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Subcellular Ionic Distribution in Liver and Heart Tissues of Cold-Acclimated Rats[1] (38191)

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Much evidence has been accumulated demonstrating that endogenous catecholamines and thyroid hormone participate in the establishment of nonshivering thermogenesis during acclimation to cold (1, 2). The importance of the liver in this process is confirmed by the higher temperature of hepatic venous blood, as compared to that found in either the portal vein or the aorta, and by the enhancement (three- to sixfold) of this gradient in cold-exposed animals (3). Moreover, the livers of acclimated animals show elevated oxygen consumption (4), again suggesting that hormonal stimulation of calorigenesis could be mediated by increased metabolic rate of the liver (1).

Thyroxine uncouples oxidative phosphorylation of liver mitochondria (5), but only at very high concentrations of the hormone (6). Whether thyroxine exerts control over the metabolic rate by varying the "tightness" of oxidative phosphorylation or if the observed uncoupling is merely a manifestation of thyrotoxicity is still unclear (7).

Mitochondria from livers of cold-acclimated rats show decreased P/O ratios (8) with evidence of uncoupled oxidative phosphorylation (9). A relative uncoupling of oxidative phosphorylation could therefore be produced by thyroxine or the catecholamines, resulting in increased heat production (via substrate oxidation) in the cold-exposed animal during phosphorylation of ADP, and thus be involved in the cellular mechanisms of acclimation to cold.

The effects of massive uptake of ionic calcium on mitochondrial structure (10) and metabolism (11) are similar to those of the calorigenic hormones. On the basis of these similarities, it has been proposed that hormonal action in nonshivering thermogenesis may occur through not yet elucidated ionic changes in the mitochondria (12).

In the present study, livers from cold-acclimated rats were analyzed for content and subcellular distribution of ions (Ca, Mg, K, Na) with the purpose of clarifying what role may be played by intracellular ionic movements in nonshivering thermogenesis. The hearts of these rats were similarly analyzed, since many forms of stress, including cold, have been shown to affect the electrolytic equilibrium of myocardial tissue in a deleterious manner (13, 14, 15).

Materials and Methods. Male Holtzman rats were used in these experiments. The controls were housed, 3 to a cage, in cubicles with an automatic 12 hr day–night cycle of illumination, at 25°, with food (Teklad diet) and water *ad libitum*. The cold-exposed rats were kept singly in metal cages within a thermo-regulated chamber (5° ± 0.5; relative humidity 50%) with feeding and illumination identical to those of the controls. The animals were daily inspected and frequently weighed. After four weeks all animals were sacrificed, with a blow on the head, at their ambient temper-

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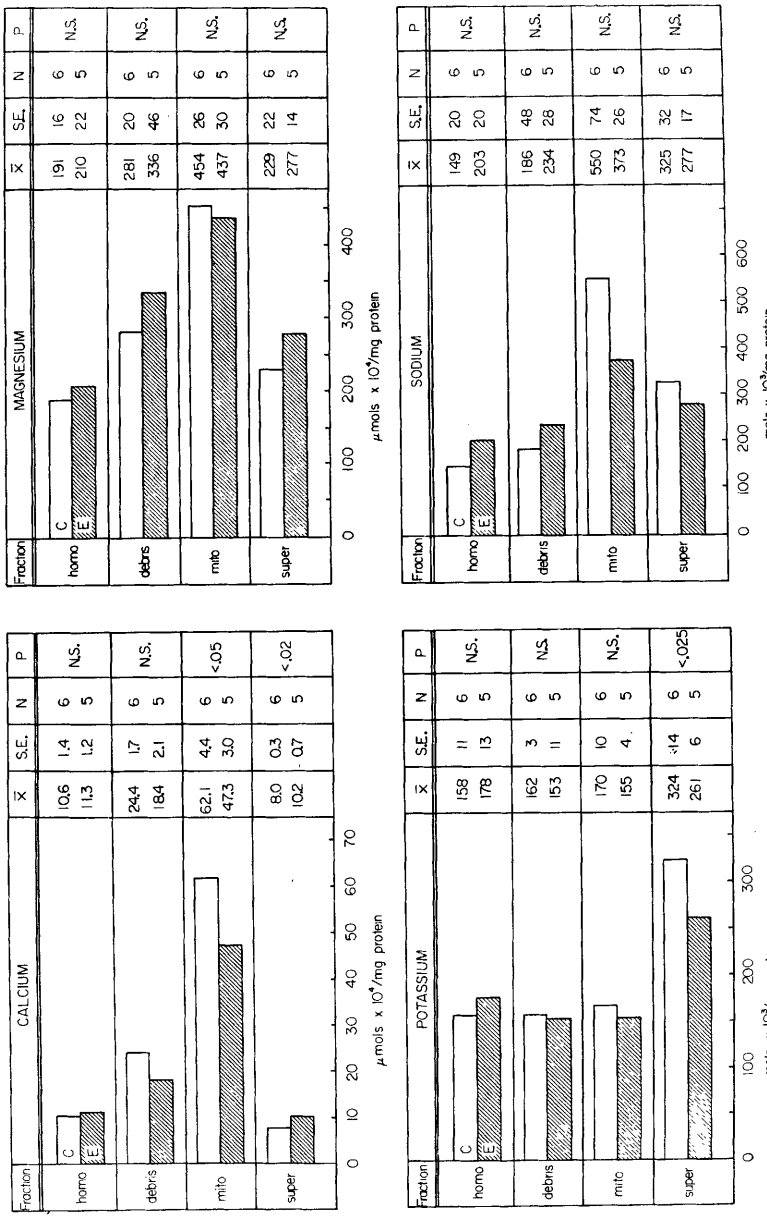

