References and Notes

1. For a general account of variables see the summaries of H.-L. Teuber in *Handbook of Physiology*, section 1, *Neurophysiology*, H. W. Magoun, Ed. (American Physiological Soc., Washington, 1960), vol. 3, pp. 1609 and 1617–1618, and of H. A. Witkin *et al.*, in *Personality through Perception* (Harper, New York, 1954), pp. 477–478. The visual performance of brain-injured soldiers is reported in W. Poppelreuter, *Die Psychischen Schaedigungen durch Kopfschuss im Kriege 1914–16* (Voss, Leipzig, 1917); in H.-L. Teuber, W. S. Battersby, and M. B. Bender, *J. Nervous Mental Disease* **114**, 413 (1951), and in H.-L. Teuber and W. Weinstein, *A.M.A. Arch. Neurol. Psychiat.* **76**, 369 (1954). Cerebral palsied and brain-injured children are subjects of reports by W. M. Cruickshank, H. V. Bice, and N. E. Wallen, *Perception and Cerebral Palsy* (Syracuse Univ. Press, Syracuse, N.Y., 1957); W. M. Cruickshank and J. E. Dolphin, *J. Clin. Psychol.* **7**, 228 (1951); H. Werner and A. A. Strauss, *J. Abnorm. Soc. Psychol.* **36**, 236 (1941); and L. Cobrinik *Am. J. Psychol.* **72**, 566 (1959). For findings of age and sex differences, see L. Ghent, *Am. J. Psychol.* **69**, 56 (1956) and H. A. Witkin, *J. Personality* **19**, 1 (1950).
2. H. Werner and A. A. Strauss, *J. Abnorm. Soc. Psychol.* **36**, 245 (1941); W. M. Cruickshank, H. V. Bice, N. E. Wallen, *Perception and Cerebral Palsy* (Syracuse Univ. Press, Syracuse, N.Y., 1957), p. 114; H.-L. Teuber, W. M. Battersby, M. Bender, *Visual Field Defects After Penetrating Missile Wounds of the Brain* (Harvard Univ. Press, Cambridge, Mass., 1960), pp. 369–377.
3. B. F. Green, A. K. Wolf, B. W. White, *Am. J. Psychol.* **72**, 503 (1959).
4. The patterns used in the experiments were printouts from computer-generated instructions. The individual dots are not symmetrical but narrow in one dimension. In order to produce square displays, more spaces had to be included in one direction than the other. Improved displays have now been prepared by photographing the face of the cathode ray output of a computer, the method first used by Green *et al.* but not available when the present experiment was being prepared.
5. For supplementary tables, giving data for each subject, order Document 7585 from the Chief, Photoduplication Service, Library of Congress, Washington 25, D.C., ADI, Auxiliary Publications Project, remitting $1.75 for microfilm (35 mm) or $2.50 for photocopies (6 by 8 inches).

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Brown Fat: Thermogenic Effect during Arousal from Hibernation in the Bat

Abstract. *In the bat* Eptesicus fuscus *the temperature of brown fat exceeded that of other tissues by about 3°C during the late stages of arousal from hibernation. Heat production seems to be a major function of brown fat in the hibernating mammal.*

Brown fat is widely distributed in many mammals and is particularly abundant in species that hibernate. It has been postulated that brown fat plays a definite but undetermined role in the hibernation process (*1*). Smith (*2*) has proposed that it serves as a heat source in cold-adapted rats, and there are some data linking it also to arousal from hibernation (*3*, *4*). During arousal, the body temperature rises from about 5°C to the homeothermic temperature of the species, so that considerable heat production is necessary in a short time. As part of a biochemical study of brown fat, we have investigated the thermogenic capacity of brown fat in the big brown bat, *Eptesicus fuscus*, during arousal from hibernation under controlled conditions.

Bats were collected locally in September (late summer colony) and in January (hibernating in caves). They were maintained in the laboratory refrigerator at 5° ± 2°C without food, but with free access to water. Temperatures of tissues were measured with a rapid-response microthermistor probe that passed through polyethylene tubes surgically implanted while the bat was under ether anesthesia. The tubes were implanted in interscapular brown fat, dorsal and lateral muscles adjacent to brown fat, ventral pectoral muscle, ventral and dorsal aspects of the caudal region, and the dorsal midline just posterior to the brown fat. Efforts to implant tubes in the thoracic cavity were unsuccessful. After surgery the animals were maintained at room temperature (22° ± 2°C) without food, but with free access to water, for 1 to 4 days and then replaced in the refrigerator. They entered hibernation without apparent difficulty and after 2 to 4 days they were placed in the cold room at 4° ± 2°C for measurement of tissue temperature. A pain stimulus for arousal was provided by a hemostat applied to the abdominal skin. Three tissue temperatures could be recorded per minute. Recordings were continued until the animal became too active to manage. Care was taken to prevent the transfer of heat from the operator to the animal during the measurement.

