect of Exercise (2 Hr Swimming) on Plasma PBI of Rats.

Treatment	PBI	Change of PBI	P
	A. 6 normal rats		
1. Control	4.2 ± .34		
		−3.1 ± .12	<.001
2. Swimming	1 ± .35		
	B. 8 thyroidectomized rats Rats received 20 μg of dl-thyroxine subcut. daily.		
			Diff. resulting from swim stress
3. Control, before thyroxine inj. on day of exp.	2.6 ± .49		
		+6.9 ± .55	
4. Control, 2 hr after thyroxine inj.	9.4 ± .41		
			−2.3 ± .72 <.01
5. Exp., before thyroxine inj. on day of exp.	3.9 ± .15		
		+4.6 ± .47	
6. Exp., after thyroxine inj. and 2 hr of swimming	8.5 ± .46		

TABLE III. Effect of Cortisone on Rat Plasma PBI.

Treatment	Duration of exp.	N	Dose, mg	PBI Initial	PBI Final	Change of PBI	P
1. Normal, fed	4 hr	6	12.5	2.5 ± .45	1.7 ± .26	— .8 ± .36	>.05
2. " "	4 days	6	12.5 × 3	2.7 ± .42	.8 ± .41	—1.8 ± .42	<.01
3. T + T*	4 "	7	12.5 × 3	2.8 ± .52	7.7 ± .40	+4.9 ± .56	<.001
4. " + 4°C for 15 hr	4 "	7	12.5 × 3	2.8 ± .52	3.9 ± .49	+1.1 ± .55	>.05

* T + T = Thyroidectomized + thyroxine.

occurred(3,4). The thyroid hormone is only one of many factors concerned in regulating the metabolic rate of tissues, and the rate at which the hormone is metabolized probably depends to some extent on the intensity with which other calorigenic influences are acting simultaneously.

For the most part, the PBI concentration in the normal group of animals remained constant unless disturbed by some experimental procedure. At the time of the experiments on the effects of cortisone, however, the initial (*i.e.*, control) values were significantly reduced (Table III, No. 1 and 2). The cause of this reduction is obscure, but whatever the cause it is apparently consistent, since the control value remained steady during the period (Table III, No. 1 vs No. 2 "initial PBI") and experimental variation as reflected by the standard error was no greater at this time than at any other. The constancy of the control values at this time therefore validates the experimental observations in spite of the fact that the actual initial value was at a somewhat unusual level.

It therefore appears that several different mechanisms operate to alter the PBI. Cold stress and exhausting exercise appear to cause an increased rate of removal of the hormone. This effect is somewhat balanced by an increase in the rate of release of thyroid hormone. In direct opposition to this sequence of events is the effect of activating the adrenal cortex. The cortical hormones appear to reduce the rate of disappearance of thyroxine and to reduce the synthesis (though not necessarily the rate of release) (2,17) of the thyroid hormone. As a result, cortisone tends to raise the PBI by decreasing its destruction and to lower the PBI by reducing the synthesis of the thyroid hormone. This antagonism between the peripheral and glandular effects of cortisone may explain the fact that the PBI is not invariably depressed by cortisone in man(3,4,16).

The depression of the plasma PBI observed after 24 to 72 hours of starvation is probably a result of reduced thyroid activity, since it has been shown that the I^{131} uptake is reduced after 48 hours of fasting(8), and that the concentration of thyroid stimulating hormone in the blood is depressed after 4 to 8 days of

starvation(15). No attempt was made to determine the effect of fasting on the PBI of the thyroxine-maintained thyroidectomized animal, however.

These experiments do not justify specific conclusions as to the effect of stress or cortisone upon the metabolic fate of thyroxine. Although it seems likely that the alterations of the rate of disappearance of thyroxine from the blood reflect changes in the utilization or catabolism of the hormone, the possible role of increased excretion as a result of alterations in renal or intestinal function cannot be ignored.

Summary. The plasma protein-bound iodine concentration of the intact rat is decreased after prolonged fasting, exhausting exercise and exposure to cold. This effect is not merely a result of suppressed thyroid function, since exercise and cold stress increase the rate of disappearance of injected thyroxine in thyroidectomized rats. Cortisone reduces the rate at which thyroxine is removed from the plasma, and can prevent the increase in the rate of disappearance of the hormone which is normally produced by exposure to a cold environment.

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