Brown Fat and Thermogenesis

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HISTORICAL BACKGROUND

Brown adipose tissue and thermogenesis are conceptually a recent association that tells a new story about the old problem of animals surviving in cold environments. In brief, the brown fat provides an internal heating jacket that overlies parts of the systemic vasculature and on signal becomes an active metabolic heater ap-
plied directly to the flowing bloodstream as it passes to and from the cooler periphery (328, 329, 338).

Brown adipose tissue appears in relation to the prevalence or intensity of the cold and is probably very general among the montane temperate and holarctic land mammals. Primarily, it is brought to bear in three situations of cold threat: 1) during the early neonatal stages as the basic organ for nonshivering thermogenesis; 2) in either transient or chronic cold exposure, as a thermostabilizing device in holding viable steady states of temperature in the thoracocervical spinal segments and the thoracic core; and 3) as a principal biothermic effector organ facilitating the arousal of the hibernator.

First described by Conrad Gesner in 1551 (122) in the interscapular area of the marmot (Muris [Marmota] alpinus), brown adipose tissue was identified with hibernation; however, its function remained obscure, the more so perhaps as the existence of this tissue became known in many nonhibernating mammals.

Some four centuries later Polimanti in 1912 (270) offered the speculation, translated as follows:

I hold, however, that the organ of hibernation in these [non-hibernating] animals, but especially in those which hibernate, is representative of a dual, very important function, intimately correlated one with the other, one for the exchange of material and the other for the regulation of heat. In fact, this fatty cushion, which surrounds all of the thorax and all of the organs which are contained in the thorax, and also all of those organs in the abdominal cavity, serves as a buffer between the external ambient temperature and the animal in hibernation, and does so in such a manner that the animal is not subjected to the very abrupt changes in temperature and tends to maintain the body temperature of the animal at a constant level.

Although Polimanti was sufficiently cited (281–283), his allusion to a thermoregulatory function was evidently not seriously examined, nor did he himself carry out supporting studies.

Hence, quite independently and about two decades later, metabolic measurements made on brown fat in vitro (112, 115, 298) showed that this tissue respired much more rapidly than did white fat. By similar means Hook and Barron (168) in 1941 made the important observation that at a bath temperature of 8°C brown fat slices from the ground squirrel (Citellus tridecemlineatus) retained a considerably greater percentage of their "control" (38°C) respiratory activity than did kidney tissue.

With revival of basic research after World War II and possibly the associated impetus toward stress syndromes and adaptive behavior, brown adipose tissue became a target organ of interest in a number of laboratories (308, 354, 365). During this period Page and associates (260) drew the important conclusion, viz., that brown fat in rats became functional only under conditions of stress, especially that of cold exposure; this group, however, remained noncommittal as to assignment of a specific function (217, 218, 260). Similarly, Timiras and Selye (365) had observed somewhat earlier the dissolution of fat globules in the interscapular fat pad of rats exposed to cold for 16–18 hr. Page's group also demonstrated the hyper trophy response of this tissue to chronic cold exposure and to thyroid administra-
tion as well (218). Considering the foregoing and other data (369, 399–401) with hormonal, environmental, and seasonal trophic responses of brown fat, Johansson (199) was able to concur with Polimanti in stating that “brown fat, at least in some animals, appears to be important in the regulation of body temperature.”

Contemporaneously, in their studies on adaptive mechanisms of heat production, Smith and Hoijer had become especially impressed with the thermogenic possibilities of the brown adipose tissue (330, 337) and certain implications were being experimentally examined (328, 329, 335, 336). The first report of their experiments (328) read in part: “It is concluded that the interscapular brown adipose of the rat at 40 days of cold-acclimation may contribute of the order of . . . 5 times the heat production of this gland in the rat at normal ambient temperature.” These findings together with supporting data from the earlier literature served to crystalize the concept that brown fat was indeed a thermogenic tissue, that its heat was transported to the thoracocervical spinal cord, the vital organs of the thorax, and locally generated in the renal areas as well (329, 333, 338); further, it had qualified as a thermogenic effector organ mediating the arousal of the hibernating marmot and other such mammals (329, 325, 336).

Among the first to engage seriously in the “thermogenic era” of brown adipose tissue were several pediatricians [e.g., Silverman (318), Dawkins and Hull (cf. 81, 177), Brück (cf. 41, 42, 397), and Heim et al. (91, 150)]. Although a paper from Harashima’s clinic in Tokyo appearing in 1949 showed a small differential rise of cutaneous temperature in the nape region of cooled neonatal infants (214), this was not interpreted in terms of a thermogenic response of brown adipose tissue until late (1965) (139). Prior to this was Silverman’s independent study (318), explicitly predicated on thermogenesis of brown fat.

Since the comprehensive review of brown fat by Johansson in 1959 (199), a number of papers have appeared treating in varying depth several aspects of the physiology and biochemistry of brown fat (1, 20, 42, 83, 90, 106, 137, 177, 193, 238, 325, 334).1

PHYLOGENETIC DISTRIBUTION

As multilocular brown adipose tissue derives from embryonic mesenchyme, its phyletic origins may stem not only from the very roots of the homeotherms but also quite possibly from their precursors among the Permian reptiles. As a possible affirmation of ontogeny recapitulating phylogeny, it is notable that the tissue is most abundant in neonates and has been described widely among mammals, which further suggests both a very early differentiation and a natural preselection of broad survival value in cold. As we have recently learned more of the physiological role of this tissue as a heat-producing organ, it has become more evident that such a system

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1 Abbreviations used here are: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NE, norepinephrine; FFA, free fatty acid; DNP, 2,4-dinitrophenol; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; \( V_{O_2} \), \( O_2/\)unit time; \( Q_{O_2}\), \( O_2/\)unit time per unit mass; ACTH, adrenocorticotropic; FMN, flavin mononucleotide.
would have developed most likely through selection under the stress of cooling environments.

Within the class Mammalia, occurrence of brown fat has been reported in many species distributed through seven orders: Chiroptera, Insectivora, Rodentia, Lagomorpha, Artiodactyla, Carnivora, and Primates (283). To Rasmussen's extensive 1924 listing of species (283), Itoh (188) has added the monkey *Macaca fuscata* (Blyth) reared in Hokkaido, and Chaffee et al. (56, 61) have studied brown fat in the rhesus monkey (*Macaca mulatta*), squirrel monkey (*Saimiri sciurea*), tree shrew (*Tupaia chinensis*), and several true shrews (*Suncus* and *Cryptotis*). Others have rapidly extended the list of rodents to include *Cerbellus* (J. Fain, personal communication), *Peromyscus* (162, 292, 293), and *Perognathus* (R. Lindberg, personal communication).

ANATOMY AND TOPOLOGY

As usually seen grossly, brown adipose tissue is readily distinguished from white fat by both appearance and topology. The color of brown fat ranges from pale buff to dark reddish brown, depending largely on seasonal, nutritional, and environmental conditions. The colors derive largely from the blood hemoglobin and to some extent from a high level of heme porphyrins (largely cytochromes) (168, 195) and flavin compounds (324). Infiltration with white (unilocular) fat turns the color toward beige. Under conditions of inanition brown fat is initially maintained at the expense of white fat, but as this is depleted, the multilocular fat vacuoles of the brown fat cells tend to disappear; as the cells shrink, the prolific vascular residuum of capillaries imparts a deep blood red color to the fat pad (75, 136, 138). For similar reasons white fat also becomes more reddish with severe inanition (378).

Anatomical studies of brown fat in various species date from a few remarks in the 17th century by Velsch (374) and Harder (140). Buffon (50) reiterated Gesner's (122) original description of the tissue as resembling that of the udder of the cow. Velsch, Harder, and others up to the time of Jacobson (191, 264, 272) identified the brown fat as thymus tissue. Reviews covering the earlier anatomical works include the comprehensive monographs by Auerbach (11), Dubois (99), and Hammar (136) and the detailed studies of Rasmussen (283), who updated Auerbach's annotated list of species and respective loci of brown adipose tissue. From this list the sites most frequently noted are those in the cervical and thoracic regions, followed in order by the axillary, interscapular, and renal regions. The inguinal locus is least frequently noted, perhaps partly because this area appears in some species to become converted into predominantly white adipose tissue at a very early stage of postnatal development (378).

Among the earlier detailed anatomical studies may be included that of Sulzer (353) on the rat (*Rattus norvegicus*) and hamster (*Cricetus cricetus*); Rasmussen (203) on the woodchuck (*Marmota monax rufescens*); Dubois (99) on the European marmot (*Marmota marmota*); Carlier (54), Auerbach (11), and Polimanti (270) on the hedgehog (*Erinaceus europaeus*); Hatai (141), Bonnot (35), and Wassermann (376) on man (adults and neonates). Johansson (199), in his paragraph on the gross anatomy of brown fat, cites 47 references. A most useful synopsis of the earlier literature, especi-
ally on physiology of hibernation, is appended by Dubois in his 1896 monograph (99).

More recently the topology of brown fat has been described in neonatal rabbits (81, 180) and mice (53), as well as in adult rats (193, 338), ground squirrels (C. tridecemlineatus) (193), hamsters (C. cricetus) (301), bats (Myotis lucifugus) (290), and the human infant (3, 77, 189, 379). Sheldon (312), who compared the distribution of brown adipose tissue in rats with that in the cat and monkey, emphasized the apparent transition of the cells from multilocular to unilocular, noting that this occurred earliest in the peripheral sections of the respective loci.

