MITOCHONDRIAL RESPIRATION IN MAMMALIAN LIVER DURING COLD EXPOSURE AND HIBERNATION

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ABSTRACT. — Studies were made of the effect of cold exposure and hibernation (1-3 months at 5°C) on succinate respiration of liver mitochondria in hamsters and chipmunks. Measurements which were made included: 1) mitochondrial respiration with excess exogenous substrate, 2) mitochondrial respiration with excess exogenous substrate plus ADP, 3) respiratory stimulation caused by ADP addition, and 4) efficiency of oxidative phosphorylation. Succinate-ADP respiration was increased in mitochondria from hibernating chipmunks and decreased (both with and without added ADP) in hibernating hamsters. Respiration and phosphorylation were unaffected in chipmunks which did not hibernate during cold exposure but succinate respiration was decreased and the P:O ratio increased in nonhibernating cold-exposed hamsters.

The purpose of this investigation was to study the effect of cold exposure and hibernation on oxidative activity of liver mitochondria isolated from various species of hibernators. A wealth of data has accumulated over the years on natural mammalian hibernation (Lyman and Chatfield, 1955) and these studies have demonstrated that the phenomenon of hibernation is not precisely the same among all mammals and differs even among various families of rodents (Lyman, 1954). Relatively few studies have been carried out, however, investigating possible differences between hibernators at the cellular and enzymatic level. As a result, our knowledge of the cellular changes effected by hibernation is limited. The greatly decreased general metabolism observed in dormant animals, however, makes the cellular metabolic events which occur during hibernation of special interest.

A limited number of studies have been carried out measuring the oxygen consumption of various tissues of hibernators, such as the liver, muscle, kidney, brain, and brown fat. Some of these studies have reported a slight reduction in the oxygen consumption of brain and kidney slices in the hibernating hamster and the hibernating ground squirrel (Hook and Barron, 1941; Kayser, 1954; South, 1958; and Meyer, 1959). Meyer also reported, however, that the respiratory activity of cardiac and skeletal muscle tissue increases by 60-120% during hibernation in the ground squirrel. Chaffee et al. (1966) stated that liver mitochondria from hibernating ground squirrels were 50% higher in the oxidation of succinate than were control mitochondria.

In many of these studies, it has been noted that certain hibernators, particularly the hamster, show an inconstancy when entering hibernation while they are exposed to cold in the laboratory (Pohl, 1965) such that some of the animals may never hibernate. Comparisons of cellular metabolism between hibernating animals and cold-exposed animals which have failed to hibernate have been made by measuring oxidative activity of the tissues of both groups of animals. Denyes and Hasset (1960) observed that in the golden hamster the respiration of liver slices actually increases after cold exposure or hibernation. Similarly, increased succinic oxidase activity of liver mitochondria was found in both cold-exposed and hibernating hamsters at 37°C (Chaffee, 1957; Chaffee et al., 1961). Inasmuch as a similar increase occurred in both hibernating and cold-exposed hamsters which did not become dormant (Chaffee, 1957), Chaffee stated that the increased metabolic capacity reflects a response to cold exposure and is not a result of hibernation itself. A study by Mokrasch et al. (1960), however, failed to confirm this view; they reported no significant alteration from the control level in the rate of oxygen consumption by liver mitochondria of hibernating hamsters.

The present study was undertaken, therefore, to learn more about the cellular metabolic changes which take place during hibernation by studying liver mitochondral activity in animals exposed to cold. The specific objectives were: 1) to compare the effects of cold exposure in two species of hibernators, *Mesocricetus auratus,* the golden hamster, and *Tamias striatus,* the eastern chipmunk, to see if any differences in cellular activity during hibernation are apparent in these two species, and 2) to compare the results obtained on hibernating animals with those obtained on animals, also exposed to cold, but which fail to hibernate.

Materials and Methods

Eastern chipmunks were trapped on the campus of The Pennsylvania State University. Eighteen of these chipmunks, both males and females, were exposed to 5°C for 1-3 months. Six of the chipmunks entered hibernation while 6 others died during exposure to cold. The remaining 6 chipmunks exposed to cold remained active and showed no signs of becoming torpid. These cold-exposed active and hibernating chipmunks (plus 6 controls maintained at an ambient temperature of 24° ± 1°C) were used to study the effect of cold on liver mitochondrial respiration.

