QUANTITATIVE ASPECTS OF BROWN ADIPOSE TISSUE THERMOGENESIS DURING AROUSAL FROM HIBERNATION[1]

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Evidence which indicates that brown adipose tissue may serve as a specialized site of heat production has recently been reviewed by Joel (1965). The relative abundance of this tissue in hibernators has led a number of workers to suggest that brown adipose tissue may play an important role in the generation of heat during arousal from hibernation. There are, however, few data available which permit one to assess the quantitative aspects of such a role for this tissue. The recent attempts of Ball (1965) to calculate these quantitative aspects from available data in the literature, involved many assumptions which only served to emphasize the need for more data. This report describes one attempt to obtain such data for the big brown bat (*Eptesicus fuscus*) during its arousal from hibernation. The bat was chosen for this study because there is some evidence (Hayward *et al.*, 1965) to indicate that hibernating bats may be the most specialized of adult mammals in terms of the magnitude of brown adipose tissue thermogenesis.

A quantitative estimate of the contribution of heat by brown adipose tissue during an arousal from hibernation can be obtained by comparing the oxygen consumption of brown adipose tissue with the total body oxygen consumption during the arousal process. Ideally, the *in vivo* respiration of brown adipose tissue would be measured. However, the small size of the bats (about 15 grams) precludes such an approach. As an alternative, we have attempted to ascertain the heat production of brown adipose tissue from measurements of its *in vitro* respiration. The respiration of liver and heart slices from these bats has also been determined for comparison with brown adipose tissue respiration, and to obtain a larger estimate of the non-muscular heat production.

The limitations involved in efforts to summate *in vitro* tissue respirations to account for total animal respiration are well known (von Bertalanffy and Pirozynski, 1953). Such limitations have been invoked in this study to explain the failure of our tissue respiration measurements to provide a satisfactory estimate of the quantitative contribution of brown adipose tissue to arousal thermogenesis.

The technique of thermography has been used to provide additional descriptive evidence of the thermogenic capacity of brown adipose tissue.

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Methods

Prior to experimentation, the bats used in this study had been hibernating intermittently for a period of approximately five months. Measurement of their total oxygen consumption during arousal from hibernation was accomplished using a closed-circuit, volumetric respirometer. Exhaled carbon dioxide was absorbed by soda lime and the decrease in pressure in the closed system was detected with a sensitive manometer. At two-minute intervals, the pressure was restored to the initial level by allowing an accurately-measured quantity of water to flow into the system, this measure being equal to the oxygen consumed in that interval. The system was designed to maintain normal atmospheric gas concentration in the bat chamber and to minimize errors due to possible slight temperature fluctuations in the system.

To provide a criterion of stage of arousal from hibernation, each bat had, previously, a small thermocouple implanted in its interscapular brown adipose tissue. During an arousal in the respiration chamber the thermocouple lead wires were led from the chamber through an air-tight seal, enabling a continuous record of brown adipose tissue temperature to be obtained. All arousals were conducted at the ambient temperature at which the bats had been hibernating (5° C.).

Immediately subsequent to each arousal, the bat was sacrificed and a weighed sample of brown adipose tissue taken for the *in vitro* respiration measurements. The total remaining brown adipose tissue, from all locations in the body, was carefully dissected and weighed, care being taken to prevent drying of the tissue during this procedure. The heart and liver from each bat were also weighed and several samples of these tissues taken for respiration measurements.