In the cat, however, while finding the lobes to be located about as in the rat, Sheldon (312) observed in both kittens and adults the presence of "well-defined characteristic lobes . . . in the mesentery and also subcutaneously in the abdominal and inguinal regions."

Although Itoh and Hiroshige (188) found brown adipose tissue richly distributed in the monkey M. fuscata (Blyth) reared in Hokkaido, the northern island, two Macaca cynomolga (Linne) flown to Sapporo from their southerly native area in Malaya "were found entirely free of brown adipose tissue." In M. fuscata the brown fat was characteristically distributed except for the occurrence of a large cervical mass "especially in the perijugular area around the thyroid." This cervical mass was confluent with lobes in the axillary fossae, but there was no apparent interscapular pad; a thoracic strip extended caudally from the neck along the aorta and extended into the abdominal region "where a fairly large amount of brown fat was found in the perirenal area. There was no indication of this tissue in the inguinal regions."

The typical distribution of brown fat in adult rodents may be described as a middorsal, thoracocervical complement of tissue extending anterioventrally by 10.220.33.3 on June 24, 2017 http://physrev.physiology.org/ Downloaded from

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![Diagram of fetal mouse with regions of brown fat shaded](http://physrev.physiology.org/)

**FIG. 1.** Sagittal section of fetal mouse; shaded areas denote regions of brown fat [from Smith (331)].
### TABLE 1. Relative brown fat content (% body wt)*

<table>
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<tr>
<th>Species</th>
<th>Brown Fat Location†</th>
<th>Control</th>
<th>Cold-Adapted Animal</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>White rat (Rattus rattus)</td>
<td>T</td>
<td>.64-.91</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>White mouse (Mus domesticus)</td>
<td>I</td>
<td>.67</td>
<td>338</td>
<td></td>
</tr>
<tr>
<td>Deer mouse (Peromyscus m. sonorienis)</td>
<td>I</td>
<td>.94</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>Newborn mouse (Mus sp.)</td>
<td>I</td>
<td>.13</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>Newborn rabbit (Oryctolagus sp.)</td>
<td>T</td>
<td>.36</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Newborn guinea pig (Cavia sp.)</td>
<td>T</td>
<td>.28</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>Hamster (Cricetus cricetus)</td>
<td>I + C</td>
<td>.64</td>
<td>277</td>
<td>57</td>
</tr>
<tr>
<td>Hedgehog (Erinaceus europaeus)</td>
<td>T</td>
<td>1.06-2.9</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Woodchuck (Marmota m. rufescens)</td>
<td>T</td>
<td>?</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>Ground squirrel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citellus lateralis</td>
<td>T</td>
<td>4.0</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>C. tridecemlineatus</td>
<td>T</td>
<td>2.95</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>Brown bat (Eptesicus fuscus)</td>
<td>I</td>
<td>0.86</td>
<td>1.91</td>
<td>323</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>5</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3.3</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td>Least shrew (Cryptotis parva)</td>
<td>T</td>
<td>2.70</td>
<td>3.35</td>
<td>61</td>
</tr>
<tr>
<td>Musk shrew (Suncus murinus)</td>
<td>T</td>
<td>.63</td>
<td>1.29</td>
<td>61</td>
</tr>
<tr>
<td>Tree shrew (Tupaia chinensis)</td>
<td>T</td>
<td>.22</td>
<td>.24</td>
<td>61</td>
</tr>
<tr>
<td>Rhesus monkey (Macaca mulatta)</td>
<td>T</td>
<td>.22</td>
<td>.21</td>
<td>61</td>
</tr>
</tbody>
</table>

* All animals adults except where indicated. †T = total brown fat; I = interscapular; C = cervical.

beneath the scapulae along the course of its thoracodorsal vascular supply toward the brachial-axillary area; there the brown fat overlies the junction of the main blood vessels and the associated nerve trunks as these emerge from the brachial plexus. Lying deeply medial in the neck under the semispinalis capitis between the the segmental level of T1 to C2 are the paired superior cervical pads, disposed bilaterally in single or double strips of brown fat.

Thoracic brown fat appears in the fetal mouse (Fig. 1) as a dorsomedial continuation of the azygos vein and covers the sympathetic ganglionic chain as well as the intercostal margins of the rib cage. This strip extends further caudally along the aorta through the diaphragm to spread out retroperitoneally over the adrenals and interrenal area including the renal pedicle and the iliac returns. More highly developed are the brown fat deposits in various hibernators, of which bats, marmots, hedgehogs, ground squirrels, and dormice are examples.

Recent explorations of brown adipose deposits in neonatal stages of various mammals have given rise to diverse anatomical descriptions. In newborn rabbits Dawkins and Hull (81) describe a “well defined mass of tissue between the scapulae and around the neck [which] usually weighs about as much as the liver (5–6% of the body weight)” (cf. Table 1). This accounts for about 75% of the total fat at that
time. They also report large amounts of interscapular fat present in the neonatal coypu and guinea pig. The newborn piglet *Sus domesticus* has little or no brown fat (82).

In view of the fact that bats undergo rapid diurnal changes in body temperature, it is notable that the adult big brown bat (*Eptesicus fuscus*) should have some 5% of its body weight in the form of brown adipose tissue, as compared to 2.5% or less in rodents (148; cf. Table 1). Moreover, the bat has most of its brown fat accumulated in a bilobed packet high on the prescapular dorsal cervical area, where its heat production may be applied most efficiently both to the neural integrating centers and to the vital organs of the thoracic cage; very possibly this geometry also protects the body core from heat losses during flight. The gross and microscopic anatomy of the interscapular “gland” of the vespertilionid bat, *M. lucifugus*, has been described in considerable detail by Remillard (290).

The American woodchuck (*M. monax rufescens*) was carefully dissected by Rasmussen, and the anatomical and histological features of the brown fat are well described in his monograph (283); this work evidently became the prototype of a similar extensive study by Coninx-Girardet (70) on the European marmot (*Arctomys [Marmota] marmota*). Each begins with a description of the intrathoracic component of four large lobes and the close relationship of the largest of these to the superior mediastinum, the superior pericardial surface, and the ramifications engulfing the thymus and the great vessels; they also note the adherence of brown fat to the trachea and the lateral sides of the esophagus. The observation that the caudal two-thirds of the lobe is bounded laterally and ventrally by the upper apex of the lung nicely confirms the identity of this lobe with the structure originally described as thymus by Velsch in 1670 (374).

The major differences between the brown fat of the *Marmota* group and that typified above by rats, mice, and hamsters stem from the enormous proliferation in the marmot of the thoracic anlage both within the thorax and in its extension cephalad into the cervical, ventrolateral, and mediastinal thoracic regions. Thus brown fat in the adult marmot is observed to encase the carotid arteries to the level of the clavicle, with distribution over the lateral aspects of the trachea, the esophagus, and entirely through the cervical region along the internal jugulars and the carotid arteries. Also seen are substantial contacts of brown fat with the thyroid gland, the trachea, and an extension into the posterior cervical mass in the posterior triangle of the neck.

The development of the superior cervical, axillary, and interscapular brown fat deposits is also somewhat different from that in the rat, since the interscapular brown fat of the adult woodchuck is derived from the thoracic anlage by an extension of the posterior cervical lobe rather than generated from an anlage in the cervical flexure as observed in the rat (cf. 138).

However, a true homologue of the superior cervical pads of the rat occurs also in the marmot as a pair of thin pads medially located under the semispinalis capitis. These lie just above the cervical vertebrae extending between C6 and T1. A further difference of the brown fat in the woodchuck from that in the rat group is its extraordinary development of the axillary lobe, which attains a mass of over half the
total brown fat as it extends mediolaterally over the thoracic wall and also covers
the deep axillary vessels and nerves; in the rat, however, the brown fat actually
overlays the thoracodorsal and deep cutaneous vessels but leaves the axillary
peripherally bare.

In the hedgehog (163) the interscapular brown fat is largely encapsulated by
connective tissue; there it occurs in considerable amount between the scapulae and
in the gaps between and under the ascending and horizontally running fibers of the
trapezius muscle. This extends medially under the rhomboideus much as in the rat.
The thoracic fat is continuous with large lobes of brown fat on the anterior part of
the neck, and thus overlays the large vessels in the thoracic cavity down to the base
of the heart.

The thymus is partially surrounded, as are the thoracic aorta and the inferior
and superior vena cava. Small islands lie in the course of the bronchi to the hilus of
the lung and even along the internal mammary artery clear to the diaphragm
(163). The allusion to the brown fat overlaying the normally bare inferior vena cava
would appear more likely if it had been made in reference to the azygos, since this is
normally covered with brown fat.

The anterior lobes also provide brown fat extending under and over the clavi-
cle, into the axillary space, and ventrolaterally along the rib cage to the 6th and
7th intercostal space; there appears to be an essentially continuous connection
throughout the thoracic regions, but without any brown fat apparent in either
the renal or groin regions (163).

