

Serum Thyroxine, Triiodothyronine, and TSH Levels in Iodine-Deficient and Iodine-Sufficient Rats before and after Exposure to Cold¹ (41123)

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Abstract. Thyroidectomized rats are unable to survive chronic exposure to cold. Severely iodine-deficient rats, on the other hand, despite very low levels of serum T₄ (<0.5 µg/100 ml), may or may not display cold intolerance. Many of these animals (25-50%) are able to maintain their body temperatures and to survive exposure to 4°. We had previously shown that serum T₃ levels are relatively well maintained in severely iodine-deficient rats, and in the present investigation we sought to determine whether survival of iodine-deficient rats in the cold could be correlated with serum T₃ levels, either initially or in response to the cold exposure. In two separate experiments we observed no correlation between initial serum T₃ levels and survival in the cold. The cold-sensitive rats showed slightly lower serum T₃ levels than the cold-resistant rats after cold exposure, but the difference was not statistically significant. Our data favor the view that thyroid hormone plays only a permissive role in the increased thermogenesis elicited in rats by chronic exposure to cold.

In a previous communication from this laboratory (1) we reported that serum T₄ levels in severely iodine-deficient rats were reduced to very low values (<0.5 µg/100 ml) after 1 month on a low-iodine diet (LID). The levels remained low with continued feeding of LID. Serum T₃, on the other hand, did not drop nearly to the same extent in rats on the same low-iodine diet, and there was considerable variability in the serum T₃ response to iodine deficiency.

In the same communication we also reported that rats that had been maintained on LID for a few months displayed a marked intolerance to cold, compared to rats fed the same diet supplemented with KI in the drinking water. The majority of the iodine-deficient rats were unable to maintain their body temperature when placed in individual cages in the cold room at 4°, and death occurred after a variable period (5-25 days). Since KI uniformly protected LID rats against cold exposure, it seemed clear that the observed intolerance

to cold was related to the decreased thyroid hormone levels in the circulation.

Some of the rats on LID, however, were able to maintain their body temperature close to the normal range, despite their very low levels of serum T₄, and these animals were able to survive the exposure to cold. Because serum T₃ levels varied considerably in the iodine-deficient rats at the onset of the cold exposure, the question arose whether survival in the cold may have involved those particular animals that displayed higher levels of serum T₃. It was also of interest to determine whether maintenance of the serum T₃ level in response to cold might be important for survival. The present investigation was undertaken to provide answers to these questions.

Methods and Materials. *Animals and diets.* Sprague-Dawley male rats weighing 110-120 g were purchased from the Holtzman Company (Madison, Wisc.). In Experiment I they received a Remington low-iodine diet supplied by Teklad Test Diets (Madison, Wisc.), containing approximately 20 ng I/g as determined by a previously described method (2). A control group received the same diet supplemented with KI in the drinking water (2 µg/ml in deionized water). The animals were maintained on the diets for 5 months before the

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start of the cold-exposure experiment. In Experiment II the rats received a Remington low-iodine diet supplied by ICN Nutritional Biochemicals, containing about 20 ng I/g. The control group received a Teklad Remington diet supplemented with 2 $\mu\text{g/ml}$ KI in deionized drinking water. The diets were fed for 4 months before the start of the cold-exposure experiment.

Experiment I. The major purpose of this experiment was to determine whether there was any relationship between the initial serum T_3 level at the time the rats were exposed to cold and their ability to survive cold exposure. There were 11 rats in the group maintained on Remington diet alone, and 12 rats in the group maintained on Remington diet + KI. Approximately 1.5 ml of blood was drawn from the jugular vein under nembutal anesthesia for measurement of the initial serum T_3 , T_4 , and TSH levels. Sera were frozen for later analysis. The rats were allowed to recover for 10 days and were then transferred to individual cages in the cold room at 4°. Rectal temperature was measured daily with a Model 46 Yellow Springs Instrument thermometer between 9 and 10 AM starting 3 days before the animals were placed in the cold. It was also of interest in this experiment to determine changes in serum T_4 , T_3 , and TSH levels in response to cold. When it became apparent that one of the LID rats would not survive, as indicated by a progressive drop in body temperature, this animal was observed closely. As soon as death occurred, or as shortly thereafter as possible, blood was taken by cardiac puncture. Animals that survived the cold, which included all of the rats on LID + KI, were killed after 37 days of cold exposure.