Oxygen consumption of tissue samples was determined at 37.2° C. by means of the Warburg manometric apparatus. The incubation medium was Krebs-Henseleit phosphate buffer (Krebs and Henseleit, 1932) modified to contain one-half the recommended calcium. In some experiments, glucose was dissolved in this medium to yield a concentration of 1.5 mg./ml. The main compartment of the vesssels contained 2.9 ml. of medium and the tissue sample. The center well contained 0.2 ml. of 20% KOH and a strip of filter paper to facilitate CO_2 absorption. The sidearm contained 0.1 ml. of a catecholamine solution dissolved in H_2O weakly acidified with HCl. The gas phase was oxygen. Brown adipose tissue was cut into small pieces with a razor blade after weighing to 0.1 mg. on a torsion balance, 15–20 mg. of tissue being used per vessel. Liver and heart were sliced with the aid of a Stadie-Riggs tissue slicer and weighed to the nearest mg. The amount of liver used per vessel was 100–175 mg. while heart samples weighed between 50–100 mg. In all cases, tissue samples were prepared and run immediately after removal from the bat. Vessels were gassed with oxygen for 10 minutes, closed, and after another five minutes for thermoequilibration, readings were taken at 10-minute intervals for 40–50 minutes. The contents of the sidearm were then added and readings continued for another 50–100 minutes.

The epinephrine used was a sample of the free base kindly supplied by Burroughs-Wellcome Co. The norepinephrine was a bitartrate preparation purchased from Calbiochem.

Bats were prepared for thermography (infrared radiography) by first shaving the hair off the dorsal aspect of their bodies. This facilitated the detection of distinct differences in the radiation of heat from the skin surfaces, these differences being largely dependent upon the temperatures of the underlying tissues. Thermo-