In addition, twenty adult male hamsters, (purchased from the Con Olson Company, Madison, Wisconsin) were exposed to the same cold temperature for 1-3 months and ten others were maintained at ambient temperature. Six of the cold-exposed animals hibernated while 3 died during exposure. The remaining cold-exposed animals remained active. The effect of cold on liver mitochondrial oxidative metabolism was then studied on 6 cold-exposed, 6 hibernating, and 6 control hamsters. All animals (both chipmunks and hamsters) were fed Purina Laboratory Chow and water *ad libitum.*

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 was quickly removed and placed into an ice-cold isolation medium. The isolation medium contained 225 mM 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. Washed mitochondria were then prepared by differential centrifugation in a refrigerated centrifuge according to the method described by Schneider (1948). The nitrogen content of the mitochondrial preparation was determined by the micro-Kjeldahl method described by Ma and Zuazaga (1942).

Measurements of respiration and phosphorylation were made polarographically at 25°C using the rotating oxygen electrode as originally described by Chance and Williams (1955) and the Oxygen Monitor System manufactured by Yellow Springs Instrument Company. The reaction medium used has been described elsewhere (Frehn and Anthony, 1965). Mitochondrial substrate respiration was measured by adding 0.15 ml of the mitochondrial suspension to 3.0 ml of the reaction medium containing substrate. After measuring mitochondrial respiration on exogenous substrate for approximately 3-4 minutes, 0.01 ml of 0.05 M ADP was added to the reaction mixture in order to measure the substrate-ADP respiration rate.

Results and Conclusions

Table 1 summarizes the results obtained on the mitochondria of control, cold-exposed, and hibernating chipmunks. Two types of measurements were made. These were: 1) the efficiency of oxidative phosphorylation as reflected by the P:O ratios; and 2) exogenous substrate (succinate) and exogenous substrate-ADP rates of respiration. Respiration values represent X±S.E. in $\mu M\ O_2$/sec/mg of nitrogen. The R_s value represents the respiratory stimulation caused by the addition of ADP. It is evident from these data that the P:O ratios remain unaltered in washed mitochondria in cold-exposed and hibernating chipmunks. Likewise, succinate respiration is unaffected in mitochondria from both groups. The R_s values shown in Table 1, however, indicate that ADP stimulated substrate respiration more in hibernating animals than in either control or cold exposed chipmunks. Further examination of the data indicates that this elevated R_s value is due to a significantly increased substrate-ADP rate of respiration in mitochondria from hibernating chipmunks.

The respiration rates and P:O ratios obtained on hamster liver mitochondria are found in Table 2. As in the results obtained on hibernating chipmunks, these data show that the P:O ratio remains unchanged from the control level in hibernating hamsters. In contrast to hibernating chipmunks, however, respiration rates are significantly reduced in mitochondria from hibernating hamsters. Succinate respiration is reduced by 33% and succinate-ADP respiration is reduced by 26%. The R_s value is the same, however, for the hibernators as for the controls. Also in contrast to the chipmunks, the results in Table 2 show that the

Table 1. Effect of Cold (1-3 months at 5°C) and Hibernation on Exogenous Substrate Respiration and Phosphorylation in Chipmunk Liver Mitochondria.

Chipmunks	N	Succinate	Succinate plus ADP	R_s	P:O
Control	6	0.77 ± .04	4.94 ± .29	6.4 ± .2	1.60 ± .05
Hibernating	6	0.83 ± .05	6.52 ± .43[1]	7.9 ± .3[2]	1.57 ± .02
Cold-exposed	6	0.81 ± .07	5.07 ± .45	6.3 ± .4	1.63 ± .04

[1] $P < .02$
[2] $P < .01$

greatest response to ADP addition occurred in mitochondria from hamsters which failed to hibernate. Examination of these data indicates that the elevated R_s value observed in cold-exposed hamsters is due to a lowered rate of substrate respiration and is not due to an elevated substrate-ADP rate of respiration.

Thus, there appears to be rather significant differences at the cellular level between hibernating chipmunks and hibernating hamsters. The most obvious difference is in mitochondrial succinate-ADP respiration. In substrate-ADP respiration, substrate and ADP are present in excess. Therefore, the main rate-limiting factor of respiration is the concentration of functional respiratory units present in the mitochondria. Thus, the elevated R_s value observed in hibernating chipmunks apparently reflects an increased concentration of functional respiratory units in liver tissue of hibernating chipmunks. In contrast, the reduced respiratory rates observed in mitochondria from hibernating hamsters indicates a decreased concentration of functional respiratory units in the liver tissue of these animals.

Although succinate-ADP respiration remains unaltered, there does appear to be significant differences in mitochondrial activity between hamsters and chipmunks which were exposed to cold but which failed to hibernate. Succinate respiration, unaltered in cold-exposed chipmunks, is slightly reduced in cold-exposed hamsters. Substrate rate of respiration may be influenced either by the availability of endogenous ADP or by the concentration of functional respiratory units. Since the concentration of functional respiratory units appears to be unchanged during cold exposure, it would appear that there is a lower availability of endogenous ADP in mitochondria of cold-exposed hamsters. This might occur in mitochondria in which phosphorylation and respiration become more tightly coupled. This conclusion is substantiated by the elevated P:O ratio found in mitochondria from cold-exposed hamsters (see Table 2).