FIG. 1. Ion concentrations in different subcellular fractions of the liver in control (C) and cold-acclimated (E) rats. $\bar{x}$ = mean value; S.E. = Standard Error of the Mean; N = number of observations; P = level of significance of the difference.

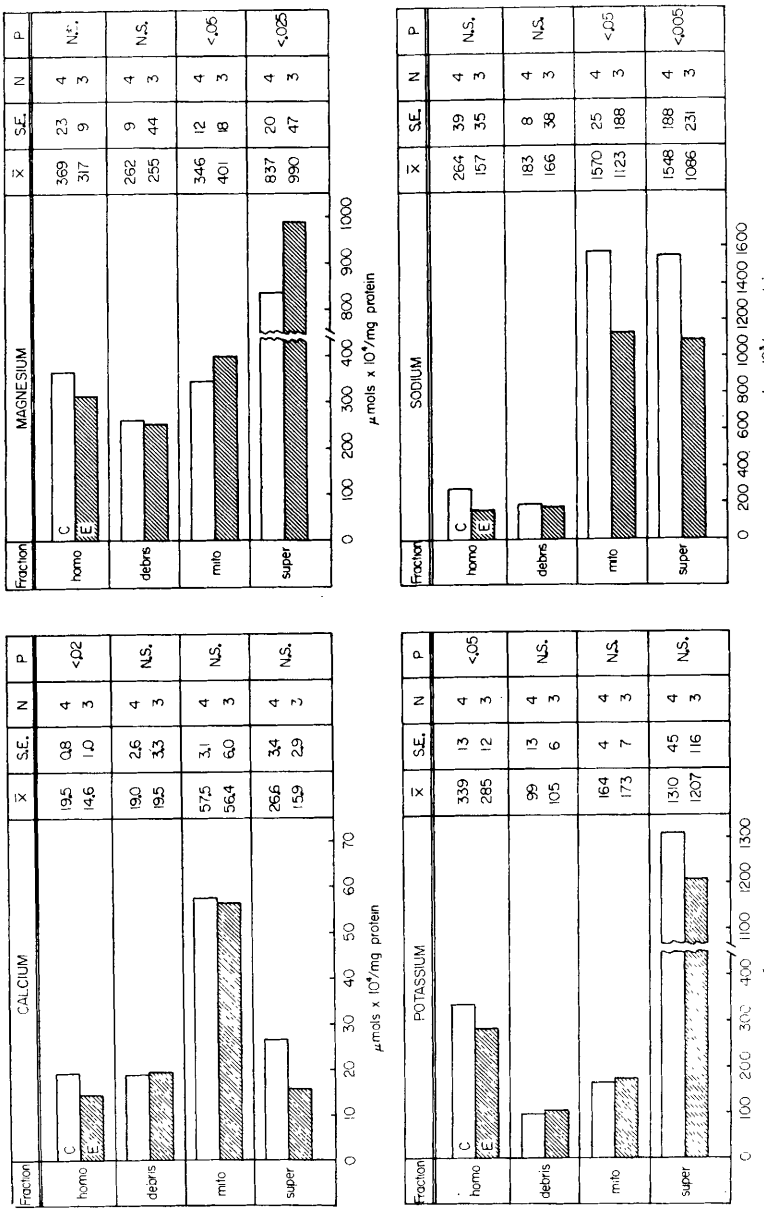

FIG. 2. Ion concentrations in the heart. For explanations, see Fig. 1.

ature. Liver and heart were quickly excised and placed in iced 0.25 *M* buffered sucrose (pH = 7.4; $NaHCO_3$, 3.2×10^{-4} *M*). All subsequent isolation steps occurred at 0°.