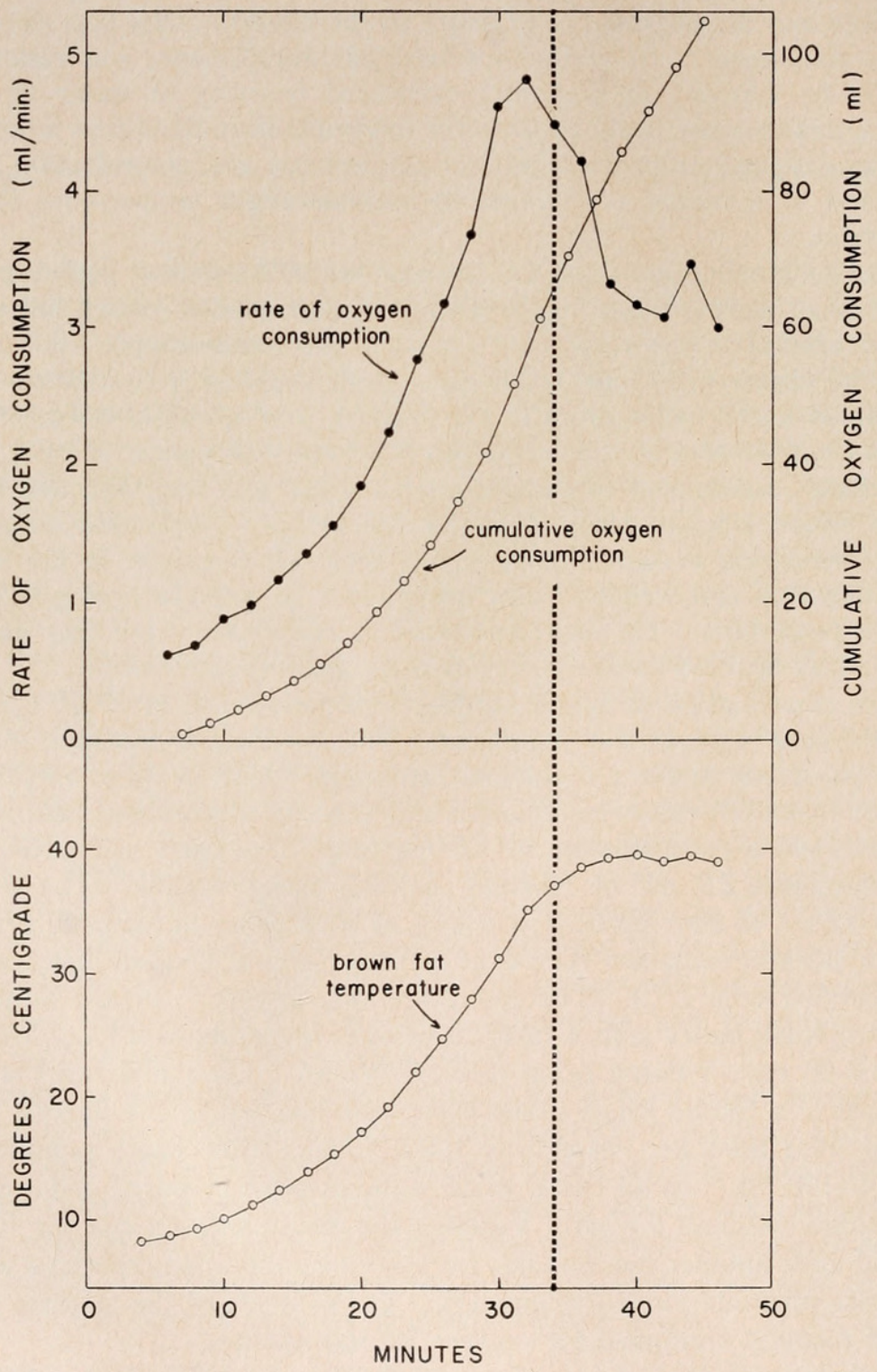

FIGURE 1. The typical patterns of oxygen consumption and brown adipose tissue (brown fat) temperature increase in the bat during arousal from hibernation. The vertical broken line intersects the curves at the time when rapid arousal is completed. Ambient temperature during the arousal was 5° C.

grams are pictorial thermal maps and were obtained using a Barnes Medical Thermograph.[3]

Results

Total oxygen consumption during arousal

For each bat, the rate of arousal from hibernation varied to a certain extent with body weight, amount of brown adipose tissue, and apparent individual behavior differences. Amongst this variation, however, healthy individuals of similar body weight demonstrated a consistent pattern of oxygen consumption during arousal. The data of Figure 1 are an example of one such arousal. The brown adipose tissue (brown fat) temperature[4] curve is included in Figure 1 to aid identification of the course of arousal. For approximately the first 15–20 minutes of arousal, there is a low and slowly-increasing rate of oxygen consumption, but

Table I

Rate of oxygen consumption of bat tissues at 37.2° C. under various experimental conditions

Tissue	Treatment	No. of animals	No. of measurements	Mean rate of O_2 consumption ± S.E. (μl./100 mg. fresh tissue/hr.)
Brown fat	Control	9	26	392 ± 42.4
	Epinephrine	9	9	1320 ± 93.9
	Epinephrine + glucose	6	6	1252 ± 107.7
Liver	Control	4	7	168 ± 5.0
	Epinephrine	3	3	160 ± 12.6
	Epinephrine + glucose	3	3	157 ± 9.3
Heart	Control	2	4	119 ± 11.6
	Epinephrine	2	2	117
	Epinephrine + glucose	2	2	114

the rate increases rapidly and reaches high levels for the 20–35-minute interval. After approximately 34 minutes (vertical broken line), the rate of oxygen consumption begins to fall to a lower level, coinciding with the approach of maximum brown adipose tissue temperature. At this point, the cumulative oxygen consumption is near 65 ml.

The mean figures for duration of arousal and total oxygen consumption for 8 bats are 36 minutes and 66.15 ml., respectively (from Table II). Assuming a low respiratory quotient (R.Q.) of 0.75 for arousal (Jansky and Hajek, 1961), this oxygen consumption represents 312 cal. of heat production, attributable to the enthalpy increase of the body mass and the heat loss during the arousal.

Tissue respiration

A representative experiment in which the oxygen consumptions of brown adipose tissue and liver were measured is shown in Figure 2. The respiratory rate of both

[3] Barnes Engineering Company, Stamford, Connecticut.

[4] The temperature of interscapular brown adipose tissue is typically about 1° C. above core temperature during arousal from hibernation (Hayward *et al.*, 1965).