During the arousal period the temperature of brown fat exceeded that of all other tissues examined (14 determinations on 10 animals). The temperature of brown fat exceeds that of other thoracic areas, including the heart itself, by about 3°C. The caudal temperature shows a distinct lag. To follow the time course of thermogenesis, we made use of the time required for the measured temperature of the brown fat to rise 10°C (T_{10}). On the average, T_{10} was 18 minutes. A warming curve (average of data from six bats) is shown in Fig. 1, in which ventral muscle temperatures are compared with those of dorsal muscle and brown fat. The differences between brown fat and ventral muscle became significant ($P = 0.01$) as early as $T_{10} = 0.3$.

Preliminary experiments on animals from which the interscapular brown fat had been removed indicated that such animals warm up at a significantly lower rate. In these individuals (two cases) temperatures in the area formerly occupied by the brown fat did not exceed that of the pectoral muscle.

Two animals were allowed to awaken at room temperature with the same pain stimulus as that used in the cold environment. In these cases the temperature of the brown fat did not exceed that of the musculature.

Our results demonstrate that brown fat has a pronounced role in thermogenesis in the big brown bat. It seems probable that the provision of metabolic heat is its main role in hibernation, at least in this species. In the bats studied the ratio of the weight of brown fat (450 mg) to that of the combined heart, shoulder, and thoracic musculature (1300 mg) was approximately 1 : 3. This indicates that the tissue could provide a significant portion of the heat necessary for arousal in the bat.

It is not known whether a thermogenic role can be demonstrated for brown fat in all hibernators, but such a function has now been shown in two different mammalian orders, Rodentia (*2*) and Chiroptera. While our experiments dealt only with arousal, it is pos-

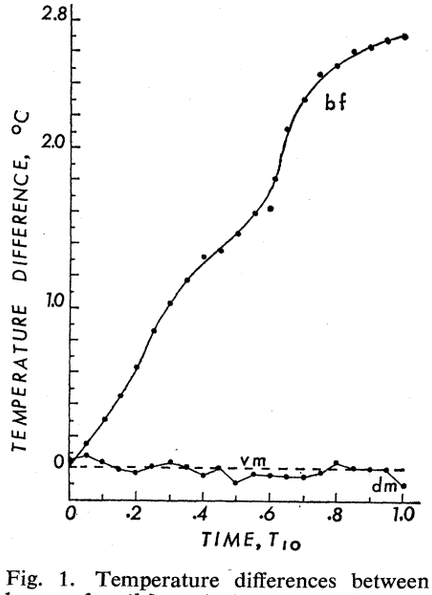

Fig. 1. Temperature differences between brown fat (*bf*) and dorsal muscle (*dm*) compared to ventral muscle (*vm*). See text for a description of T_{10}.

sible that provision of heat to maintain temperature at an optimal level during hibernation could also be of importance. This might be particularly important at subfreezing ambient temperatures (*5*).

Our experiments were first attempted in late July and early August, but it was not possible to arouse the animals in the cold. This is in accord with the work of Menaker (*6*), who demonstrated that the summer animals are in a state of hypothermia as distinct from the winter animals in hibernation (*7*).

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References and Notes

1. B. Johansson, *Metabolism* **8**, 221 (1959); C. Kayser, *The Physiology of Natural Hibernation* (Pergamon Press, New York, 1961), pp. 254–258.
2. R. Smith, personal communication.
3. R. Bullard and G. Funkhouser, *Am. J. Physiol.* **203**, 266 (1962); P. Suomalainen and A. Herlevi, *Arch. Soc. Zoolog. Botan. Fennicae "Vanamo"* **5**, 72 (1951).
4. Since this work was completed and submitted for publication a report describing a thermogenic role for brown fat in the marmot arousing from hibernation has been published. [R. E. Smith and R. J. Hock, *Science* **140**, 199 (1963)].
5. K. Zirm, *Z. Naturforsch.* **11b**, 535 (1956).
6. M. Menaker, *J. Cell. Comp. Physiol.* **59**, 163 (1962).
7. We thank Dr. G. E. Folk for the loan of the temperature recorder and for his many helpful suggestions. This work is a portion of a dissertation to be submitted by one of us (R.L.S.) to the University of Iowa in partial fulfillment of the requirements for the Ph.D. degree. Supported by a grant from the American Cancer Society (No. Q-246.)