Experiment II. This experiment was similar to Experiment I except that it was designed more specifically to determine whether changes in the T_3 or the T_4 level in response to cold exposure played any role in survival of severely iodine-deficient rats. In this experiment, unlike in Experiment I, we did not wait for the cold-sensitive animals to die before taking a blood sample. It was evident from Experiment I, as well as from previous experiments, that a progressive decrease in body temperature to 35° or

less in a cold-exposed rat was almost a certain indication that the animal would not survive. In this experiment, therefore, when the body temperature of a cold-exposed rat reached 35° the animal was killed, and blood was taken for measurement of serum T_3 , T_4 , and TSH. There were 12 rats on Remington diet alone and 11 rats on Remington diet + KI. The experiment was terminated on the 20th day of cold exposure.

Measurement of serum T_4 , T_3 , and TSH. Serum T_4 , T_3 , and TSH were measured by radioimmunoassay. The procedures for T_4 and T_3 have been described previously (3). Serum TSH was determined with the NIAMDD kit. Results were expressed in terms of a standard TSH preparation (RP-1 from NIH) containing 0.22 U/mg. Terminal blood samples were taken from the abdominal aorta under pentobarbital anesthesia. Serum samples were frozen before analysis. Samples that were to be compared with each other were assayed simultaneously to minimize interassay variability.

Statistical analysis. Data were analyzed by the unpaired Student *t* test, and *P* values <0.05 were considered to be statistically significant.

Results. Effect of cold exposure on body temperature and survival of iodine-sufficient and iodine-deficient rats. Figure 1 shows the body temperatures of the rats in Experiment I at daily intervals after the onset of cold exposure. Results are shown separately for three groups: (i) the control rats on the iodide-supplemented diet, (ii) the cold-resistant iodine-deficient rats, and (iii) the cold-sensitive iodine-deficient rats. The rats receiving the iodide-supplemented diet showed a slight but significant mean increase in body temperature with continued cold exposure, from a value of 37.48 ± 0.17 on Day 0 to 38.07 ± 0.33 on Day 37 ($P < 0.001$). We have reported a similar observation in a previous study (1). The iodine-deficient rats that survived the cold exposure also maintained their body temperature very well, but at a slightly lower level than the iodide-supplemented group. The initial body temperature for this group was 37.58 ± 0.084 , and on Day 37 it was 37.42 ± 0.15 . This group did not display the