TABLE II

Calculation of the percentage of the total oxygen consumption for arousal that is attributable to brown fat

Bat	Body weight (g.)	Duration of arousal (min.)	Weight of brown fat (g.)	Maximum metabolic rate of brown fat (μl. O_2/100 mg./hr.)	Total O_2 consumption for arousal (ml.)	O_2 consumption of brown fat for arousal (ml.)	Percentage of total O_2 consumption by brown fat
A	15.6	39	0.446	1500	78.05	4.35	5.57
B	14.2	39	0.453	1890	84.16	5.57	6.61
C	13.3	44	0.415	995	68.64	3.03	4.41
E	14.5	26	0.432	1490	51.88	2.79	5.38
G	14.8	22	0.584	1390	52.30	2.98	5.69
H	13.6	38	0.467	1420	71.33	4.20	5.89
J	15.6	38	0.643	1235	67.44	5.03	7.46
K	12.7	42	0.334	1070	55.39	2.50	4.52
Means	14.3	36	0.472	1374	66.15	3.81	5.69

tissues was unaltered by the addition of glucose to the medium. Addition of epinephrine, 1 μg./ml., caused a 500% increase in the rate of oxygen consumption of brown adipose tissue but was without effect upon liver. In other experiments in which the action of epinephrine on brown adipose tissue was compared at concentrations of 0.1, 1.0 and 10 μg./ml., it was found that a maximum response was obtained at 1.0 μg./ml. A comparison of norepinephrine and epinephrine at con-

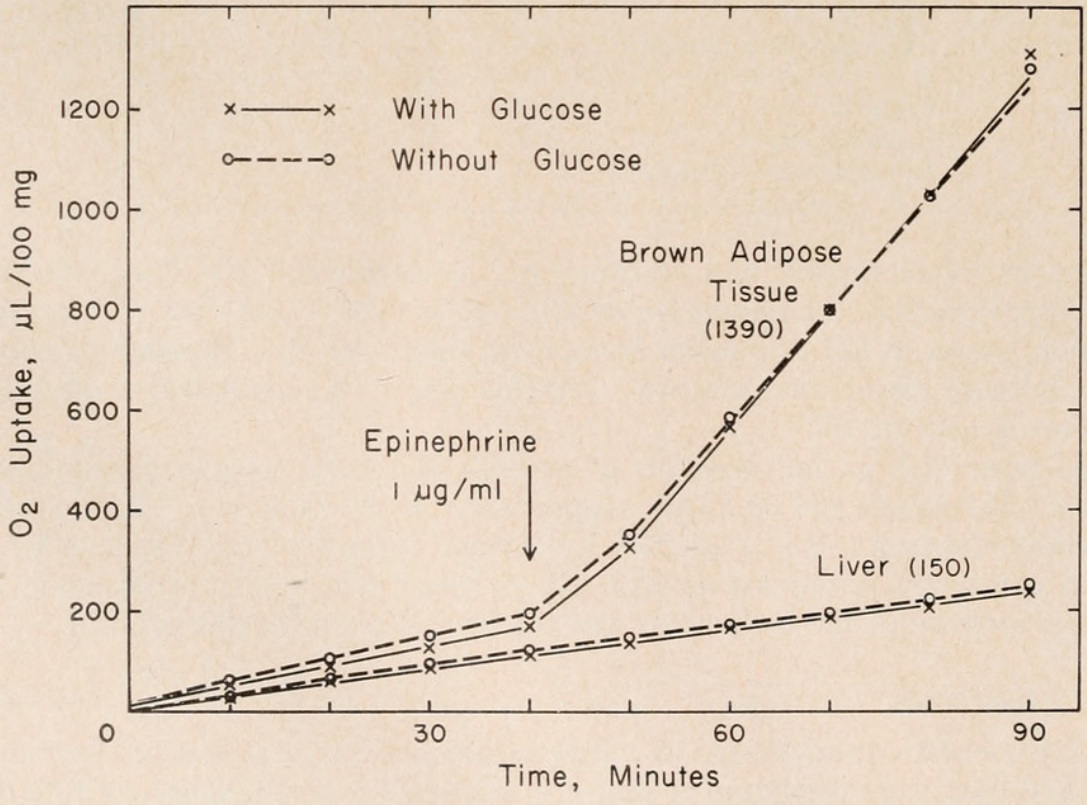

FIGURE 2. A representative experiment in which the oxygen consumptions of brown adipose tissue and liver of the bat show the effects of epinephrine and glucose addition. Numbers in parentheses are rates of oxygen consumption (μl./100 mg. fresh tissue/hr.).

centrations of 0.1 μg./ml. also showed that no significant difference in their action could be observed. Epinephrine at a concentration of 1 μg./ml. was therefore employed routinely in the series of experiments performed.