Distribution of brown fat in the human infant has recently been checked by
Aherne and Hull (3) on a very large series of autopsy material (394 cases). They
state:

In all the infants brown adipose tissue was found at the following sites:
(a) An interscapular mass . . . lies in a thin diamond-shape sheet between the shoulder
blades. It is separated from the subcutaneous white adipose tissue by a discontinuous fibrous
layer. When replete with fat it has a yellowish-brown colour; depleted it is much darker.
It has a fine lobular structure.
(b) Many smaller masses of brown adipose tissue are present around the muscle and
blood vessels of the neck. The main mass follows the course of the internal jugular vein and
common carotid artery.
(c) Extensions from the adipose tissue of the neck pass under the clavicles to rather
large deposits in the axillae.
(d) Further extensions accompany the great vessels entering the thoracic inlet. From
these, fine fingers of brown adipose tissue spread out from the midline with each inter-
costal artery. Similar deposits lie among the internal mammary vessels. Many discrete,
moderately large masses lie in the mediastinum between the oesophagus and the trachea.
(e) In the abdomen discrete masses of brown adipose tissue accompany the aorta and
lie in relation to many structures on the posterior abdominal wall such as the pancreas,
autonomic ganglia and chromaffin tissue. By far the largest abdominal mass envelopes the
kidneys and adrenals.

Interestingly, the amount of brown fat noted by Aherne and Hull (3) appears
more abundant than that observed in Japanese neonates (189). The distribution
and composition of these anlagen are thus strongly reminiscent of those described
by Rasmussen in woodchucks, where in most of these sites large unilocular cells of


### TABLE 2. Blood flow through brown adipose tissue

<table>
<thead>
<tr>
<th>Animal*</th>
<th>Flow, ml/min per g tissue</th>
<th>% of Cardiac Output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA rat (in warm)</td>
<td>.67</td>
<td>.32</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td>.31</td>
<td>.32</td>
<td>216</td>
</tr>
<tr>
<td>WA rat (cold-stressed)</td>
<td>1.18</td>
<td>.31</td>
<td>192</td>
</tr>
<tr>
<td>CA rat (in cold)</td>
<td>2.33</td>
<td>1.35</td>
<td>192</td>
</tr>
<tr>
<td>CA rat (in warm)</td>
<td>.83</td>
<td>.5</td>
<td>192</td>
</tr>
<tr>
<td>WA rat + NE</td>
<td>1.78</td>
<td>1.69</td>
<td>216</td>
</tr>
<tr>
<td>CA rat + NE</td>
<td>2.67</td>
<td>2.48</td>
<td>216</td>
</tr>
<tr>
<td>Newborn rabbit (warm)</td>
<td>.87</td>
<td>7.05</td>
<td>150</td>
</tr>
<tr>
<td>Newborn rabbit (cold-stressed)</td>
<td>3.04</td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>Newborn rabbit + NE</td>
<td>3.60</td>
<td>20.13</td>
<td>255</td>
</tr>
</tbody>
</table>

*WA = warm-acclimated, CA = cold-acclimated animals.

the white adipose type are admixed with the brown fat cells; however, the mediastinal and paraaortic deposits appear more purely of the multilocular brown adipose type (3, 189). This last, incidentally, is also characteristic of many adult rodents that have been examined.

### VASCULATURE OF BROWN ADIPOSE TISSUE

**Microcirculation and Vascularity**

During embryonic development brown adipose tissue is a primordia closely associated with an exquisitely ramified vascular system, and, as these derive from a common mesenchymal origin, the course of the vasculature is topologically identifiable with that of the proliferating brown adipose tissue throughout embryonic and early neonatal development (138). Intravascular injections of these tissues show a profuse distribution of arteriolar loops, basketlike reticular fibers, and capillary networks surrounding the cells (77, 158, 164, 378).

More so than in other tissues, the vascular system of brown fat is literally the main extracellular compartment surrounding the cells. Thus, in baby rabbits (150) blood vascular exchange at rest is 90% of tissue volume per minute and 300% in response to cold. Notably this accords with data on rats that have shown blood flow in brown fat of warm-adapted rats to be doubled on acute exposure to cold, whereas in the cold-acclimated animal (in the cold) the flow is almost four times that of warm-adapted rats (at room temperature) (cf. Table 2). Also one may note that in percent of cardiac output, the range of blood flow through brown adipose tissue is subject to wide variation from low levels of about 0.5% in the warm-adapted rat to about 20% in the newborn rabbit treated with norepinephrine (Table 2).
Using $^{51}$Cr-tagged red cells, Hausberger and Widelitz (145) have shown the vascularity of the interscapular brown fat pads of normal rats to be four to six times higher than that of white adipose tissue and hence comparable with that of resting skeletal muscle.

A further consideration of the vascular transfer functions of brown fat has been approached by Aherne and Hull (3) on necropsy material from infants of gestational age ranging from 29 to 40 weeks and postnatal up to 4 weeks. Their microanatomical description of the vascular bed confirms observations on the hedgehog (163) and on human infants (77, 376). Aherne and Hull (3) state: “Each adipose cell is held in a basket of fine reticulin fibres,” noting further that capillarity per cell is high with cell to vascular surface arranged so that for each fat-depleted cell a third of its surface will be in contact with an equal area of capillary wall. The differentiating lobules display “a rich plexus of capillary blood vessels to which the developing adipose cells adhere” (3).

The “mature” ratio of capillary surface per cubic millimeter of tissue has already been reached by 28 weeks of gestation, with further development involving increase only of capillary cross section (3). For the full-term infant, the volume of the brown adipose cell was estimated at 31,000 $\mu^3$, including a nucleus of about 280 $\mu^3$, cytoplasm of 16,300 $\mu^3$, and the enclosed fat 14,500 $\mu^3$. If depleted of fat this cell would be reduced in volume by perhaps 50%, and the ratio of capillary surface to cell volume would increase. These ratios range roughly from 50 to 100 mm$^2$/mm$^3$, which compare reasonably well with those adduced from the older literature (245).

Similarly, comparing these with the data of Hoepke and Nikolaus (163) on seasonal hibernational changes in diameters of brown adipose cells in the hedgehog, it is also obvious that mass transfer functions can be highly sensitive to the attending regression in cell size (Table 3). Hence, while the brown fat cell surface-to-volume ratio changes from 0.24 $\mu^{-1}$ in fall to 0.60 in spring, the change in transfer function is even more impressive if one assumes the absolute vascular surface area per cell to remain constant. Thus the fall-to-spring ratio of cell volumes (15.6) represents a mass transfer function by the factor (cell surface/volume) of 0.60/0.24, i.e., 2.5; this brings the net transfer coefficient in the spring potentially up to some 39 times greater than that in the fall. In practical terms, these trends may determine TABLE 3. Surface-to-volume ratios of brown fat cells of the hedgehog (Erinaceus)*

<table>
<thead>
<tr>
<th>Cell Diameters, $\mu$</th>
<th>Surface Area, $\mu^2$</th>
<th>Volume, $\mu^3$</th>
<th>Surface:Volume, $\mu^{-1}$</th>
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<tbody>
<tr>
<td>Fall</td>
<td>20 $\times$ 30 (~25)</td>
<td>1963</td>
<td>8182</td>
</tr>
<tr>
<td>December</td>
<td>20 $\times$ 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>20 $\times$ 22 (~22)</td>
<td>1510</td>
<td>5576</td>
</tr>
<tr>
<td>February</td>
<td>20 $\times$ 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>March-April</td>
<td>20 $\times$ 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>8 $\times$ 12 (~10)</td>
<td>314</td>
<td>524</td>
</tr>
</tbody>
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* Calculated from the cell diameters reported by Hoepke and Nikolaus (163); cells taken to be spherical.
FIG. 2. Biothermic flow chart of vascular geometry in respect to brown adipose tissue lobes (shaded areas). Transmural thermal conduction shown by indicators for countercurrent exchanges, respectively, \(-C\) for negative feedbacks, \(+C\) for positive feedbacks, and \(=C\) for null, net exchange zero. Vascular loops in parallel describe convective exchange through visceral regions and in series between heart and lungs and in series-parallel with brown fat-spinal cord and gut-liver interface with the environment \((E)\) by thermal exchange at surface through conduction \(h_{ce}\), evaporation \(q_{ev}\) and radiation \(q_{r}\).

the physiological limits either of resistance to cold or of facility of restitution as the hibernator emerges from somnolence into the rites of spring.

**Gross Vascular Supply**

In the rat, brown fat of the brachial, cervical, and interscapular sites is supplied mainly by the axillaries via the cervical trunk and thoracodorsal artery. Venous drainage from the interscapular pads is of a dual nature, i.e., bilaterally by the thoracodorsal veins returning into the subclavian and by the large central veins that empty, usually on the left side, directly into the inner vertebral plexus at \(T_4\) and commonly also at \(T_5\) and \(T_6\) by superior venae. These returns are
FIG. 3. Drawing from corroded plastic replica of venous vasculature from adult rat showing major drainage from cervical and interscapular brown fat regions [from Smith and Roberts (338)].

further conveyed from the sinus into the azygos via the large 4th thoracic vein and the adjacent intercostals. Within the thorax, both of these routes are largely overlaid with brown fat.

The deep cervical pads are also supplied by arteriovenous couplets leading off the dorsal cervical vessels; analogous with the interscapular pads, the cervical pads are also bilaterally drained into the inner vertebral sinuses, but on each side at two points (i.e., at C2-3 and C6), the latter segment coinciding with the origin of the vertebral veins draining usually into the precaval return.