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Cell Culture Perfusion Chamber: Adaptation for Microscopy of Clonal Growth

Abstract. *Silicone rubber O-rings used as gaskets in Sykes-Moore cell culture perfusion chambers are permeable to carbon dioxide. When small numbers of cells are planted in such chambers, loss of carbon dioxide leads to an undesirable increase in* p*H and inhibits cell multiplication. Substitution of Teflon O-rings prevents this loss and allows a constant carbon dioxide tension to be maintained within the chamber. The rate of growth and plating efficiency of small numbers of cells planted in the modified chambers have been found to be comparable to those observed in conventional culture vessels. The necessary additional parts of the chamber are described, and the modifications in procedure outlined.*

For studies of cell growth and multiplication by flying spot ultraviolet cytophotometry, we at this institute needed a perfusion chamber in which small numbers of cells could be maintained in rapid growth, to ensure the spatial isolation of individual cells desirable for the spectrophotometric procedures to be employed (*1*). Although the Sykes-Moore modification of the Rose chamber (*2, 3*) proved convenient in other respects, no increase in cell number was observed with inocula smaller than 10^4 cells, even in media which support clonal growth according to the technique of Puck *et al.* (*4*). When growth medium based on Earle's saline and containing phenol red was added to the chambers, it was observed that the *p*H increased promptly, reaching a value of about 8. If such sealed chambers were placed in an atmosphere with elevated CO_2 tension, a rapid decline in *p*H followed. Chambers charged with as few as 50 cells could be maintained at proper *p*H with a controlled CO_2 incubator; under these conditions clonal growth was obtained. It was concluded that CO_2 was lost through the silicone rubber O-ring used as the sealing gasket, a conclusion supported by the observations of others (*5*) that elastomers used in making O-rings are more permeable to CO_2 than to other gases. The Rose chamber (*2*), when equipped with a gasket of Silastic silicone rubber sheet, behaves similarly.

Table 1. Comparison of clonal growth characteristics of C14 FAF28 Chinese hamster cells in petri dishes and in modified perfusion chambers. Three dishes and three chambers were set up with replicate inocula, and incubated at 37°C for 7 days.

Characteristic	Petri dish	Perfusion chamber
Number of colonies	26	26
Number of colonies	40	26
Number of colonies	29	29
Mean colony number	31.6	27.0
Inoculum, cells	50	50
Plating efficiency	.63	.54
Mean diameter (in mm) of 30 colonies*	.605	.852
S.E. of mean	.032	.041

* Average diameter of each colony was estimated microscopically from measurements of two diameters at right angles; a filar micrometer eyepiece was used.

To avoid the restrictions which would result from having to work with the chambers in a special atmosphere, O-rings having low permeability to CO_2 were required. Teflon was selected, since it has low gas permeability, is inert and nontoxic, and is available commercially in the form of O-rings of the required size (*6*). When equipped with Teflon O-rings, the chambers do allow satisfactory clonal growth of cells.

The Teflon O-rings make necessary certain modifications in the technique of assembling and filling the chambers. Since Teflon is too rigid to admit hypodermic needles by puncture, holes are bored with a No. 67 jeweler's twist drill (0.032-inch in diameter) held in a pin vise. Such holes make a snug working fit on 20-gauge hypodermic needles (0.035-inch in diameter) for filling or perfusion. When the holes are not in use, they are stoppered with stainless-steel taper pins. These may be machined from stainless-steel rod, to give a taper at one end of about 1:15, the diameter at the small end being reduced to about 0.032 inch to enter the hole (see Fig. 1).

The rigidity of the Teflon also requires a high torque in the assembly of the chamber, to ensure a satisfactory seal against the cover slips. To avoid damage to the cover slips from irregularities in the metal parts, it is useful to place paper washers between glass and metal to serve as cushions. Such washers may be cut from Whatman No. 1 filter paper with the aid of a cutting tool like that shown in Fig. 1. The die is pressed against a Plexiglas block by means of an arbor press.

The parts of the chamber are assembled in the order shown in Fig. 1. A taper pin is placed in one of the holes in the O-ring to ensure alignment with the holes of the outer metal ring before the parts are tightened. The assembled chambers and the taper pins are then sterilized separately by dry heat.

The sterile chambers may be filled with a cell suspension by inserting a 20-gauge hypodermic needle attached to a syringe, and injecting the required volume while the chamber is held in a vertical position, with the empty hole upward. A taper pin is inserted in the latter, and the assembly inverted. The syringe may then be withdrawn and a second taper pin inserted in its place. After the cells have attached to the cover slip, connection to a perfusion system may be made in similar fashion.

The ability of the modified chambers