Calorigenesis of Brown Adipose Tissue in Cold-Exposed Rats* (32782)

YUSUKE IMAI,[1] BARBARA A. HORWITZ,[2] AND ROBERT EMRIE SMITH
Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, California 95616

The multilocular brown adipose tissue has been shown to participate in the thermal response of arousing hibernators (1, 2) and newborn mammals exposed to cold (3, 4). Brown fat from adult rats also responds to chronic cold exposure of the animal by increasing its mass and the respiratory rate *in vitro* (5). Moreover, in rats acutely subjected to low temperatures, direct recordings have demonstrated an increase in heat production of this tissue (6, 7). However, in view of the small proportion of brown fat relative to the total body mass of the adult rat, the quantitative significance of the heat produced by this tissue in adult nonhibernators has been questioned (1, 8).

The present study was thus undertaken to evaluate brown fat thermogenesis *in vivo* in relation to the total heat production of rats during acute exposure to cold.

Materials and Methods. Rats (417 ± 4 gm) exposed to 5°C for 3–4 weeks and returned to 26°C for 2 weeks were anesthetized (Na Pentabarbitol, 80 mg/kg of body weight). Cu-constantan recording thermocouples (TC) were placed over the transiently exposed interscapular fat pad, and on both the inner and outer surfaces of the skin over the pad. TC were also fixed upon the left thoracodorsal artery and the deep central venous drainage; another was inserted approximately 5 cm into the colon.

An hour after anesthesia, these preparations were placed in a closed chamber and total oxygen consumption was continuously recorded[3] at a constant temperature (4 or 25°C).

The heat production of the brown fat was calculated as the heat (q_{cv}) transferred from the interscapular pad to the perfusing blood (Eq. 1) plus that lost from the tissue to the skin (q_{cn}), as defined by equation 2, plus the heat (q_f) required to raise the brown fat temperature from its initial to final value (equation 3).

(1) $$q_{cv} = mFh_b(T_{v-a})t,$$

where F = blood flow; h_b, specific heat of blood; T_{v-a}, temperature difference between venous and arterial blood; m = mass of interscapular pad, and t, time.

(2) $$q_{cn} = \lambda A(\Delta T/d)t,$$

where λ = conductivity; A, dorsal surface area of interscapular pad; ΔT, brown fat-outer skin temperature; d, distance from brown fat to outer skin surface; and t, time.

(3) $$q_f = mh_f(\Delta T_f),$$

where m = mass of interscapular pad; h_f, specific heat of brown fat; and ΔT_f, final — initial brown fat temperature.

Values of the constants in the preceding equations were taken as F = 34.62 ml hour^{-1} gm tissue^{-1} (9); $\lambda = 3.33 \times 10^{-2}$ cal cm^{-1} min^{-1} deg.$^{-1}$ (cf. 10); h_b = 0.9 cal. deg.$^{-1}$ ml^{-1}; h_f = 0.9 cal deg.$^{-1}$ gm tissue^{-1}; m = 0.32% gm body weight (5). The surface area, A, and the distance, d, were estimated in the experimental animals as 2.86 ± .13 cm^2 and 5.5 ± .3 mm, respectively.

Assuming from earlier data (5) that (a) the interscapular pad would be 29.4% of the total brown fat and (b) that heat production rates of the tissue were essentially uniform in all locations, the total thermogenesis of the brown fat was calculated as:

$$\frac{\text{(heat production of the interscapular pad)}}{0.294}. \quad (4)$$

These values were then referred to those

* Supported in part by NASA Research Grant NsG 721/050-07-044 and USPHS Research Grant 7 R01 HD 03268-01.

[1] Present address: Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

[2] Postdoctoral Fellow, USPHS (1-F2-GM-13,445-01).

[3] Two Med-Science volume meters were attached in parallel to be used alternatively on the animal chamber.