Liver parenchyma and ventricular muscle were weighed and homogenized in a Potter or Virtis "23" homogenizer, respectively. After removal of an aliquot, the whole "homogenate" was centrifuged and washed 2 times at $600g \times 10$ min. The resulting pellet constituted the "debris" fraction. The supernatant fluid was centrifuged and washed twice at $10{,}000g \times 20$ min, to obtain the "mitochondrial" pellet and "supernatant" fraction. The aliquot, pellets and final supernatant fraction were suspended in enough Tris Buffer (pH = 7.2; 0.05 *M*) to bring the final protein concentration to 2–5 mg/ml.

Calcium and magnesium contents were determined by a slight modification (16) of the titrimetric method of Walser (17). Potassium and sodium were measured with an automatic flame photometer. Protein determinations were performed with a modification of the method of Folin and Ciocalteu (18).

Results. Liver. Exposure to cold produced a statistically significant decrease in mitochondrial calcium concentration, with a simultaneous increase in that of the supernatant fraction (Fig. 1). A decrease in potassium concentration of the supernatant fluid was the only change noted for that ion. No statistically significant changes were observed in magnesium or sodium contents.

Heart. The distribution of ions in the myocardium of control and cold-exposed rats is shown in Fig. 2. Both calcium and potassium decreased in the whole tissue homogenate of the experimental rats. This change was accompanied by a similar, although marginally significant ($.05 < P < .10$) decrease in calcium concentration of the supernatant fraction. Magnesium increased in both the supernatant and mitochondrial fractions, while in these same samples, the sodium content decreased.

Discussion. The electrolytic changes observed in the livers of the cold-acclimated rats of this study do not support the hypothesis that ion-induced uncoupling of mitochondrial oxidative phosphorylation in the liver is the major mechanism operative in the maintainance of nonshivering thermogenesis.

In the cold-acclimated animals, calcium was lost from the hepatic mitochondria and only slightly increased in the supernatant fraction, with no indication of that massive calcium accumulation which is known to be necessary for induction of mitochondrial uncoupling of oxidative metabolism (10, 19). Thus, Lehninger's contention (12) that hormonal acclimation to the cold may result from calcium-induced changes in mitochondrial metabolism, is not supported by the present data.

It is known that uncoupling induced by means other than massive loading results both in a decreased mitochondrial ability to accumulate calcium (20) and in a loss of the ion previously sequestered (21). The lower calcium concentrations found in the liver mitochondria of our rats could be an indication of a decreased mitochondrial calcium sequestering ability, which was caused by uncoupling of oxidative phosphorylation. This nonion induced uncoupling has been reported to occur in livers of cold-acclimated animals (8).

Cold stress (13), thyroxine (22) and the catecholamines (14, 15, 23, 24) are known to either induce myocardial necrosis or sensitize the experimental animal to the development of this cardiopathy. This pathological change seems to depend on the disruption of cardiac metabolism caused by massive accumulation of calcium by the heart mitochondria (14, 15, 25). The changes in ionic distribution seen in the myocardia of the animals in the cold were, however, not suggestive of a similar mechanism being active in the cold-acclimated state.

Magnesium has been found to be essential for survival in the cold (26) and is known to protect mitochondria from uncoupling (27). Sodium, on the other hand, has been implicated in the development of cardiac necroses (28, 29, 30). The changes in cardiac magnesium and sodium seen in this study suggest that any mechanism resulting in uncoupling of liver mitochon-

dria during cold-acclimation would not be likely to have induced a comparable cardiac mitochondrial uncoupling with concomitant inefficiency of ATP production.

In the light of the present data, it is interesting to note that, while acute cold stress may be deleterious to the heart (13, 26), acclimation to stressor agents can afford significant protection against the development of myocardiopathy (31). Since our rats were acclimated to the cold by a more than adequate duration of exposure (32), a significant degree of protection against stress-induced cardiopathy (through increased magnesium concentrations in their hearts) might be predicted.

Summary. Liver and heart tissues from cold-acclimated rats were analyzed for content and subcellular distribution of the ions calcium, magnesium, potassium and sodium. The data provided indirect evidence of uncoupling of liver mitochondria in the cold-acclimated state, while casting doubt upon the hypothesis that massive calcium accumulation is the primary factor in the maintainance of non-shivering thermogenesis. The observed ionic changes in the heart suggest a protective mechanism, involving magnesium, against cold-induced cardiopathy.

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