These arrangements place the inner vertebral sinuses in a series-parallel relationship with the vascular supply and venous returns from the brown fat of the interscapular and superior cervical lobes draining from the plexus into the thorax (Fig. 2).

This central venous drainage into the thorax was described originally in the rat and hamster by Sulzer in 1774 (353), and in some of our figures (338; Fig. 3) it has been labeled “Sulzer’s vein”; were this term to be used it should designate a plexus involving the one or more superior extrathoracic portions of what Greene (124) describes as the fourth thoracic vein.

The vascular drainage of the interscapular brown fat of the human fetus has been described by Aherne and Hull (3); more complex than in the rat, the collecting veins from the interscapular tissue in the human infant are “numerous,” and
as they drain toward the midline, by perforating the trapezoid aponeurosis just lateral to the vertebral spines, they join the drainage of the back musculature and so form the external posterior vertebral plexus. By transverse anastomoses the resulting bilateral venous plexus empties into the “rich venous plexuses that surround the spinal cord, which in turn drain to the jugular or azygos veins depending upon the segmental level.” In functional topology, however, this appears to be essentially the same system as that found in the rat.

INNERRVATION

Despite a good deal of earlier histological work (54), the neuroanatomy of brown fat is not extensively described. However, by use of such functional criteria as topologic heat and nutrient exchange, one may delineate neuronal connections and also levels of nervous control. Thus Dubois in 1892-96 (99) recorded the time course of the warming of hibernating marmots after systematic surgical interventions at selected levels of the central nervous system, beginning with decerebration. He found no effect on the arousal upon successive removal of cerebral hemispheres, corpus striatum, the optic thalamus (couche optique), and corpora quadrigeminae. After ablations of the midbrain, the necessary artificial respiration proved incapable of sustaining the thermogenesis for the arousal.

Further, Dubois (99) found that sectioning of the spinal cord at C₄ permitted respiration but prevented thermogenesis, whether in a hibernating marmot or a refrigerated rabbit. At this level, however, whereas section of only the anterolateral quadrant of the cord did not interfere with normal rewarming of the anterior region, hemisection at C₄ resulted in symmetrical thermogenesis and normal diaphragmatic respiratory function but flaccidity of the ipsilateral thoracic musculature. However, at C₄ he also made an anterior transection, whereupon arousal of the cephalic part developed up to 28–30°C, but the colonic temperature failed to rise above 17°C (Fig. 4). The inference drawn here is that after this section, necessary shivering could not be induced (see below). Notably, after complete section at C₄, electrical stimulation of the distal butt of the cord induced an increase in the rate of rewarming from the hibernating state. After transection between C₇ and T₁ the rewarming was slower, but on electrical stimulation of the distal butt there was some temperature rise, but none at all on stimulation of the central portion.

Results of these and lower segmental transections can be adduced as evidence that arousal requires an intermediation of the spinal cord at the level of C₄. Also, transection at C₇-T₁ slowed but did not abolish the rewarming, and sectioning at T₁-T₅ had no effect. Hence, in retrospect one may attribute innervation of the brown fat to have been derived from spinal tracts emerging from the cervical and first five thoracic segments and a sympathetic outflow from ganglia at least as far caudally as T₄.

In terms of biothermic control, the essence of Dubois’ neurological results has been recently brought into focus by the classical experimental series of Brück and the Winnenbergs on the guinea pig (42, 48, 395). They have demonstrated a temperature sensorium in the thoracocervical segments (C₆-T₁) of the spinal cord. By
controlled warming of these segments in a cooled, shivering animal, they induced inhibition of shivering (Fig. 5). Failure to inhibit shivering after ventral transsection of the cord at C₅ indicated communication to higher centers by tracts located ventral to the level of the anterior horns. From the neuronal aspect, this introduces a new component in the thermoregulatory control system; thus Brück (42) proposes to place shivering under a chain involving: thermoreceptor from the skin—central relay system (synaptic transmission)—efferent motor activity increasingly inhibited as cervical cord temperature rises. This finding (48, 395) evidently brings in an integrating control function by which heat from the brown fat may override the shivering mechanism even under conditions of a hypothermic core temperature.

Anatomically, innervation to brown fat appears to be of the mixed type carry-
FIG. 5. Effect of artificially warming the vertebral canal (lumbar and cervical) on shivering, the rate of oxygen consumption, and the temperatures of the brown fat, skin, and colon of a 3-week-old guinea pig (48).

There is general agreement that spinal nerves C₃, C₄, and C₅ supply the cervical and interscapular brown fat pads in rats together with medial branches from C₁ to C₃ that communicate with cranial nerves X and XII and the sympathetic system (124). Also, according to Clément (66), interscapular brown fat receives innervation from the first five sympathetic branches of the thoracic chain. Sidman and Fawcett (316) refer to "six fairly large nerves [entering] each brown fat body at its ventromedial surface" and running in the interlobular septa, with smaller branches taking the course of the major interlobular blood vessels.

Rasmussen (283), describing in woodchucks (M. monax rufescens) the most dorsal cervical lobes as lying deep medially under the semispinalis capitis, notes that the branches of C₃–₅ cross the deep surface of these lobes. This relationship also appears substantially the same in other rodents such as rats, hamsters, mice (Peromyscus), and squirrels (citellids), wherein the innervation of the deep dorsal cervical lobes may be homologous across many species (cf. 11, 283).

Of the hedgehog (Erinaceus), Carlier (54) states "The nerve-supply of the axillary part of the gland is derived from the cutaneous branches of the 3rd, 4th and 5th intercostal nerves, which are of considerable size and have a large gan-
glion [presumably the superior thoracic] developed in connection with them. The remainder of the organ derives its nerves from the cutaneous branches of the cervical and upper dorsal nerves."

Carlier also described and well illustrated in axillary brown fat the innervation of the arteries; in this he confirmed Beale (21) on the presence of a plexus of non-medullated nerve fibers between the tunica adventitia and tunica media. Carlier (54) demonstrated further a "second fine plexus of nonmedullated fibers between the media and intima, the two plexuses being connected at somewhat wide intervals by short communicating fibres that pierce the tunica media." This minor plexus appeared much finer than that of Beale and exhibited small ganglion cells at intervals. Carlier (54) stated, "The function of this plexus is undoubtedly vaso-motor." The ganglion, containing a multitude of small pyriform unipolar ganglion cells with very long stalks, is lucidly described as to its neuronal microanatomy (54).

Electron microscopy has demonstrated the abundance of intercellular non-medullated nerve endings in brown fat (254). Thus, Napolitano refers to them as "frequently observed" in the connective tissue septa separating the glandlike lobules, and axons may occur in close apposition to brown fat cells. He also shows an electron micrograph in which a "naked axon" (i.e., fiber not enclosed by a Schwann sheath) is located in a lacuna formed by the plasma membrane of the brown adipose cell. He remarks: "The resulting morphologic relationship is similar to a Schwann cell with its C-fibers. Such intimate neural relations have not been observed in white adipose tissue" (254).

Fluorescent microscopy has confirmed the existence of generous adrenergic innervation to the brown fat (79, 89, 393), again showing individual nerve fibers running between brown fat cells (79). Intracellular fluorescence and its depletion after reserpine treatment were "taken to indicate the presence of noradrenaline both in autonomic nerve fibres and within brown fat cells" (79). However, this is not confirmed from recent work by Wirsen and Hamberger (393), who, with more refined techniques, found: "almost all brown fat cells are enclosed by a delicate network of adrenergic terminals," but no apparent intracytoplasmic intrusions.

The sympathetic supply to the brown fat has been somewhat clarified from results of section or ablation of neural components and topological observation of the functional impairment of the tissue lobes. Thus, by unilateral section of the innervation to the interscapular fat, first Hausberger (142) and later Sidman and Fawcett (316) demonstrated that the nutritional exchange of the tissue depends on an intact nerve supply. From this it could have been inferred that sympathetic activity was essential to the observed effects, as was indeed suggested by Beznák and Hasch (26) and Clément (66). It was Correll (71, 72), however, who most succinctly demonstrated that stimulation of the innervations to the interscapular brown fat as well as the epididymal (white) fat pads was followed by release of free fatty acids from the tissues.

In young rabbits Hull and Segall (179, 181) have also demonstrated that the cervical sympathetic reaches the cervical brown fat pads via nerves following branches of the carotid artery and fibers also from the stellate ganglion proceeding
along the subclavian and axillary arterial supply to this tissue. This work also shows that stellate ganglionectiont inactivates the cephalic portion of the interscapular pad (innervated via T₁, T₂) albeit the mixed innervation from C₃ to C₅ is presumably present.

Sympathetic control of brown fat overlying the supra- and ilioinguinal regions is probably derived from the least (lowest) splanchnic nerve, which in the rat "becomes closely applied to the abdominal aorta at the level of the renal arteries, curves around the aorta, passing ventral to the ilio-lumbar vessels, and meets the nerve from the opposite side at the bifurcating of the aorta in a ganglion . . ." (124).