A summary of this series of experiments is given in Table I. In the absence

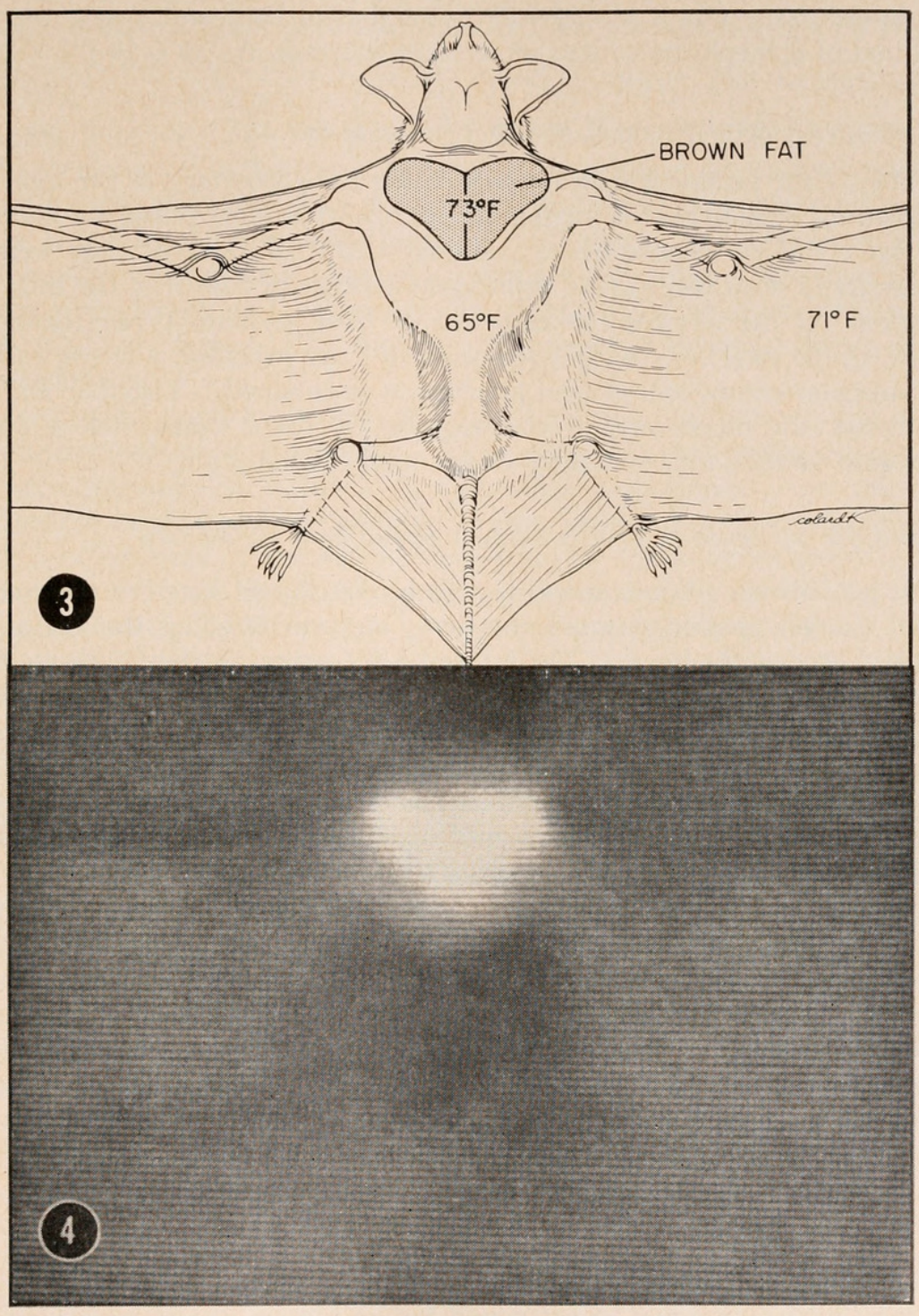

FIGURE 3. Illustration of a bat as positioned for thermography, showing the location of the interscapular brown adipose tissue and the major temperatures prevailing at the commencement of thermographic scanning.