Thus, differences in cellular metabolic activity were noted between hamsters and chipmunks in both the cold-exposed and hibernating groups. These results suggest, therefore, that

TABLE 2. Effect of Cold (1-3 months at 5°C) and Hibernation on Exogenous Substrate Respiration and Phosphorylation in Hamster Liver Mitochondria.

Hamsters	N	Succinate	Succinate plus ADP	R_s	P:O
Control	6	0.54 ± 0.02	2.17 ± 0.13	4.0 ± .1	1.34 ± 0.03
Hibernating	6	0.36 ± 0.02[1]	1.51 ± 0.03[1]	4.3 + .2	1.40 ± 0.03
Cold-exposed	6	0.47 ± 0.02[2]	2.18 ± 0.06	4.7 ± .1[1]	1.46 ± 0.03[3]

[1] $P < .001$
[2] $P < .05$
[3] $P < .02$

differences between hibernators do exist at the cellular and enzymatic level. Inasmuch as mitochondria from cold-exposed animals showed a different respiratory activity than mitochondria from hibernating animals, the results do not appear to agree with earlier reports (Chaffee, 1957) that the altered metabolic capacity observed in hibernating animals merely reflects a response to cold exposure and is not a result of hibernation, itself. It should be noted, however, that the study by Chaffee was carried out at 37°C whereas in the present study, mitochondrial activity was measured at 25°C. Thus, it would appear desirable that additional studies be performed at various temperatures.

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Literature Cited

Chaffee, R. R. 1957. Mitochondrial oxidative and phosphorylating enzyme systems in mammals during cold-exposure and hibernation. Thesis, Harvard University.

———, F. L. Hough and C. P. Lyman. 1961. Mitochondrial oxidative enzymes and phosphorylations in cold exposure and hibernation. Am. J. Physiol. 201:29-32.

———, E. T. Pengelley, J. R. Allen, and R. E. Smith. 1966. Biochemistry of brown fat and liver of hibernating golden-mantled ground squirrels (*Citellus lateralis*). Canad. J. Physiol. Pharm. 44:217-223.

Chance, B., and G. R. Williams. 1955. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J. Biol. Chem. 217:383-393.

Dennes, A., and J. Hasset. 1960. A study of liver, diaphragm and kidney in cold-exposed and hibernating hamsters. In Mammalian Hibernation. Ed. by C. P. Lyman and A. R. Dawe. Bull. Mus. Comp. Zool., Harvard Univ. Cambridge. 124:437-456.

Frehn, J. L., and A. Anthony. 1965. Cellular respiration and phosphorylation during cold exposure in rats. Trans. Illinois State Acad. Sci. 58(4): 263-267.

Hook, W. E., and E. S. G. Barron. 1941. The respiration of brown adipose tissue and of kidney of the hibernating and non-hibernating ground squirrel. Am. J. Physiol. 133:56-63.

Kayser, C. 1954. L'increment thermique critique de la respiration *in vitro* de tissu renal de Hamster ordinaire (*Cricetus cricetus*), reveille en ete et en sommeil en hiver. C. R. Acad. Sci. 239:554-556.

Lyman, C. P. 1954. Activity, food consumption and hoarding in hibernators. J. Mamm. 35:545-552.

———, and P. O. Chatfield. 1955. Physiology of hibernation in mammals. Physiol. Rev. 35:403-425.

Ma, T. S., and G. Zuazaga. 1942. Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Industrial and Engineering Chem. Analytical Ed. 14:280-282.

Meyer, M. P. 1959. Tissue slice respiration in wild mammals with special regard to effects of growth in the opossum and hibernation in the thirteen-lined ground squirrel. Doctoral Thesis, University of Wisconsin.

Mokrasch, L. C., H. J. Grady, and S. Grisolia. 1960. Thermogenic and adaptive mechanisms in hibernation and arousal from hibernation. Am. J. Physiol. 199:945-949.

Pohl, H. 1965. Temperature regulation and cold acclimation in the golden hamster. J. Appl. Physiol. 20:405-410.

Schneider, J. 1948. Intracellular distribution of enzymes. III. The oxidation of octanoic acid by rat liver fractions. J. Biol. Chem. 176:259-266.

South, F. E. 1958. Rates of oxygen consumption and glycolysis of ventricle and brain slices obtained from hibernating and non-hibernating mammals as function of temperature. Physiol. Zool. 31:6-15.

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