Dubois (99) sectioned bilaterally the sympathetics in the neck, with no hindrance to normal arousals. However, an excision of the inferior cervical and first thoracic ganglia in a marmot aroused to 33 C, the animal returned in some hours to a profound torpor. In a rabbit the same procedure prior to refrigeration also led rapidly to hypothermic death. On marmots beginning to arouse, this treatment initially slowed and shortly suppressed the rewarming, with ensuing death in cold torpor. Conversely, excitation of the sympathetics in the "thoracic region" by "two needles" produced an accelerated rewarming. In some measure these last results adumbrate the findings of Correll (72) and more directly those of Hull and Segall (179, 181).

CYTOLOGY AND ULTRASTRUCTURE

Differentiated brown fat cells can be histologically distinguished from mature white fat cells by their smaller size [25-40 μ in diameter (3, 54, 163, 283, 290, 312) as opposed to 17-120 μ (289)], polygonal (110, 136, 163, 175, 217, 283, 361) rather than oblate spheroidal shape, larger relative cytoplasmic volume (217, 242), and richer blood supply (242). Most characteristic of these cells is the dispersion of numerous small lipid inclusions in the cytoplasm (multilocular) rather than enclosed as a single large lipid vacuole (unilocular) (70, 75, 129, 136, 175, 217, 234, 242, 283, 312, 361, 382); moreover, in the brown fat cell the nucleus is generally spherical (about 5-8 μ in diameter) and centrally located in the cytoplasm rather than flattened against the periphery as seen normally in the white fat cell (3, 54, 70, 73, 217, 229).

In further contrast to the white fat cell, the brown fat cells possess a high concentration of mitochondria (Fig. 6) that are distributed throughout the cytoplasm and vary in shape and size (18, 230). These differ strikingly from mitochondria found elsewhere, as they exhibit a complex internal structure characterized by numerous, closely packed, regularly arranged cristae that extend fully across the particle (cf. Fig. 7; 2, 73, 229, 230, 253, 255). The numerous lipid droplets, which seem to be distributed randomly throughout the cell (253), are often closely associated with mitochondria (229, 323), and the absence of the mitochondrial limiting membrane has been noted at points of contact between the mitochondria and these lipid vacuoles. Although Lever (229), Lindberg et al. (231), and Smalley (323) have
FIG. 6. Section through interscapular brown fat of a cold-adapted rat. Fixed in 2.5% glutaraldehyde, postfixed in 1% osmic acid, embedded in Maraglas; lipid intentionally removed during dehydration. N = nucleus; L = lipid vacuole; M = mitochondrion. X 8470
FIG. 7. Mitochondria in the interscapular brown fat of a cold-adapted rat. Fixed in 2.5% glutaraldehyde, postfixed in 1% osmic acid, embedded in Maraglas. × 43,400
noted the absence of any limiting membrane surrounding the lipid vacuoles. Cotte and his colleagues (73, 268) report that in rats after starvation and refeeding the lipid droplets were always surrounded by membranes and appeared to be regenerated from the interior of the smooth endoplasmic vesicles. These latter findings (73, 268) support the view of Oda et al. (259), who contend that lipid accumulation in brown fat is intimately related to the endoplasmic reticulum.

In the brown fat cells, however, the endoplasmic reticulum and Golgi zones are relatively sparse (229, 253, 268), although the granular cytoplasm contains some free ribosomes (231, 253) and at times considerable glycogen (243, 291). Mature white adipose cells, on the other hand, generally do not contain detectable amounts of glycogen.

DEVELOPMENT OF BROWN ADIPOSE TISSUE

Cells of Origin

The origin of brown as well as white adipose tissue has been the subject of some controversy over the years. In 1870 Toldt (366) proposed that fat cells originated from a specific organized area within the connective tissue. This view was not shared by Flemming (116, 117), who felt that fat cells were fibroblasts modified by the deposition of lipid; i.e., adipose tissue was derived directly from loose connective tissue and could be converted back into connective tissue when the cells lost their lipid. Observations indicating that fat cells were derived from fibroblast-like cells (22, 65, 118) were interpreted as support for Flemming’s concept. However, Hammar’s report of two distinct types of adipogenesis presented an intermediate position (136). He described “secondary adipose tissue formation” to be characterized by fat deposition in connective tissue cells that showed no particular lobular arrangement, whereas the “primary adipose tissue formation” occurred in groups of connective tissue cells that were arranged in lobules before the lipid droplets were deposited. From Hammar’s studies it was concluded (75) that brown adipose tissue was derived from a specific organized group of cells while white fat originated from connective tissue.

Subsequent work by Wassermann and his colleagues (376-378), as well as by Simon (321), has supported Hammar’s conclusion in part. These investigators, who have shown that the first adipose cells in the human embryo appear from distinct formations within the connective tissue (in the perivascular region), maintain that these formations, the “primitive fat organ,” give rise to both white and brown fat cells, the end product of differentiation being determined by the final location of the cells (321, 376, 378).

Although views regarding the origin of adipose tissue still vary the weight of the embryological evidence to date favors the concept that brown fat, at least, does derive from specific mesenchymatous precursors.
Various developmental stages of human fetal interscapular fat were described first by Hatai (141) and then by Bonnot (35) and Shattock (311). Recent detailed descriptions have been provided by Aherne and Hull (3) and Simon (321). Briefly, the primitive fat cells begin to differentiate from reticular cells at the time the capillary bud penetrates the lobule (26–30 weeks). Adipogenesis appears to be associated with the proliferation of the capillary, with the result that all stages of differentiation may be seen in the developing lobule; i.e., the most mature cells may be found close to the central axis of the lobule, while the youngest are more peripherally located. The mitochondria in the primitive fat cell increase in number and size, whereas the endoplasmic reticulum remains sparse, although free ribosomes have been seen. The lipid droplets, which initially appear near the center of the cell, are not transformed from mitochondria (321) as had been concluded by Sheldon (312), and these vacuoles contain lipids that are insoluble in acetone or chloroform. Simon (321) has emphasized that the occurrence of lipid vacuoles in the primitive fat cell corresponds with loss of the capacity of this cell to divide. As the droplets increase in number they tend to fuse and surround the nucleus. At this stage the lipid inclusions are soluble in acetone, and the cell has taken on the appearance characteristic of mature brown fat cells (moruloid) (321). Development of human brown adipose cells, however, is not complete at term, for by the 3rd–5th postnatal week, the amount of cytoplasm per cell has increased to over 150% of that seen at birth (3).

The descriptions of the developmental pattern in the rat (314) indicate that by the 18th day of gestation the cells that aggregate to form the interscapular brown fat body contain a few cytoplasmic glycogen granules and one or two fat droplets. During the next few days increases occur in the cytoplasmic volume, the number of glycogen granules, and both the number as well as the size of lipid droplets. However, mitotic figures are still evident throughout the tissue, and Sidman (314) has concluded that in the embryonic rat brown fat develops through mitotic division and accretion of newly differentiated adipose cells from the mesenchyme, resulting in an increase of the cytoplasmic volume. In the rat at birth the brown adipose tissue still contains little fat, but during the next week the lipid vacuoles continue to enlarge and coalesce; the fat content per cell increases and connective tissue septa divide the body into irregular lobes that surround the blood vessels. In the 2nd postnatal week a reticular network originating from the septa and vessel walls envelopes the individual fat cells. A similar developmental pattern has been observed in the rabbit, although at birth the brown fat pad already contains a considerable amount of lipid (177).

In the newborn rat, mouse, rabbit, cat, monkey, and human the interscapular fat pad contains not only the multilocular brown fat cells but also unilocular cells that increase in relative number after birth (177, 210, 312, 314, 320, 321, 370; Cameron and Smith, unpublished data). The occurrence of these unilocular cells, as well as the fact that the developing white adipose cell may pass through a multilocular stage, has led many investigators to argue that the moruloid brown fat cell
is simply an early (embryonic) obligatory stage in adipogenesis that may remain postnatally to varying degrees in different species \((1, 11, 34, 70, 75, 100, 138, 269, 312)\). Aside from the embryological studies, this view has been reinforced by the apparent transformation of multilocular brown fat to unilocular white fat cells by experimental denervation \((142, 316)\) and under certain culture conditions \((314)\). However, changes from unilocularity to multilocularity have been observed in white fat cells with low fat content \((65)\). Furthermore, a recent report on the effect of altitude acclimatization on fat tissue of rats suggested that the cells replacing the destroyed white fat cells in the inguinal pads might represent immature brown fat tissue, since there were both lobulation and cytoplasmic granulation in the new cells; however, the authors did note the existence of cytological differences between these and "normal" brown fat cells \((31)\).

Thus, a large proportion of the histological observations has been interpreted as support for the "embryonic" nature of brown fat. The main lines of evidence for this view have been the presence of a multilocular stage in white fat differentiation, the occurrence in the embryo and newborn of unilocular cells in regions containing predominantly brown fat, and the apparent replacement of brown fat cells by unilocular white fat cells with aging and under certain experimental conditions.