FIGURE 4. Thermogram of the dorsal surface of a bat during its arousal from hibernation. The higher the temperature and intensity of infrared radiation from the skin surface, the brighter is the image.

of any added stimulation, the mean value for the rate of oxygen consumption of rate of brown adipose tissue and was without effect upon liver or heart slices. In experiments not reported here, it was found that there was no significant difference between the respiratory rate of brown adipose tissue sampled before and after arousal from hibernation.
brown adipose tissue was 2.33 and 3.29 times that of liver and heart, respectively. The addition of epinephrine caused an average increase of 350% in the respiratory

Relative oxygen consumption of brown adipose tissue

In Table II the pertinent data for calculating the percentage of the total oxygen consumption for arousal that is attributable to brown adipose tissue are summarized for each bat. Brown adipose tissue averaged 3.30% of the total body weight and, based upon its *in vitro* respiration, utilized an average of 5.69% of the total oxygen consumption. If the oxygen consumptions of liver and heart (averaging 4.31% and 1.16% of the total body weight, respectively) are included, a larger estimate of the non-muscular component of heat production is obtained. Together with brown adipose tissue, the mean oxygen consumption by these tissues for a 36-minute arousal would be 4.55 ml., or 6.88% of the total consumption.

Thermographic evidence of brown adipose tissue heat production

In the bat, brown adipose tissue is widely distributed between the muscles of the dorsal thoracic region, around the neck, and surrounding the major vessels entering and leaving the heart. The largest depot occurs in the interscapular fossa and extends to the back of the head and sides of the neck. The tissue lies just beneath the skin in the general position illustrated in Figure 3. To obtain the best contrast pattern on the thermogram, the following procedure was used. A hibernating bat was stimulated to arouse, and during this process was kept in a cold environment of 6° C. (42° F.) until the stage when the brown adipose tissue temperature was approaching the ambient room temperature of 22° C. (71° F.). It was then removed from the cold and held in the scanning field of the thermograph. The wing membranes rapidly equilibrated with room temperature. Within 2–3 minutes, the skin temperature over the brown fat passed room temperature, but the rest of the body was still cold. Infrared scanning was commenced when the major temperature areas were those shown in Figure 3. Scanning took approximately three minutes, during which time the bat had to be held immoble. The resulting thermogram (Fig. 4) shows a conspicuously-delineated "hot" area that coincides exactly with the shape of the underlying brown adipose tissue.

Discussion

An average of only 6.9% of the total oxygen consumed by the whole bat during arousal from hibernation can be accounted for by measurements on brown adipose tissue, liver, and heart respiration *in vitro*. Even this value is high since the *in vitro* measurements were made only at 37° C., a temperature approached by the tissues only at the end of the arousal period. This is such a surprisingly low percentage of the total that it raises several questions.

First, if the data are accepted, one must still account for the remainder of the oxygen consumption. Muscle, because of its relatively large mass, would seem to be the main tissue to be considered. Rough dissections of muscle from several bats indicate the total muscle mass of an average bat to be about 4 grams. If, as our data would suggest, we attribute at least 75% of the total O_2 consumption during a 36-minute arousal period to muscle, then we obtain a rate of respiration for muscle of $\frac{0.75 \times 66.15 \text{ ml. } O_2}{4 \text{ g.} \times 36 \text{ min.}} = 0.345$ ml. O_2/g. min. The corresponding rate, as measured here, is 0.224 for brown adipose tissue and 0.02 for heart muscle. It is difficult to reconcile this very high rate of respiration for skeletal muscle with the indication that shivering is an unimportant feature of the arousal from hibernation of the bat, since the results of Hayward and Lyman (in press) show that there is no difference in the arousal time when bats are curarized. Moreover, if muscle was consuming oxygen at this rate, it should be producing over 50% more heat than brown adipose tissue. The thermogram presented certainly does not validate such a conclusion.

