CELLULAR RESPIRATION AND PHOSPHORYLATION DURING COLD EXPOSURE IN RATS

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ABSTRACT. — The effect of cold exposure (4 weeks at 2 to 5°C) on the oxidative phosphorylation and respiration of liver mitochondria and liver homogenates in rats was studied. The studies were made polarographically using the Clark type oxygen electrode. Respiration was measured with added exogenous substrate (succinate), exogenous substrate plus ATP, and exogenous substrate plus ADP. No alterations were observed in mitochondria from cold-exposed rats. The ADP:O ratio was significantly lower and the succinate-ADP respiratory rate was significantly higher in homogenates from cold-exposed rats.

The experimental work described here was designed to investigate in detail the effect of cold exposure on certain of the tissue metabolic parameters that characterize cold acclimation. The pursuit of this study was encouraged by the conflicting results previously reported in measuring these parameters. For example, Smith and Fairhurst (1958) suggested that oxidative phosphorylation ,that is, the conversion of ADP (adenosine diphosphate) into ATP (adenosine triphosphate), which is normally coupled to cellular respiration, may become partially uncoupled in the mitochondria of cold-exposed animals. Several studies of oxidative phosphorylation using liver homogenates and unwashed liver mitochondria from cold-exposed rats tend to support this hypothesis (Hannon, 1959; Panagos et al., 1958; Lianides and Beyer, 1960). Other studies using liver mitochondria from cold-exposed rats have failed, however, to demonstrate an uncoupling of oxidative phosphorylation (Patkin and Masoro, 1960; Chaffee et al., 1961; Frehn and Anthony, 1962.)

In an attempt to explain these apparently contradictory results, Patkin and Masoro (1960) suggested that oxidative phosphorylation, a mitochondrial event, may be regulated by extra-mitochondrial factors. This suggestion was based on the finding of Smith (1960) that an uncoupling of oxidative phosphorylation in cold-exposed rats occurs only in systems where other cell components are present in addition to the mitochondria. Thus, it is presumed that these extra-mitochondrial factors may be removed on preparing washed mitochondria (Smith, 1960).

Aldridge and Stoner (1963) measured oxidative phosphorylation in cold-exposed rats, using both washed and unwashed liver mitochondrial preparations. In contrast to the finding of Smith and Fairhurst (1958), however, they found that the liver mitochondria from these cold-acclimated rats did not differ from normal rats in their P:O ratios (number of moles of ADP phosphorylated per gram atom of oxygen consumed) in either the washed or unwashed preparations.

In addition to the studies on oxidative phosphorylation, additional investigations have suggested that augmented rates of tissue oxygen consumption may contribute toward increased non-shivering thermogenesis during cold exposure (Hannon, 1960). In support of this suggestion, some investigators using tissue homogenates or tissue slices (Hannon, 1958; Clark et al., 1954; You and Sellers, 1951) have found an increased rate of tissue oxygen consumption in cold-exposed rats. Others, using mitochondrial preparations, have failed to show such an increase in the rate of tissue respiration (Smith and Fairhurst, 1958; Chaffee et al., 1961; Frehn and Anthony, 1962). Aldridge and Stoner (1963) observed no increase in oxygen consumption in either washed or unwashed liver mitochondrial preparations from cold-exposed rats.

It is becoming increasingly evident that the *in vitro* determination of the metabolic responses of cold-exposed animals is markedly influenced by the method of tissue preparation. The present study was, therefore, undertaken with the aim of attempting to help clarify the conflicting reports by further investigating the differences between liver mitochondrial and homogenate respiration during cold exposure in rats.

Materials and Methods

Ten adult female strain CFE (Carworth Farms) rats were exposed to a continuous cold environment of 2 to 5°C for 3 to 5 weeks. These animals (plus 10 controls maintained at an ambient temperature of 24° ± 1°C) were used to study the effect of cold on liver mitochondrial respiration. A second group of six rats was exposed to cold for 4 weeks to study the effect of cold on liver homogenate respiration. Again, six controls were maintained at ambient temperature.

Washed mitochondria were prepared according to the method described by de Duve et al., (1955). The entire procedure for the isolation of the mitochondria was carried out in a cold room (1 to 2°C). Animals were killed with a sharp blow on the head, and the liver quickly removed and placed into an ice-cold isolation medium. The isolation medium contained 225mM mannitol, 75 mM sucrose, and 0.1 mM EDTA. Homogenization of the tissue was carried out by forcing a rotating Teflon pestle to the bottom of the grinding tube.

The tissue used for the study on rat liver homogenate respiration was prepared by subjecting the homogenized tissue suspension to slow speed centrifugation (500 x g for 8 minutes) in order to remove erythrocytes, nuclei and unbroken cells. Measurements of respiration and phosphorylation were made polarographically, using the rotating oxygen electrode as originally described by Chance and Williams (1955) as well as the Clark oxygen electrode as described by Strickland et al., (1960). The reaction medium contained 45mM mannitol, 15 mM sucrose, 40 mM KCl, 20mM $MgCl_2$, 20 mM phosphate buffer, pH 7.3, and 10 mM succinate. Ten microliters of 0.05 M ADP were added to determine the ADP plus substrate respiration rate and efficiency of oxidative phosphorylation. In addition, 20 microliters of 0.1 M ATP were added to the homogenates during the reaction to determine the substrate plus ATP rate of respiration.