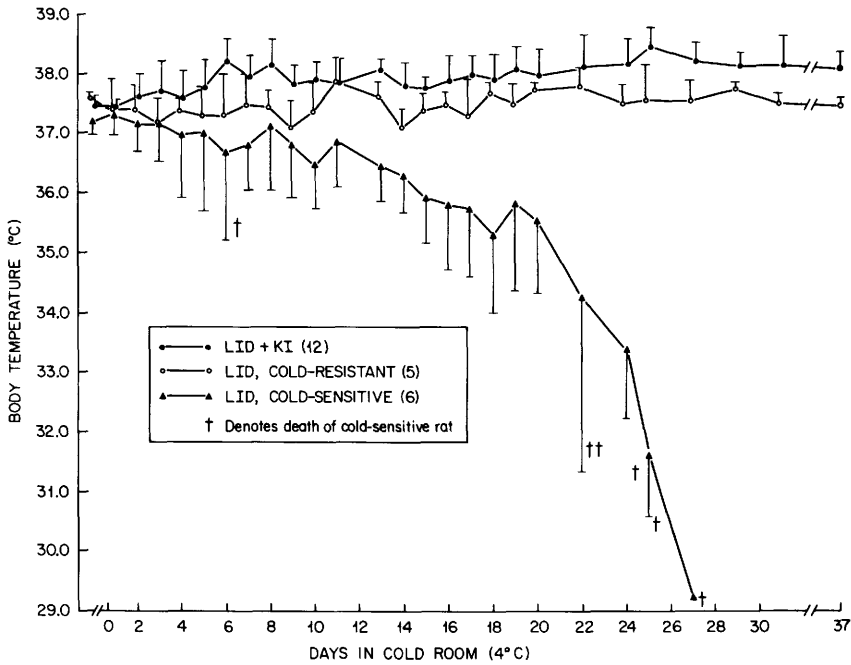


FIG. 1. Body temperature of iodine-sufficient and iodine-deficient rats in Experiment I at daily intervals after rats were exposed to a temperature of 4°. In the case of the LID + KI and cold-resistant LID groups all the animals survived the cold exposure. Thus, for these groups each point on the curve represents the mean \pm SD for a constant number of animals (the starting number for each group). However, the curve for the cold-sensitive LID group represents surviving animals. Therefore, the number of rats in this group starts with six at Day 0 but falls off each time a death occurred. The last point on the curve, at 27 days, represents the last surviving rat in this group.

increase in body temperature with cold exposure that was observed with the iodine-sufficient rats. However, the fact that they were able to maintain a normal body temperature in the cold environment contrasts with the response of the cold-sensitive rats. In the latter group the rats showed a progressive fall in body temperature, and death occurred after the body temperature had dropped to 34° or less.

Relationship between serum T_4 and T_3 levels and response to cold exposure in iodine-deficient rats. Table I shows serum T_4 , T_3 , and TSH levels for the rats in Experiment I before and after cold exposure. Comparison of the cold-sensitive and cold-resistant iodine-deficient rats shows that there was no significant difference between these groups in their initial serum T_3 levels. Neither was there any difference in serum T_4 or TSH. The initial serum T_3 levels in the cold-sensitive iodine-deficient rats were not

significantly different from those in the iodide-supplemented group, all of which survived the cold exposure. It hardly seems likely, therefore, that cold sensitivity in the iodine-deficient rats can be attributed to a low initial serum T_3 level. The serum T_3 levels after exposure to cold are also shown in Table I. The T_3 level in the cold-sensitive group was slightly lower than that in the cold-resistant group, but the difference was not statistically significant. The serum TSH level, initially very high in the iodine-deficient rats, showed a decrease after cold exposure. The magnitude of the decrease was similar in the cold-sensitive and cold-resistant animals. Because of the great variation in the TSH levels the decrease in each group was only of borderline significance. However, when data from the two groups were pooled the decrease in serum TSH was highly significant ($P < 0.01$).

The results of Experiment II are shown in

TABLE I. SERUM T₄, T₃, AND TSH LEVELS IN IODINE-DEFICIENT AND IODINE-SUFFICIENT RATS BEFORE AND AFTER EXPOSURE TO COLD (MEAN ± SD)

Serum component	Before or after cold exposure ^a	Iodine-deficient rats		Iodine-sufficient rats
		Died during cold exposure	Survived cold exposure	
T ₄ μg/100 ml	Before	0.35 ± 0.13 (6)	0.38 ± 0.13 (5)	5.6 ± 1.2 (11)
	After	0.15 ± 0.07 (5)	0.15 ± 0.06 (5)	2.1 ± 0.9 (12)
T ₃ ng/100 ml	Before	39 ± 7 (6)	33 ± 6 (5)	40 ± 15 (11)
	After	25 ± 5 (5)	30 ± 4 (5)	35 ± 10 (10)
TSH ng/ml	Before	6460 ± 3380 (6)	6537 ± 2800 (5)	286 ± 118 (12)
	After	3718 ± 774 (5)	3422 ± 1297 (5)	314 ± 108 (12)

^a Blood was taken from the jugular vein of all rats, and after a recovery period of 10 days they were transferred to the cold room at 4°. See text for further details. Figures in parentheses indicate number of rats in group. The experiment was terminated after 37 days of cold exposure.

