with 2*N* HCl; the neutral lipid fraction was obtained by extraction with benzene. The sterol acetates were isolated by preparative TLC of the lipid extracts, acetylation, and further preparative TLC. The fraction from the vegetative portions, 1.5 mg, had radioactivity of 174,000 count/min while the radioactivity of the tuber fraction, weighing 8.1 mg, was 109,000 count/min.

Both fractions gave three spots by TLC on Anasil B. The upper and lower spots had the same mobilities as β-sitosterol acetate and stigmasterol acetate, respectively, while the middle, much fainter spot corresponded to cholesterol acetate. Gas liquid chromatography gave three peaks, which corresponded to these three sterol acetates. Attempts to dilute the sterol acetate fractions with cholesterol acetate and then to purify to constant radioactivity showed that recrystallization does not remove the other sterol acetates and even purification by the dibromide is inefficient in this respect. Therefore the two sterol acetate fractions were combined, and by preparative TLC on Anasil B most of the β-sitosterol and stigmasterol acetates was separated so that a middle fraction enriched in cholesterol acetate remained. Reversed-phase TLC (paraffin oil and ethyl cellosolve-propanol-methanol-water, 45:8:25:22) on Kieselguhr G of a hydrolyzed portion of this fraction gave a spot having the same mobility as cholesterol and a lower spot corresponding to β-sitosterol and stigmasterol. To remove the rest of the other sterol acetates, this fraction (3.0 mg) was diluted with 4.2 mg of pure cholesterol acetate and repeatedly subjected to preparative TLC, adding more carrier as necessary and each time recovering the middle fraction from the previous chromatogram. After six such treatments, cholesterol acetate (2.7 mg, 1240 count/min) which was pure by thin-layer and gas-liquid chromatography was obtained. This was diluted with 33.9 mg of cholesterol acetate and hydrolyzed to cholesterol, which had a radioactivity of 9220 count/min per millimole. Two purifications through the dibromide lowered the radioactivity to 5430 count/min per millimole. Another such purification and recrystallization from ethanol-water did not change this activity.

Both of the plants studied contain sapogenins having 27 carbon atoms. Since cholesterol, unlike the other common plant sterols, also is a 27-carbon compound, the possibility exists of a biogenetic relationship with the sapogenins. In this connection it is interesting to note that we found neither cholesterol nor sapogenins in a sterol extract from *Xanthium pennsylvanicum*, although β-sitosterol and stigmasterol were present (*7*).

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Brown Fat: Thermogenic Effector of Arousal in Hibernators

Abstract. *Earlier indications that brown fat has a thermogenic role in rats exposed to cold suggested that certain comparable functions in hibernating marmots be investigated. From the results it appears that arousal of the animal by cold is induced by sympathetically activated thermogenesis in areas of brown fat so located, relative to the vasculature, that heat is transferred to the thoracic structures and cervical spinal regions.*

In previous reports (*1*, *2*) and discussions (*3*) evidence has been presented which indicates that multilocular brown adipose tissue in rats and other mammals has a thermogenic role. In experiments in the rat it has been shown that this thermogenesis is under autonomic control and becomes activated in response to an acute drop in ambient temperature. The brown fat, because of its location relative to the vascular system, is then able directly to heat the blood as it flows toward the thoracic organs and the cervical spinal regions. As applied to the hibernator (*2*), these findings give meaning to some early observations of Raphael Dubois (*4*), made in 1895, to the effect that the hibernating marmot could be rendered incapable of arousal, either through ligation of the subclavian veins or through spinal transection at segment C_4. Likewise, demonstration that brown fat has a thermogenic role in the hibernator would give metabolic support to the suggestion of Polimanti (*5*) that this tissue serves as a thermal insulator of the body cavity. Such a demonstration, moreover, would reinstate "hibernating gland" (*6*) as an organ discretely useful to the hibernator, a status largely moot since 1551 when Gesner first described the interscapular brown fat in the marmot (*7*).

Hence, the experiment reported here had a dual objective: to demonstrate (i) that thermogenesis could be elicited in vivo at sites of brown adipose tissue in a hibernator, and (ii) that the degree of this activity could be selectively enhanced by exposure of the hibernator to a critically low environmental temperature.

Our study was conducted at an altitude of 3800 m at the Barcroft Laboratory of the University of California's White Mountain High Altitude Research Station. Into each of three adult marmots *(Marmota flaviventris)* (Nos. 2, 5, and 6), deeply hibernating at ambient temperature of 6°C, iron-constantan thermocouples sealed into stainless steel hypodermic cannulae were inserted percutaneously into four sites of brown adipose tissue—the interscapular "hibernating gland," the mid-dorsal area between the paired superior cervical pads, and the axillary areas adjoining the left thoracic region and the right foreleg. Cannulae were inserted subcutaneously at "neutral" sites—a mid-dorsal site in about the T_{10}-L_2 region and also, in two of the marmots (Nos. 2 and 6), a mid-ventral site in the area of the xiphoid process. The cannulae were attached to a standard six-channel (Speedomax) recording device; deep rectal (about 7 cm) temperature and air temperature were read from calibrated thermistors. Respiratory rates were determined visually.