The concept that brown fat is an early stage in white fat development, however, is not universally shared \((3, 177, 183, 252, 283, 327)\). In his studies of the brown adipose tissue of the woodchuck Rasmussen \((283)\) was unable to find any transition cells indicating that brown fat differentiated into white fat, although such transition stages have been reported by other workers \((11, 70, 136, 312)\). Moreover, preliminary work involving transplantation of murine brown fat (interscapular pad) to the area of the kidney capsule indicated the presence of some unilocular cells in the implants 8–15 days after transplantation \((114)\). It is not clear, however, whether these unilocular forms in the implants, which were essentially denervated preparations, were derived from existing brown fat cells or even that they actually possessed the ultrastructural characteristics of white fat cells. Furthermore Smalley's group \((326, 327)\) recently examined the development of inter- and subscapular brown adipose tissue in hamsters and have noted that these areas, which are occupied by brown fat cells in the adult, contain only white fat cells in the newborn. Interestingly, the development of these "signet-ring" (white fat) cells occurs without the appearance of recognizable multilocular cells. At 3 days of age the scapular adipose cells are primarily unilocular with clusters of immature brown fat cells becoming apparent \((326)\). By 9–10 days of age these immature brown fat cells comprise about 50% of the tissue. Lipid droplets are visible in these cells at 15–18 days and the area has taken on the characteristics of the adult brown adipose tissue prior to 30 days of age. On the other hand, although the early developmental stages of the inguinal, epididymal, and ovarian adipose tissues are similar to those in the scapular region, in the former areas \((327)\) immature brown fat cells fail to appear, and the unilocular cells seen at birth mature to form the white adipose tissue in the adult. The results
of this study have led to the conclusions that in the hamster brown fat cells arise from the reticuloendothelial system, are laid down in a preexisting white fat matrix, and are not immature forms of white fat cells (326, 327).

Consistent with the view that brown and white fat cells are distinct cellular types is the observation that, in newborn rabbits and in man, the unilocular cells present in areas that at one time had contained multilocular cells differ considerably from those of the adult white adipose tissue in displaying less fat and more cytoplasm (81).

With the advent of refined electron-microscopic techniques, more subtle distinctions have been drawn between the brown fat cells and the multilocular form of white fat. In particular Napolitano's examination of fat pads in developing rats (252) has indicated that the mitochondrial ultrastructure characteristic of brown adipose cells is not similar to that of the mitochondria in white fat cells during any stage of their development; i.e., in the mitochondria of white adipose cells the cristae are less numerous, fail to extend across the organelles, and have no consistent orientation with respect to the mitochondrial axis. Similar ultrastructural differences have been noted in the rabbit by Hull (177). Moreover, since the number of fat vacuoles in brown fat cells seems to depend on extracellular conditions (3, 75), it has been suggested that electron-microscopic examination of the unilocular cells in the “brown-fat regions” may reveal ultrastructural differences between these cells and white fat in other areas of the body (177). Finally, it has been argued that neither the presence of unilocular cells in predominantly brown fat areas nor the replacement of multilocular by presumptively white fat cells necessarily indicates a transformation of brown to white fat; rather, however, it may represent an actual replacement resulting from hyperplasia of the white adipose tissue (177).

It thus appears that a critical electron-microscopic comparison of the unilocular adipose cells in the brown fat pads as well as a reexamination of the sequential changes leading to the appearance of these unilocular cells may settle the controversy over the embryonic nature of the brown fat tissue.

A problem related to the development of the brown adipose tissue concerns the ability of the differentiated brown fat cell to undergo mitotic division. As mentioned above, Simon (321) has reported that the developing human fat cell loses its capacity to divide at approximately the same time that the lipid is deposited, although mitotic figures have been observed in the interscapular body of the developing rat for several days after the lipid droplets first appeared (271, 314). The existence of any cell division in the brown adipose cells of adult rats had been denied by earlier investigators (70, 138). Furthermore, Cameron and Smith (53) injected tritiated thymidine into rats exposed to 6°C for varying lengths of time and examined the distribution of label 1 hr after injection; their data were adduced as evidence that brown fat cells originated from reticuloendothelial precursors rather than from existing brown fat cells. Although the cold exposure induced a hyperplastic increase in the mass of the brown fat, the early radioautographs showed DNA synthesis to be present only in the reticuloendothelial cells within the vascular spaces of the tissues, never in the brown fat cells. Later, however, labeled adipose and reticuloendothe-
lial cells were detected in rats exposed to cold for 72 hr, injected in the cold, and sacrificed 24 or 96 hr later (Fig. 8). These investigators also considered the absence of mitotic figures in the brown fat cells to be highly significant, but the difficulties in visually identifying mitosis in small and intensely staining nuclei have been pointed out (225).

A more recent radioautographic examination of brown fat proliferation in cold-exposed rats (184) has essentially confirmed the time sequence of events reported by Cameron and Smith (53). Furthermore, Hunt and Hunt (184) also observed no proliferative activity in the brown fat cells per se, this being confined to the vascular endothelium and extravascular fat "precursor cells." Hence it appears that the hyperplastic response of brown adipose tissue in cold-exposed rats involves proliferation of new brown fat cells through cytogenesis from progenitor cells rather than from existing brown fat cells. However, this does not actually preclude a turnover of brown fat cells via mitotic division. That such mitotic events occurred was concluded by Hellman and Hellerström (156), who injected tritiated thymidine into rats and by radioautography noted the frequency of radioactive nuclei in the brown fat. They explained the observed decrease in frequency of labeled nuclei (i.e., from 40% initially to 10-15% 5 months after injection) as a dilution effect resulting from cell division. It is not apparent from their published photographs, however, as to whether the label was mainly in brown fat cells or in the reticuloendothelial cells. If in fact the nuclei of brown fat cells were labeled, a dilution effect would probably reflect mainly the cell turnover, since little if any proliferation of brown adipose tissue would be expected as the animal aged under the non-stressful maintenance conditions after injection. Furthermore, one might argue that dilution resulting from division of labeled reticuloendothelial cells (cf. 53) rather than of brown fat cells led to the decreased frequency of labeled nuclei. This possibility involves not only the proliferation, differentiation, and turnover of the endothelial progenitor cells but also the degradation of existing brown fat cells. In lieu of further data the life cycle of a fat cell remains obscure, both in its origin and whether it can undergo cell division.
TABLE 4. Relative composition of brown adipose tissue in adult animals*

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<td>3.4</td>
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<td>42.5</td>
<td>14.2</td>
<td></td>
<td></td>
<td>263</td>
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</table>

* Values given as % wet weight; BAT = brown fat; WAT = white fat. Hibernating species were active at time of sacrifice; laboratory rats were not cold-exposed; wild rats and hamsters were maintained in outdoor cages. † Values obtained during nonhibernating season.

COMPOSITION

Histochemical data on the composition of brown adipose tissue have indicated that in general the differences between brown and white fat are more quantitative than qualitative (e.g., 110, 242). A survey of the analyses of the water, lipid, and lipid-free dry material found in the interscapular pad of the adult rat (Table 4) emphasizes the scatter of values obtained by different investigators. In these studies the lipid content varied from 36 to 71% of the wet weight of the tissue as compared to 58–88% for white adipose tissue. The data on the relative water content range from 23 to 51% of the wet weight of the brown fat and from 10 to 30% of the white fat, whereas the lipid free dry material constitutes 6–16% of the interscapular mass but only 1–12% of that of the white fat.

Compared with that of laboratory rats, the brown fat of adult nonhibernating hamsters, bats, hedgehogs, and wild rats (Table 4) shows somewhat higher (43–68%) water and lower (15–43%) lipid content. Although these differences may reflect specific variations that relate to the potential for hibernation, the values obtained for the laboratory rat most likely represent analyses on the mixture of multilocular and unilocular adipose cells typical of the unstressed adult animals. Affirming this also are the compositional similarities between the brown fat of the hamster, bat, and wild rat.

The cells of white adipose tissue contain more total lipid (64, 86, 242, 256) and a greater percentage of neutral fat (64, 67, 242, 342) than brown fat cells; but in brown adipose cells there are greater amounts of water and protein (217, 301) as well as higher percentages of phospholipids (64, 110, 119, 241, 242, 256), cholesterol
(75, 110, 113, 241, 242, 367), and, in some species, saturated fats (64, 67, 70, 256, 341, 342).

The triglycerides comprise over 85% of the total lipid in the interscapular fat pad of adult rats (64) and bats (383) and approximately 75% in that of the ground squirrel (342) and mouse (256). Chromatographic techniques have extended the range of histochemical work by identifying the specific fatty acid components of the various lipid fractions. Thus, of the brown fat triglycerides from various animal species, the glyceride fraction is mainly composed of unsaturated fats, with oleic and linoleic acids predominating (Table 5). Of the saturated fatty acids in brown fat, palmitic acid appears to be the most prevalent (Table 5). At the present time, however, the relationship between the fatty acid composition and the function of the brown fat has not been defined.

The high phospholipid concentrations in brown adipose tissue, relative to those in white adipose tissue, presumably reflect the greater number of mitochondria. Phosphatidylcholine and phosphatidylethanolamine account for 67-68% of the phosphatides in both the brown and white adipose tissue of the mouse (341) and 56% in brown fat of bats (383). In mice the phosphatide contents of brown and white fat differ primarily in respect to the polyglycerophosphatide and sphingomyelin fractions. Of these the latter is over four times more concentrated in white than in brown fat (13.0% compared with 3.1%); conversely, the polyglycerophosphatide concentration of white fat is only a fourth that of the brown fat (3.9% vs 13.6%) (341). Since polyglycerophosphatide appears to be concentrated in the mitochondria of tissues (237), the differences in phosphatide composition are probably attributable to the respective mitochondrial frequencies.