Table II. The iodine-deficient rats in this experiment received a different Remington diet from those in Experiment I, and the T₃ values at the onset of the cold exposure were significantly higher than those in Experiment I. However, as can be seen in Table II, there was no difference between the cold-sensitive and the cold-resistant animals, again leading to the conclusion that cold sensitivity did not involve an initially low serum T₃ value. After the cold exposure the serum T₃ level dropped much more markedly than in Experiment I. This was observed both in the cold-resistant and the cold-sensitive animals. As in Experiment I, the serum T₃ level was lower in the

cold-sensitive group, but the difference was not statistically significant. It does not seem likely from these results that cold sensitivity involved a decreased ability to maintain an adequate serum T₃ level after cold exposure. However, because of the wide variation in the serum T₃ levels and the small number of animals this possibility cannot be excluded.

Response of iodine-sufficient rats to cold. Tables I and II also show data obtained with iodine-sufficient rats, which received Remington diet supplemented with KI in the drinking water. All of these animals survived the cold exposure. In both experiments serum T₄ levels decreased sig-

TABLE II. SERUM T₄, T₃, AND TSH LEVELS IN IODINE-DEFICIENT AND IODINE-SUFFICIENT RATS AFTER EXPOSURE TO COLD (MEAN ± SD)

Serum component	Before or after cold exposure	Iodine-deficient rats		Iodine-sufficient rats
		Body temperature below 35°	Body temperature above 35°	
T ₄ μg/100 ml	Before	0.46 ± 0.23 (4)	0.55(1)	4.7 ± 0.67 (10)
	After	0.61 ± 0.19 (9)	0.43 ± 0.12 (3)	2.7 ± 0.82 (9)
T ₃ ng/100 ml	Before	60 ± 21 (8)	59 ± 5 (3)	56 ± 11 (11)
	After	30 ± 17 (9)	43 ± 14 (3)	31 ± 15 (11)
TSH ng/ml	Before	4915 ± 2691 (4)	—	332 ± 392 (9)
	After	2028 ± 1474 (3)	—	501 ± 423 (9)

Note. Blood was taken from the jugular vein of all rats, and after a recovery period of 10 days they were transferred to the cold room at 4°. See text for further details. Figures in parentheses indicate the number of rats in group. Some of the serum samples were lost in this experiment, thus accounting for discrepancies in the number of samples analyzed. The experiment was terminated after 20 days of cold exposure.

nificantly after cold exposure ($P < 0.001$). Serum T_3 showed a slight, nonsignificant decrease in Experiment I but a highly significant ($P < 0.001$) decrease in Experiment II. Serum TSH levels showed no significant change after chronic cold exposure in either experiment.

Discussion. It is well known that thyroidectomized rats are unable to survive continuous exposure to cold (4). Severely iodine-deficient rats, however, may or may not survive chronic exposure to 4° , as reported previously (1) and confirmed in the present investigation. We and others have demonstrated that serum T_4 levels are greatly depressed in severely iodine-deficient rats (1, 5, 6). Serum T_3 levels, on the other hand, are relatively well maintained, though they may be quite variable. In the present investigation we sought to determine whether survival of iodine-deficient rats in the cold was related to serum T_3 levels, either initially or in response to the cold exposure.

In two separate experiments we observed no correlation between initial serum T_3 levels and cold survival. There was no indication that the serum T_3 level at the onset of cold exposure was higher in cold-resistant than in cold-sensitive rats. The results were less clear with regard to the ability of the rats to maintain the serum T_3 level after cold exposure. The cold-sensitive rats showed somewhat lower serum T_3 levels than the cold-insensitive group after exposure to cold, but the difference was not statistically significant.