Results and Conclusions

The results of the study of the effect of cold exposure on respiration and P:O ratios in washed liver mitochondria are shown in Table 1. Respiration values represent X ± S.E. in μM O_2/second/mg. of nitrogen. The R_s value represents the respiratory stimulation with ADP. Succinate was used as a substrate. It is evident from these data that

TABLE 1.—Effect of cold (3-5 weeks at 2-5°C) on exogenous substrate respiration and phosphorylation in rat liver mitochondria.

Rats	N	Succinate	Succinate plus ADP	R_s	P:O
Control	10	0.83 ± .04	6.11 ± .20	7.4	1.67 ± .04
Cold-exposed	10	0.79 ± .03	5.95 ± .20	7.6	1.62 ± .03

the P:O ratio remains unaltered in washed mitochondria from cold-exposed rats. Likewise, it appears that the exposure of rats to cold has no effect on the succinate respiration of liver mitochondria. In addition, the R_s values shown in Table 1 indicate that ADP stimulates respiration in the cold-exposed group to the same extent as in the controls.

To determine whether a supression of P:O ratios in cold-exposed rats occurs when other cell components are present in addition to the mitochondria, studies were also made on homogenates. These data are summarized in Table 2. In contrast to the results obtained with washed liver mitochondria, it was found that the P:O ratios are significantly decreased in liver homogenates from cold-exposed rats.

The measurements which were made on liver homogenate respiration are also shown in Table 2 and include: (a) substrate (succinate) respiration, (b) substrate plus ADP respiration, and (c) respiration in the presence of excess substrate plus ATP. Previous work has shown that ATP stimulates homogenate respiration in the presence of excess exogenous substrate (Ziegler et al., 1963). This stimulation probably is due to an increased ADP concentration resulting from the dephosphorylation of the added ATP. The ratio of the substrate-ATP rate of respiration to the substrate rate of respiration provides a measure of the ATPase activity of the cells.

The results suggest that homogenate respiration in the presence of excess substrate (succinate) is unaffected by cold exposure. Likewise, exposure to cold does not appear to cause any alteration in the ATPase activity of the liver tissue. This is indicated by the fact that homogenates from control rats are stimulated by addition of ATP to the same extent as are the cold-exposed rats. These results are in agreement with previous reports that ATPase activity does not change during cold exposure (Hannon, 1959).

The data in Table 2 also show that the addition of ADP to the tissue homogenates stimulates sub-

TABLE 2.—Effect of cold (4 weeks at 2-5°C) on exogenous substrate respiration and phosphorylation in rat liver homogenates.

Rate	N	Succinate	Succinate plus ATP	Succinate plus ADP	R_s	P:O
Control	6	0.28 ± .01	0.33 ±.01	0.85 ± .03	2.6	2.02 ± .02
Cold-exposed	6	0.28 ± .01	0.34 ±.01	1.04 ± .03*	3.1*	1.74 ± .06*

* $P < .01$

strate respiration to a greater extent in the cold-exposed animals than it does in the controls. Thus, the maximum rate of tissue homogenate respiration (i.e., respiration in the presence of excess exogenous substrate and ADP) is significantly greater in the cold-exposed rats. This increase is in contrast to the unaltered succinate-ADP respiration observed with washed mitochondria.

Thus, the finding in the present study that the P:O ratios are decreased only in liver homogenates from cold-exposed rats and not in washed mitochondria supports the finding of Smith (1960) that such a suppression of P:O ratios occurs only in systems where other cell components are present in addition to the mitochondria. The results also agree with earlier reports that an increased rate of respiration can be observed in liver slices and homogenates from cold-exposed rats (Hannon, 1960; Clark et al., 1954), but that no increase occurs when the measurements are made using mitochondria (Patkin and Masoro, 1960; Chaffee et al., 1961). These data indicate that there is an increased concentration of functional respiratory units in liver tissue of cold-exposed rats. They also indicate that respiration, like oxidative phosphorylation, is influenced by some extra-mitochondrial factors which may be washed out in preparing purified mitochondria.

It is of interest to compare these results with those obtained by Aldridge and Stoner (1963) who found no change in P:O ratios or rates of respiration in either washed or unwashed mitochondria from cold-exposed animals. One explanation would be that the extra-mitochondrial factors, which need to be present in order to demonstrate these changes in cold-exposed rats, may be lost even in the preparation of unwashed mitochondria.

Despite the lack of change in substrate respiration and ATPase activity, it would appear that adaptive changes at the cellular level do occur in the rat during cold exposure which result in an accelerated rate of cellular respiration and a decrease in the efficiency of oxidative phosphorylation. It is further suggested that these results are most easily demonstrated when whole tisue homogenates rather than washed mitochondria are used. These adaptions presumably aid the rat by increasing its capacity to produce heat and thus maintain its normal body temperature in the cold.

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