Studies on Protein-Turnover Rates in Cold-Acclimated Rats[1] (34567)

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(Introduced by H. Sobel)

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It has been established that the mechanisms involved in the acclimation of rodents to cold include altered organ size and cellular enzymatic activity (1, 2). Also, there are shifts in carbohydrate (3) and fat (4) metabolism. However, protein metabolism in temperature-acclimated animals has not been so extensively studied (5). Much work has been done on nitrogen excretion in acclimating animals (6–8) and on enzymes associated with amino acid metabolism (9, 10). Also, Trapani (11) has investigated turnover rates of globulins in immune responses of cold-acclimated animals. However, studies especially designed to measure the rate of "whole body protein recycling" in temperature-acclimated animals have not been made. "Whole body protein recycling" as used in this paper refers to the net rate of amino acid→protein→amino acid turnover. The importance of a speeding up of this cycle is that it could serve as a means of increasing the intracellular levels of ADP and AMP and decreasing levels of ATP, which would thus stimulate cellular metabolism (12), and thus contribute to total heat production in the cold. The need for such an extramitochondrial ATP-wasting system has been previously pointed out (13). The following studies support, but do not prove, the validity of this hypothesis.

In this paper data are presented on the rates of whole-body disappearance and protein incorporation of labeled selenomethionine in rats kept for prolonged periods in the cold (6°). In addition, the immediate effects of cold exposure on selenomethionine incorporation and loss have been investigated.

Materials and Methods. Female Long-Evans rats, weighing 220 ± 5 g were divided into three groups with 16 rats in group I, 15 in group II, and 4 in group III. Group I (controls) was kept at room temperature (26°), group II (cold-acclimated) was exposed to cold (6°) for 10 weeks before the start of the experiment and kept there during the experimental period. Group III (cold-exposed) was exposed to 6° 1 day before the beginning of the measurements to see if short-term cold exposure results in different rates of selenomethionine loss. The method used for assessing the total body ^{75}Se selenomethionine turnover rate was essentially that of Mende and Viamonte (14) as modified by Yousef and Johnson (15).

To measure the relative extent of incorporation of labeled selenomethionine into protein of liver and kidney, 18 rats were divided into two groups. Group A (10 animals) was kept at 27° for 10 weeks and group B (8 animals) was kept at 6° for the same period. All animals then received 2.0 μCi of ^{75}Se selenomethionine intraperitoneally. Twenty-four hours later the animals were sacrificed and samples (1–2 g wet weight) of liver and kidney were taken. These were homogenized in ice-cold 10% TCA and samples were processed as previously described (15).

To study the characteristics of the initial

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TABLE I. Total Body Protein Turnover Rate and Degree of Incorporation of Selenomethionine in Liver and Kidney.

Group	Ambient temp.	K (day)	T½ (day)	% Total selenomethionine of homogenate present in TCA-precipitated proteins	
				Liver	Kidney
I	27°, 10 weeks[a] (16)	0.0461 ± 0.0009	15.2 ± 0.42[b]	—	—
II	6°, 10 weeks[a] (15)	0.0679 ± 0.0006	10.2 ± 0.87	—	—
III	6°, 1 day (4)	0.0501	13.5 ± 1.22	—	—
A	27°, 12–14 weeks (10)			88.3 ± 3.4	85.6 ± 3.1
B	6°, 10–12 weeks (8)			89.0 ± 4.2	87.8 ± 3.9

[a] Period of exposure to temperature prior to the injection of ^{75}Se methionine; numbers in parentheses represent number of animals.

[b] ± standard error.

rate of incorporation of ^{75}Se selenomethionine into various tissue proteins in cold-acclimated and control rats, another study on two additional groups of rats was made. Group C consisted of eight rats which were kept at 26° and group D, eight rats which were kept at 6° for 2 months. Each animal received 5.0 μCi of ^{75}Se selenomethionine and one rat from each group (C and D) was sacrificed every 15 min for a period of 2 hr. The radioactivity of tissue samples of liver, kidney, muscle, and brown fat were assayed using previously described methods (15).

The data were statistically analyzed using the Student *t* test.

Results. The data in Table I indicate that about 90 % of the labeled selenomethionine present in whole homogenate of liver and kidney is incorporated into proteins. This is true in both the controls and cold-acclimated rats (groups A and B).

A comparison between groups I and II indicates that cold-acclimation results in a significant increase in the protein fractional turnover rate (K). Acute exposure to cold (group III) results in a slight increase in K over control values (group I).