Nonshivering thermogenesis is thought to play a major role in adaptation of mammals to chronic cold exposure (7). This response is mediated by an elevated secretion of norepinephrine, arising at least in part from increased sympathetic nervous system activity. Although the mechanisms involved are still not well elucidated, the major sites of the calorogenic action of norepinephrine are thought to be brown fat and skeletal muscle (8). The role of thyroid hormones in nonshivering thermogenesis is probably only permissive, since it has been shown that the increased metabolic rate of chronically cold-exposed rats can be achieved without an increased rate of

thyroid hormone secretion (9). In severely hypothyroid (thyroidectomized) rats maintained at 4° the level of norepinephrine in heart muscle and in adrenal medulla has been shown to be very low before death occurs (10). It has been suggested that under this condition the rate of biosynthesis of norepinephrine may be insufficient to compensate for its high rate of secretion (11). Presumably, therefore, a similar tendency to a lowered rate of biosynthesis of norepinephrine occurs in severely iodine-deficient rats. These rats have been shown to be mildly hypothyroid by measurements of oxygen consumption and liver mitochondrial α -glycerophosphate dehydrogenase (Okamura and Taurog, unpublished observations). It may be suggested, therefore, that the cold-resistant iodine-deficient rats in this and in our previous study (1) comprised those animals which, despite some lowering of thyroid hormone function, were able to maintain norepinephrine synthesis at a rate required for increased nonshivering thermogenesis. Other differences between cold-resistant and cold-sensitive rats may also be envisaged, such as the amount of brown fat, and the sensitivity of brown fat and other tissues to the calorogenic action of norepinephrine. Further studies are required to examine these possibilities.

The effect of chronic cold exposure on serum T_4 , T_3 , and TSH in the iodine-sufficient rats in the present study is also of interest. As far as we are aware, ours is the only study in which all of these indices of thyroid function were measured simultaneously in chronically cold-adapted rats. It is generally agreed that thyroid hormone production rate is increased in rats that are acclimated to cold (12). However, there is evidence that this increase involves only an increased fecal excretion of T_4 and not an increased rate of delivery of T_4 to the peripheral tissues (12, 13). There is disagreement in the literature on the effect of chronic cold exposure on serum PBI or T_4 levels. A decrease in PBI or serum T_4 has been reported by several groups of investigators (14–17), whereas others have reported no change (13). Very recently van Hardeveld *et al.* (18) reported that serum T_4

levels declined after 1 week exposure of rats to 4° but returned to normal after 4 weeks. The initial decrease in serum T₄ could be prevented by the addition of KI to the drinking water (15 µg/ml), and they suggested that the initial decline in serum T₄ could be attributed to increased iodine loss from the circulation. They imply that the decreased serum T₄ levels reported by previous investigators arose because of insufficient iodine intake. The results obtained in the present study do not support this view. In two separate experiments we observed a very significant decrease in serum T₄ in euthyroid rats exposed to 4° for several weeks that were receiving KI in the drinking water (2 µg/ml). It seems unlikely that the iodine intake of these rats was insufficient to support an increased utilization of thyroid hormone. Perhaps other factors such as the nature of the diet and the age and strain of the rats account for the differences between our results and those of van Hardeveld *et al.* (18).

Our findings on the change in serum T₃ levels in euthyroid rats exposed to 4° for several weeks also differed from those of van Hardeveld *et al.* (18). They reported a significant increase in serum T₃ in rats exposed to cold for 4 weeks. An increase was also reported by Reichlin *et al.* (17). However, we observed either a slight decrease (Experiment I) or a profound decrease (Experiment II) in serum T₃ in our cold-exposed rats. These discrepancies may also involve differences in diet, age, and strain of the rats used in the different investigations. It seems apparent that multiple factors are involved in the regulation of serum T₄ and T₃ levels in rats acclimated to cold.

Serum TSH levels in the present study showed no significant change in iodine-sufficient rats in response to chronic cold exposure. However, there is abundant evidence that acute exposure to cold stimulates secretion of TSH within 10–30 min, most likely through activation of the hypothalamic pituitary axis (19). The results of the present study indicate that elevated serum TSH levels are not sustained with chronic cold exposure. However, this does not exclude the possibility that the rate of TSH secretion is increased in chronically cold-exposed rats, since the metabolic clearance of TSH might also in-

crease under these conditions. Such a possibility is suggested by the observation (Experiment I, Table I) that the initially very high level of serum TSH in the iodine-deficient rats decreased significantly after the animals were placed in the cold.

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