On histochemical grounds, brown adipose tissue of the guinea pig appears richer than white adipose tissue with respect to α-amino acid groups and mucopolysaccharides as well as amino acid oxidase, α-naphthol oxidase and cytochrome oxidase (241, 242). Similar comparisons in other species have indicated that brown adipose tissue contains higher concentrations of succinic and lactic dehydrogenases [bats (119, 120); rats (110)], alkaline phosphatase [rat (110)], cytochrome c [rat (195)] and cytochrome oxidase [rat (195)].

The enzymes involved in the synthesis of glycogen from glucose (hexokinase, phosphoglucomutase, and phosphorylase) have been demonstrated in brown fat (76, 244). Creasy and Gray (76), using various in vitro systems, noted the effect of inhibitors and activators on these enzymes in the brown fat and muscle of rats; they concluded from the similar response patterns that glucogenesis in brown adipose tissue occurs by processes analogous to those in muscle.

The necessary enzymes for glycolysis via the hexose-monophosphate shunt (24, 326) as well as the Embden-Meyerhof pathway (326) are present in brown adipose tissue as are those associated with the TCA cycle (326, 364, 389).

In keeping with its high respiratory capacity, brown adipose tissue contains large concentrations of diphosphothiamine (168), which is required in keto-acid oxidations, including that of pyruvate to the acetyl radical; likewise abundant is coenzyme A (363), which is essential for activation of the acetyl moiety and ascorbic acid as well.

Brown fat also possesses an abundance of the electron transport components,
### TABLE 5. Fatty acid composition of brown adipose tissue glyceride fractions

<table>
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<tr>
<th>Fatty Acid</th>
<th>Newborn Rabbit</th>
<th>Newborn Human</th>
<th>Rat</th>
<th>Mouse</th>
<th>Adult Hamster</th>
<th>Ground Squirrel</th>
<th>Woodchuck</th>
<th>Brown Bat</th>
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<td>BAT</td>
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Note: Numbers in parentheses are standard deviations.
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<th>% Unsaturated</th>
<th>Glyceride, % total lipid</th>
<th>Phospholipids, % total lipid</th>
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* BAT = brown fat; WAT = white fat; reference numbers in parentheses. Nonhibernating species were not cold-exposed; hibernating species were active when sacrificed. † R. E. Smith, unpublished data.
certain of which presumably contribute to the brown color of the tissue (195). Greatly extending the early work of Hook and Barron (168), Joel and Ball (195) have demonstrated the presence of large amounts of cytochromes $a$, $a_3$, $b$, $c_1$, and $c$ in the interscapular fat pads of the rat, with the concentration of cytochrome $c$ (per g of lipid-free dry wt) being comparable to the highest values reported for any other tissue of this organism. Similarly, in the rat the cytochrome oxidase activity of the brown fat is quite high (364; Jansky, personal communication), this activity exceeding even that of the heart, which is generally considered to be metabolically the most active organ (Jansky, personal communication).

The content of coenzyme Q$_9$ (ubiquinone) also appears to be extremely high (6 moles/mole of cytochrome $c$) in both the mitochondrial fraction and the supernatant fat layer of the centrifuged homogenate of brown adipose tissue (195). As Joel and Ball (195) have pointed out, although the additional amount of ubiquinone in the fat layer may actually occur in vivo in the fat vacuoles, its presence is more plausibly explained by a leaching process during isolation of the mitochondria. If the appearance of ubiquinone in the fat layer were indeed an artifact of isolation, the mitochondria in vivo would contain a 13-fold molar excess of coenzyme Q over cytochrome $c$ (195). High coenzyme Q levels have also been noted in brown fat of the bat (325).

In addition to these electron transport components, Smalley (323) has also reported high concentrations of flavoproteins in brown fat of bats and has attributed the color of this tissue to the FMN-indole complexes present.

The high rates of fatty acid synthesis observed in this tissue (19, 69, 107, 310, 343, 345) indicate that the brown fat cells contain all the enzymes necessary for this anabolic activity, and in fact recent work has demonstrated that the brown fat has an extramitochondrial system capable of synthesizing fatty acids from acetate (345). Moreover, rat brown fat possesses both citrate cleavage and malic enzymes, which have been proposed to participate in lipogenesis (207, 394). Further, glycerokinase, which catalyzes the phosphorylation of glycerol, has been noted in brown fat of rats (134, 368) and ground squirrels (193), but this enzyme has not been detected in the brown fat of newborn rabbits (82, 177).

With respect to the catabolism of triglycerides and fatty acids, Fawcett's qualitative histochemical examination of rat adipose tissue (110) indicated that the lipase concentration in the brown fat was higher than that in the white fat; however, this difference was not confirmed in the histochemical preparations from bat adipose tissue (120). Neither chemical nor histochemical analyses of the specific lipases involved in the observed brown fat lipolytic activity have been reported, although the existence of a hormone-sensitive lipase in brown fat cells is implied from the results of experiments concerned with the effect of endocrines on lipolysis (see below). Brown adipose tissue does contain long-chain fatty acid dehydrogenases (310, 396) of which the activity in bovine brown fat is approximately three times higher than that of the corresponding enzymes in the perirenal and mesenteric (white) fat deposits (396).

Also reported to be in brown fat are catalase (396), acid phosphatase (271), and microsomal NADPH cytochrome $c$ reductase (57, 63), as well as a material with antitryptic activity (375).
Of probable importance are the reports of the high activity of monoamine oxidase, catechol-O-methyltransferase, and other enzymes associated with the metabolism of biogenic amines. Correspondingly high are the levels of several of the amines themselves: norepinephrine (27, 79, 317, 349, 350, 381, 387, 393), histamine (27, 349), and serotonin (27, 28, 349).

Brown adipose tissue also contains several steroid compounds that are not detectable in white adipose tissue; thus cortisone, corticosterone, and deoxycorticosterone have been identified in brown fat of the rat and guinea pig (275, 284, 285, 296, 402), cortisone and 17-hydroxycorticosterone in the mouse, and corticosterone and deoxycorticosterone in brown fat of hamsters (277). Highly active androgenic materials have also been extracted from brown fat of the woodchuck (357) and the male bat (213).

CHANGES IN COMPOSITION AND MORPHOLOGY

Both the morphology and chemical composition of brown adipose tissue can be influenced by age and by various exogenous factors such as temperature, season, and diet.

Age

In general the amount of brown fat relative to body weight decreases with age [rats (144, 312), laboratory mice (360; Cameron and Smith, unpublished data), deer mice (Roberts and Smith, unpublished data), guinea pigs (46), rabbits (80, 177), humans (3)], although Aronson et al. (10) have reported that in hamsters the ratio of the interscapular fat pad to body weight was constant in animals over 20 g irrespective of age. However, in both the inguinal region and the interscapular pad of these animals the amount of white fat increased with age and body weight to the extent that in 80-g hamsters unilocular cells comprised 50% of these tissues but were absent in animals under 20 g. Thus, the apparent constancy of the weight of the interscapular fat pad relative to body weight may be attributed to the proportionate increase in the amounts of white fat invading the brown fat areas. Similarly, in many species the multilocular brown fat areas present at birth are gradually overlaid, to varying degrees, by unilocular cells [hamster (10), rat (53, 312), guinea pig (46, 47, 241), rabbit (177), mouse (Cameron and Smith, unpublished data), cats (312), humans (3)].

Thus multilocular fat is less prominent in adult man, cats, and laboratory-raised rodents and rabbits. Among wild animals, however, appreciable amounts of brown adipose tissue are found, not only in adult hibernators such as the marmot (99, 199, 283, 335, 336), hedgehog (e.g., 283), bat (146, 148, 283, 290, 329), hamster (10, 283, 301), and ground squirrel (126, 168, 172, 283, 342, 398), but also in wild rabbits (200) and many nonhibernating wild rodents [rat (301), deer mouse (162, 292, 293), and domestic mouse (e.g., 11, 283)].

The changes in chemical composition in the interscapular brown fat of growing rats have been described by Hausberger and Gujot (144), Schierer (301), Sidman (314), and more recently Hahn et al. (135) and Benjamin et al. (25). As the sparsely
vacuolated brown adipose cells of the newborn rat accumulate fat rapidly over the first 4–7 days, deposition of lipid, together with increasing amounts of water and nonlipid dry material, contributes to the observed increase in cell size (301) and weight of the tissue (135, 144, 261, 301, 314). Concentration of the nonfat dry material, however, remains relatively constant with age, whereas the water decreases and the lipid content increases (Fig. 9).

In the rabbit, where the brown fat cells of the neonate already contain a considerable amount of lipid material, the lipid concentration decreases after the 1st day of life, reaching a minimum 7 days after birth. Subsequently, the interscapular multilocular cells are replaced by unilocular fat (80, 82). In the interscapular area of the newborn hamster, however, the presence of unilocular cells at birth is associated with a high lipid level. As these cells disappear and immature brown fat cells appear during the first 13–14 days postpartum (see above), the total lipid content of the interscapular pad drops from 40% to 23%. This trend is reversed as the brown fat cells accumulate lipid, and by 35 days of age 56% of the tissue weight is referable to fat (326).