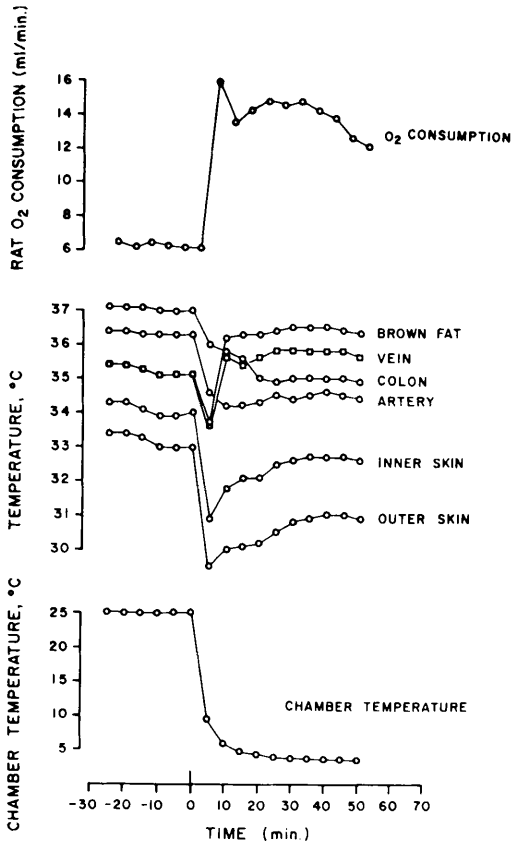

FIG. 1. Effect of cold exposure on oxygen consumption of one experimental rat.

of the intact rats as obtained from their total body oxygen consumption during the cold exposure (caloric equivalent, 4.74 cal/ml of O_2.

Results. The effect of cold exposure on the oxygen consumption of one of the six experimental rats, as well as the temperatures of the six areas of the body monitored in these animals, are illustrated in Fig. 1.

At air temperature of 25°C, the temperatures of interscapular pad (T_f) and venous outflow (T_v) were quite similar, while the arterial temperature (T_a) was 0.5–1.0°C higher than T_v and 0–1.0°C higher than T_f. The colonic temperature (T_b) averaged 1.35 ± .17°C higher than T_f.

Initially upon exposure to cold, T_v, T_a, T_f, and T_b usually fell while the rate of total oxygen consumption rose. Within an average time of 10.0 ± 3.6 min, T_f had begun to increase, and by 13.8 ± 1.7 min had reached temperatures equal to or greater than the control values (at chamber temperature of 25°C). During this period, T_v also rose, to be followed within 7.5 ± 1.4 min (18 min after cold exposure) by an increase of T_a and sometimes T_b.

After the chamber temperature reached 4°C (usually within 10–15 min, T_v–T_a remained relatively constant for each animal, averaging 1.05 ± .05°C (for n = 66 five min intervals). During the same time intervals, the inner skin surface was 2.96 ± .28°C warmer than the outer surface while the brown fat temperature averaged 5.85 ± .19°C higher than that of the outer skin.

Shivering was observed in almost every experiment. It began near an average T_f of 34.8 ± 0.9°C and T_b of 34.4 ± 0.9°C (25.4 ± 9.5 min after placement in the cold chamber) and was maintained intermittently throughout the cold exposure.

The rate of heat production of the interscapular pad, as calculated from Eqs. (1) + (2) + (3), averaged .102 ± .007 kcal/hour, whereas that for the total brown fat (from Eq. 4) was .347 ± .013 kcal/hour. In comparison to the total caloric output of the rat (Table I) the interscapular pad accounted for 2.4% of the total body heat production, or 8.2% in terms of the total brown fat.

Discussion. The calculation of thermal output of the total brown fat as 8.2% entails the assumption that the heat production estimated for the interscapular pad was representative of that of the brown fat in other regions of the body. The fact that the qO_2, i.e., "heat production", *in vitro* (per mg of nitrogen) of the interscapular pad does not deviate significantly from the average qO_2 of the other brown fat regions of the body (5) was adduced as justification for the extrapolation.