Data on the time-dependent incorporation of selenmethionine into liver, kidney, brown fat, and skeletal muscle are summarized in Fig. 1. It is clear that within 2 hr after injection, liver and kidney incorporate selenomethionine into newly formed protein more rapidly than do brown fat and skeletal muscle. This rate is strikingly faster in liver and kidney of cold-acclimated animals (group C), reaching its peak between 30 and 60 min after injection as compared to 70 to 100 min in the controls (group D) (Fig. 1). Rates of incorporation into muscle and brown fat were not different in the cold-acclimated and control groups during the first 2-hr postinjection period.

Discussion. The use of total body selenomethionine turnover rate as an index of protein turnover rate has been discussed recently by Yousef and Luick (5). The results of the present study (Table I) are in agreement with their findings with respect to a faster whole body loss of selenomethionine in cold-acclimated animals.

At first glance one might suppose that this faster loss results from less actual incorporation of selenomethionine into protein because there is more dietary methionine competing for incorporation into newly synthesized protein. However, the data (Fig. 1) show there is actually a faster initial incorporation of the isotope into liver and kidney protein, which is evidence that protein synthesis in these

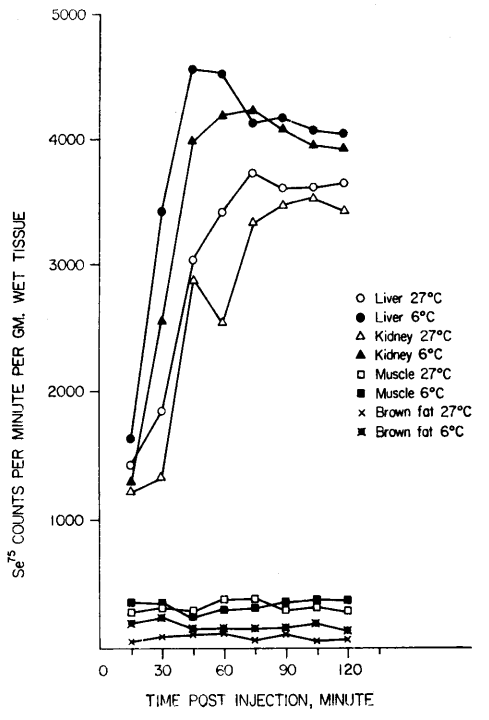

FIG. 1. Comparison of the rate of selenomethionine incorporation into tissues of cold-acclimated (group C, 6°) vs. control (group D, 27°) rats.

organs is faster in the cold-acclimated than in the control rats. Furthermore, cold-acclimated rats are in a steady state with respect to nitrogen balance (6, 16) indicating that protein synthesis and degradation rates are equal. Therefore, with respect to these organs, the data are in keeping with the hypothesis that a faster protein turnover rate is induced in the cold-acclimated animals. The thermogenic significance of such an increased turnover lies in the fact that this should result in a more rapid conversion of ATP to ADP and to AMP, since energy would be required to build protein from constituent amino acids which would then be degraded hydrolytically by intracellular cathepsins.

In looking at the rate of selenomethionine incorporation into brown fat proteins one is first struck by the relatively low amount of incorporation in comparison with liver and kidney. Thus, this type of recycling would presumably not be of any significance in brown fat thermogenesis. Also, it is apparent that there is no major difference between cold-acclimated and control animals in this respect in the brown fat or muscle.

Earlier findings (17) have shown an alteration in the free amino acids of cold-exposed animals in plasma and liver, but no significant changes in the free amino acids of the muscle. Perhaps brown fat is similar to muscle in this respect and the kidney behaves like liver. The data seem to indicate that the changes which occur in the liver and kidney protein turnover rates may contribute significantly to changes seen in the total body protein turnover rate of cold-acclimated rats.

The data on the effect of acute exposure to cold (group II in Table I) are presently inconclusive because of the small number of animals used in this study. However, further studies along these lines should establish both the time course of development of an increased selenomethionine turnover rate after cold exposure and the temporal relationship between this and the onset of nonshivering thermogenesis in the cold-acclimating animal.

Summary. Studies were made on rates of catabolism and of tissue protein incorporation of intraperitoneally injected ^{75}Se selenomethionine in cold-acclimated rats. The results of these studies indicate that both rates are increased in the cold. This could be indicative of an increased protein turnover rate in the cold-acclimated rat. Incorporation of labeled selenomethionine was faster in both kidney and liver. It is suggested that the rate of protein recycling may increase in the cold and that this could act as an intracellular respiratory stimulus by causing a more rapid conversion of ATP to ADP and to AMP.

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