Observations were made first in the laboratory for 40 to 90 minutes at an ambient temperature of 6.8° to 7.0°C. Subsequently two of the animals (Nos. 2 and 6), were taken out-of-doors and

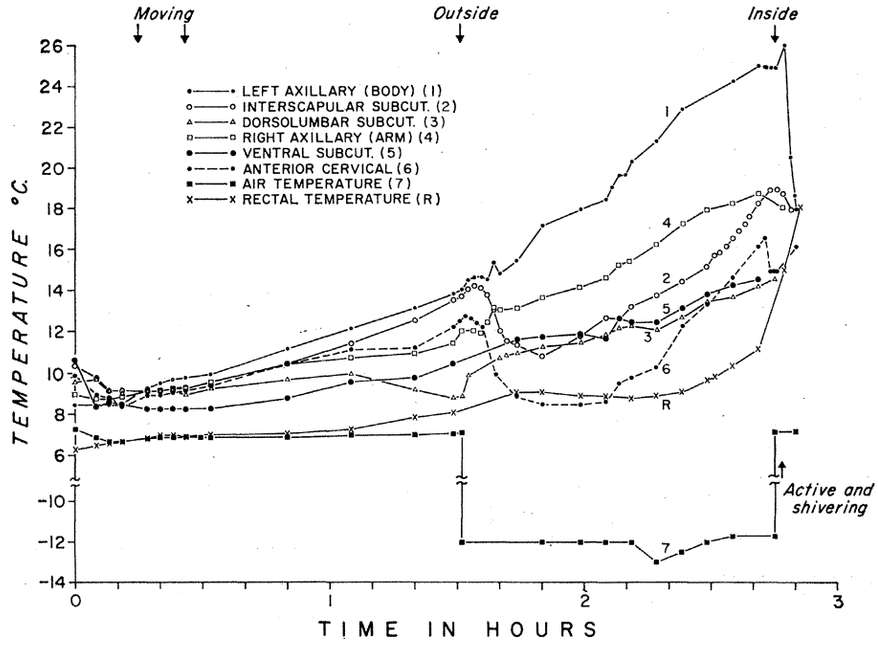

Fig. 1. Temperatures observed in marmot No. 2 during arousal from hibernation.

further exposed to a temperature of −12°C, with wind velocity at about 15 to 20 knots. Marmots Nos. 5 and 6 were litter mates about 20 months old, weighing around 1.5 kg; marmot No. 2 was about 30 months old and weighed about 2.5 kg. All had been in hibernation for over 6 weeks prior to the experiment; however, during the experiment only marmot No. 2 remained in torpor long enough to be adequately observed out-of-doors.

Satisfactory records were obtained for each of the marmots throughout the period of gradual arousal in the laboratory at 7°C. The data show clearly that both the absolute temperatures and the rates of change were higher in the areas of brown fat than elsewhere. The rectal temperatures remained at 6° or 7°C throughout the first 60 minutes; marmot No. 2 showed a rise of 1° between 60 and 90 minutes (Fig. 1).

At 90 minutes marmot No. 2 was gently taken out-of-doors, where the ambient temperature was −12°C, without handling or interruption of recordings. The immediate response in the areas of brown fat (see Fig. 1) was a rise in temperature at the axillary sites and a fall in temperature at the interscapular and superior cervical sites. The latter decreases amounted to about 4°C during the initial 20 minutes of exposure. During this period there were increases of 2° and 1°C, respectively, in temperatures at the ventral subcutaneous and the rectal sites, together with transient elevations of temperature at the axillary loci. The peaks of the latter elevations coincided very closely with the inflection points of the initial drop at the interscapular and cervical sites.

About 40 minutes after transfer of animal No. 2 out-of-doors, the ambient temperature fell to −13°C; at this time the rate of rise of temperatures in the areas of brown fat, especially at the interscapular and superior cervical sites, began to increase. At the same time there was a new thermal rise in both the rectal and the subcutaneous sites; this rise was sustained.

Changes associated with the onset of the shivering phase of arousal were observed mainly with marmot No. 2, which (see Fig. 1) was returned to the laboratory (ambient temperature, 7°C) just as this stage was developing. While onset of the shivering was marked by a rapid rise in rectal temperature, from 11° to 20°C in 10 minutes, a more striking shift in the opposite direction occurred in the thoracic axillary site, where the temperature dropped from its maximum of 28°C down to 20° in less than 3 minutes. At the same time the temperature at the other axillary site and at the interscapular site also approached the 20° level. Again, however, temperature at the latter site and in the superior cervical and thoracic axillary areas went through transient phase changes, much like those observed earlier. While the significance of these is yet to be established, it appears likely, by analogy with current findings on the vascular system of the rat (*2*), that the changes reflect vasomotor modulation of a discrete nature in the routing of the vascular supply from the interscapular and cervical sites. However this may be, it is quite obvious that the shivering phase coincides with a time of redistribution and general mixing of blood from the colder parts of the body with that from topologically discrete warm sites. It was evident that movement of the hind limbs in these animals was still impaired. Apparently the general circulatory perfusion of this region had not yet been fully restored; possibly full restoration required the thermogenic impact of local shivering. Subcutaneous temperatures at the two "neutral" sites were around 16°C at this time.

These observations indicate that during hibernation brown adipose tissues are selectively activated by exposure of the animal to extreme cold and that the arousal response so induced derives its thermal support initially from metabolically produced heat in these tissues. This heat is transferred by vascular convection directly to the cervical cord, the thoracolumbar sympathetic outflows, and the cardiorespiratory organs of the thorax. The rapid changes in temperature within the brown adipose tissues appear to be consonant with a strong vasomotor component perhaps operating through modulation in the routing of the vascular supply from the cervical and interscapular sites (*8*).

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