Since the measured caloric output of the rat included the heat production resulting from shivering, the calculated value of 8.2% for the thermal contribution of the brown fat is lower than it would have been if compared

TABLE I. Brown Fat Thermogenesis during Cold Exposure (4°C).[a]

	Rate of heat production (kcal/hour)
Intact rat[b]	4.24 ± .40
Interscapular pad	
dq_{cv}/dt	.043 ± .007
dq_{cn}/dt	.058 ± .004
dq/dt	.102 ± .007
Total brown fat	
dq/dt	.347 ± .013
	Contribution of brown fat (% total heat)
Interscapular pad	2.4 ± .1
Total brown fat	8.2 ± .2

[a] Means ± SE where n = 6. Definition of symbols: dq_{cv}/dt = rate of convective heat transfer; dq_{cn}/dt = rate of conductive heat transfer; $dq/dt = dq_{cv}/dt + dq_{cn}/dt$. Since q_f is essentially a time-independent step function, $dq_f/dt = 0$.

[b] From a caloric equivalent of 4.74 cal/ml of O_2.

only to the nonshivering thermogenesis of the animal.

Moreover, the heat production by the brown adipose tissue should be evaluated not only in terms of the amount of heat evolved, but also with respect to its distribution to the body heat sink. As has been previously emphasized (12), the topology and vascular relationships of the brown fat masses facilitate local application of their heat specifically to the vital organs of the thorax (5), the upper spinal cord (5,11), and the autonomic sympathetic chain (5).

Summary. Six adult rats, exposed to 5°C for 3–4 weeks and returned to a 26°C room for 10–14 days, were acutely prepared by insertion of thermocouples to measure temperatures of the colon, the interscapular brown fat, the arterial and venous blood perfusing the interscapular pad, and the inner and outer surfaces of the skin overlying the pad. From the temperature changes observed when the rats were reexposed to cold, the heat produced by the interscapular pad was calculated and extrapolated to that of the total brown fat. These values compared to the concurrently measured total caloric output indicated that during cold stress the intrinsic metabolism of brown adipose tissue accounted for 8.2% of the total heat production.

1. Joel, C. D., in "Handbook of Physiology," Renold, A. E. and Cahill, G. F., Jr., eds., pp. 59–85. Am. Physiol. Soc., Washington, D. C., 1965.
2. Horwitz, B. A., Smith, R. E., and Pengelley, E. T., Am. J. Physiol., 214, 115 (1968).
3. Dawkins, M. J. R. and Hull, D., J. Physiol. (London) 172, 216 (1964).
4. Hull, D. and Segall, M. M., J. Physiol., (London) 181, 449 (1965).
5. Smith, R. E. and Roberts, J. C., Am. J. Physiol. 206, 143 (1964).
6. Donhoffer, Sz., Sardy, F., and Szégvari, Gy., Nature 203, 765 (1964).
7. Donhoffer, Sz. and Szelenyi, A., Acta Physiol. Acad. Sci. Hung. 28, 349 (1965).
8. Jansky, L., Federation Proc. 25, 1297 (1966).
9. Hannon, P., as cited in Ref. 5.
10. Kleiber, M., "Fire of Life," p. 378. Wiley New York, 1961.
11. Brück, K. and Wünnenberg, W., Arch. Ges. Physiol. 290, 167 (1966).
12. Smith, R. E., Science 146, 1686 (1964).

Received Sept. 13, 1967. P.S.E.B.M., 1968, Vol. 127.

The Lecithinase (α Toxin) Activity of Strains of *Clostridium perfringens** (32783)

MITSURU NAKAMURA AND WILLIAM R. CROSS
Department of Microbiology, University of Montana, Missoula, Montana 59801

Nygren (1) has postulated that food poisoning due to *Clostridium perfringens* depends on the production of phospholipase C (lecithinase C, α toxin) by this organism, whereas Smith (2) has suggested that the ingestion of large numbers of the organism is responsible. Dack *et al.* (3) after feeding human

* This investigation was supported by a research grant (UI-00291-02) from the National Center for Urban and Industrial Health, Public Health Service,