In considering possible reasons for this apparent discrepancy, the reliability of the measurements must be examined. For example, perhaps the measurement of total oxygen consumption is too high. For this measurement, however, all conceivable errors would result in underestimation rather than overestimation of the true rate. In addition, a theoretical calculation of the heat required to warm a 14.3-g. bat from 5° to 35° C. at an ambient temperature of 5° C. verifies the experimental results. If we assume an average specific heat of 0.9 cal./g.° C. for the total tissue of the bat, then 0.9 cal./g.° C. × 14.3 g. × 30° C. = 386 cal. will be required for arousal. This value is undoubtedly too high since brown adipose tissue warms to 35° C. prior to all other tissues (Hayward *et al.*, 1965). Partly offsetting this consideration, however, will be the heat loss of the environment during arousal. A not unreasonable value would thus seem to be 300 ± 25 calories, which is comparable to the average of 312 calories from our experimental results. This calculation indicates that the measured total oxygen consumption is within the expected range and can be acquitted of possible major error.

Next, one may question the validity of the *in vitro* measurements of tissue oxygen consumption. The mean respiratory rate of 1374 μl. O_2/100 mg./hr. observed here for bat brown adipose tissue when stimulated by catecholamine is as high or higher than that reported for this tissue in other species. Joel (1965) gives an average value of 719 μl. O_2/100 mg. fresh tissue/hr. for brown adipose tissue from another hibernator, the ground squirrel (*Citellus tridecemlineatus*). This value was obtained in the presence of 1 μg./ml. of norepinephrine and was raised to 1260 if 10 μmoles of succinate were also present. In the rat, a value of 725 was observed by Joel (1965) when the brown adipose tissue was stimulated by the addition of 1 μg./ml. epinephrine. Smith and Roberts (1964) report values of 643 for brown adipose tissue from cold-acclimated rats and 232 from normal rats. These authors did not study the effect of catecholamine additions. The rates found here for heart and liver slices are not greatly different from those reported for rat tissues (Long, 1961, p. 795).

Lastly, one can offer the explanation that the measured *in vitro* rates fall far short of those that do occur *in vivo*. Certainly the measured rate for heart slices

does not reflect the rate exhibited by a heart actively beating *in vivo*. Values for perfused hearts are much higher (Fisher and Williamson, 1961). However, even if the *in vivo* rates of heart and liver respiration were 10-fold those measured here, they would still account for only 12% of the total oxygen consumption.

Dynamic metabolic conditions are characteristic of brown adipose tissue during arousal from hibernation (Joel, 1965, p. 84). We are led to suspect that brown adipose tissue heat production during arousal may be considerably greater than our respiratory data would indicate, despite their high value, and that the necessary conditions to achieve such rates *in vitro* have not yet been achieved.

We appreciate the critical advice given by Dr. C. P. Lyman on the design and conclusions of this study.

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Summary

The *in vitro* respiratory rates of brown adipose, heart, and liver tissues were studied in the bat (*Eptesicus fuscus*) to determine their contribution to the heat necessary for arousal from hibernation. The mean oxygen consumption of the whole animal for arousal from hibernation was 66.2 ml. of which 5.7% is estimated to be utilized by brown adipose tissue, and 1.2% by heart and liver combined. The maximum respiratory rate of brown adipose tissue when stimulated by epinephrine was 134 μl. O_2/100 mg. fresh tissue/hr. Despite this high *in vitro* respiratory rate, it seems inadequate, on the basis of other evidence, to account for the heat production expected for brown adipose tissue during arousal from hibernation. A thermogram of a bat arousing from hibernation is presented which provides pictorial evidence of the large thermogenic capacity of brown adipose tissue. It is concluded that the conditions necessary to measure the maximum respiratory rate of brown adipose tissue, such as it occurs during arousal from hibernation, have not yet been achieved.

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