Although the lipid content of the brown fat of the laboratory mouse initially falls after birth, this trend is soon reversed (360) and the concentration of 29% (relative to wet wt) at 4 days of age rises to 59% by the 10th week (86). Glycerides of the interscapular brown fat from mice of various ages increase from 20% of the fresh tissue weight at birth to 40% during the first postnatal days, gradually thereafter rising to the adult level of 60% (341). In the ground squirrel Citellus lateralis no differences were observed in the glyceride levels of the brown fat in either the young or adult animals (86). In brown fat of mice a decrease in the degree of saturation of the glyceride fatty acids was noted between 14 and 63 days of age (from 48.4% to 39.9%), but in 80-day-old mice the saturated fatty acids constituted 42.5% of the glyceride fraction. In contrast to this age response of brown fat, the concentration of saturated glycerides in white adipose tissue steadily fell from
44.2% in the 14-day-old mice to 30% in the 80-day-old animals. The most significant changes in the distribution of the brown fat glyceride fatty acid components were found in the concentrations of lauric (3.4% at 14 days to .2% at 80 days), palmitic (9.0% to 2.8%), and stearic (7% to 12.5%) acids (341).

Somewhat less dramatic changes in the triglyceride make-up of hamster brown fat have been reported (326). Actually, only slight differences were apparent between the 5- to 12-day composition and that of the adult except for the linoleic levels, which increased from 12.9% to 16.1%. On the other hand, the triglyceride stearic acid content remained relatively constant (7%) until 13-14 days of age (9%) when it began to increase, reaching a maximum at 17-18 days (13.5%). Although a value of 13% was still observed at 23 days, the triglyceride in the adult tissue contained only 8.5% (326). Since this increase in stearic acid content takes place as the immature brown fat cells begin to accumulate lipid (ca. 15 days) and occurs in the total lipid and phospholipid fractions as well as in the triglycerides, it has been associated with the development of multilocularity. However, these hamsters were weaned at 14 days of age and it is not yet clear to what extent dietary changes may have influenced the results (326).

The phosphatide concentration as a function of fresh weight of brown fat in the mouse falls from about 3.5% at birth to 1.5-2% in the adult (341). However, in the rat the amounts of phosphatide and lipid-free dry substances are paralleled throughout the growth of the brown adipose tissue (221), a finding consistent with the view that the small quantity of phosphatides (relative to the total amount of lipid) is located in the cytoplasm and therefore varies with the fat-free dry material as the rat ages (220).

Likewise, although the amount of cytochrome per fresh wet weight of the interscapular brown fat decreases during the growth of the rat, the concentration relative to the weight of the lipid-free dry substance remains constant (195).

There is some evidence that the steroid content of the brown fat changes with age. Ptak (278) has isolated two unidentified, highly polar steroids from the interscapular gland of newborn rats; but in 3- to 6-week-old animals, as well as in adults, only one such highly polar steroid was found, whereas the moderately polar corticosterone and deoxycorticosterone compounds appeared. The author interprets the absence of these latter substances in the tissue of the newborn rats as being related to the small amount of lipid present. That is, regardless of whether the steroids are synthesized in the brown fat (cf. 279) or accumulate there from other sources, Ptak contends (278) that the low lipid content in newborn tissue is unfavorable for retention of steroids that are not highly polar (i.e., weakly soluble in a lipid-poor environment).

Littrell (233) and Hunter (185) have demonstrated esterase activity in the brown fat of the 15- to 20-mm mouse embryo; this had not been observed at the 5- and 10-mm stages. Potter and Eastlick (271) have reported both acid and alkaline phosphatase activity in brown fat of 16-day embryonic mice. The acid phosphatase was detectable through the 2nd day after parturition as well as in the adult, but no convincing histochemical evidence was obtained for the retention of alkaline phosphatase activity after the 19th day of gestation (271).

Very few data are available on the effect of aging on the activities and concen-
trations of most of the enzymes in brown adipose tissue. In laboratory mice, succinic dehydrogenase activity appears to decrease during the first postnatal days. This activity then rises to a new maximum at about 2 weeks of age, thereafter falling only slightly over the next 2 months (360). Hahn et al. (132, 133) have reported that citrate cleavage enzyme and acetyl-CoA synthetase, enzymes of fatty acid synthesis, exhibit relatively high activities in the fetal rat. These activities, however, as well as that of the hormone-sensitive lipase, are low during the suckling period but begin a slow increase with weaning (age 15–18 days), reaching adult levels by the 30th day. Conversely, the activity of lipoprotein lipase, presumably involved with the uptake of fat into the tissue, remains high after parturition, falling transiently at the time of weaning. These changes after birth are most probably related to the high-fat diet of neonates before weaning (132, 133). Additionally, the glycerokinase activity of the brown fat appears to be higher in the newborn compared with the adult rat (134). Moreover, the in vitro release of glycerol from brown adipose tissue of fetal rats is greater than that of newborn and 10-day-old animals, although the sensitivity of this response to norepinephrine does not appear to be significantly affected (133). On the other hand, norepinephrine administered in vivo to infant rats failed to increase the fatty acid content of the tissue, although such an effect was observed in the adult (135). This observation correlates well with the increasing activity of hormone-sensitive lipase of this tissue with age (see above).

Considerable variation in enzyme activity has been found in the developing brown fat pad of the hamster (326). Malic dehydrogenase activity at 5–8 days increased from 1.7 μm NADH oxidized/mg tissue per hr to 12.8 by the 18th day, falling thereafter to 7.8 at 35 days and 2.0 in the adult. The rise in activity from 11 to 18 days was thought to reflect increasing mitochondrial numbers and/or dietary changes associated with weaning (326). Similarly, the glucose 6-phosphate dehydrogenase activity increased sixfold by the 20th day postpartum, but slowly fell to 1/5 of this value (i.e., twice the 5- to 10-day activity) in the adult. On the other hand, the aldolase activity gradually rose uncynvously until the 15- to 18-day period, whereupon it sharply decreased, so that at 35 days the activity was 1/3 that of the 5- to 6-day activity and the adult was down to 1/10 of this activity (326).

Benjamin et al. (25) have reported for rats 38–647 days old a fall of 27% in the incorporation of acetate into mixed lipids by the interscapular brown fat and a 92–97% decrease in uptake by the white fat depots. However, one may note that by 20 months of age the nitrogen content of the interscapular pad has fallen to one-half that in the 44-day-old rat (25) and has also acquired a considerable amount of white fat; hence, it appears that the activity in the brown fat cells themselves may have remained unchanged with age or even increased rather than decreased, with infiltration of white cells being responsible for the observations on the interscapular pad.

Similarly, the results of all aging studies on the composition and chemical activity of brown fat areas in most unstressed nonhibernators must be carefully interpreted to account for the increasing number of white fat cells in these regions.
Season

No seasonal variations in the lipid content and morphology of brown fat cells of well-nourished rats were seen by von Hansemann (138), although annual changes in the composition of the brown fat of nonhibernating hamsters (C. cricetus) and wild rats (R. norvegicus), maintained in outdoor cages after capture, have been noted by Schierer (301). Unfortunately, Schierer did not report the respective weights of either the animals or the brown adipose tissue, thus presenting only the amounts of water, lipid, and lipid-free material in terms of the tissue wet weight. No obvious qualitative differences appear between the seasonal response patterns in the proportions of these quantities in the two species, and in lieu of an adequate estimate of error it is difficult to evaluate the monthly variations observed.

Buchalczyk and Korybska (49) have also reported seasonal fluctuations of the weight of brown fat in shrews (Sorex araneus). Their data show that the relative weight of the interscapular gland from sexually mature adults is maximal in April (female tissue weighing about 50% more than that of males), falls in May, and is somewhat elevated in June. The tissue weights in the males then decrease in July and remain low until November, when they begin to rise. In the females the ratio of tissue to body weight exhibits a slow rise from July to October and then falls off in November. These different seasonal responses of the males and females during the autumn months may be related to the diminished sexual activity during this period; however, no correlation between weight of brown adipose tissue and pregnancy was found.

In sexually immature adults the relative weight of the brown fat decreases sharply from May to July, leveling off until November, when it begins to increase. From December to February the tissue weight varies slightly and is maintained at a higher level than during the summer, but between March and April the weight of the fat pad is quite high, particularly in the females. These annual variations, however, are confounded by the age of the animals examined; e.g., the high May and June ratios obtained were derived from shrews that had not been out of the nest for more than 2–3 weeks (49).

Hibernation

In contrast to the paucity of data concerning the seasonal rhythms of morphological and compositional changes of brown fat in nonhibernating animals, a number of detailed accounts of the changes occurring during hibernation are available.

Carlier (54) noted that the “hibernating gland” of the hedgehog was largest at the beginning of hibernation (about 3% of total body wt) and decreased gradually through the winter period, becoming only 0.9% of the body weight by April, the end of the hibernating season. Microscopic examination of the brown fat from October animals revealed the uniform histological character of all the lobes of the fat body. Although the number and size of the fat vacuoles in the cells varied, the nucleus remained spherical and the cytoplasm abundant. By January less lipid was
present in the cells, making apparent the cytoplasmic "network" and isolated granules (presumably mitochondria). After cessation of hibernation in the spring most of the cells seemed to have disappeared, while the remainder were described as being in an advanced stage of "fatty degeneration." Carlier reports that by June a few fibrous cords of tissue remained, but by the end of this month the gland had begun to increase in size.

Although a decrease in cell size during hibernation of the hedgehog has also been reported by other investigators (e.g., Table 3), the degree of cell degeneration seen by Carlier (54) has not been confirmed (11, 138, 163).

Analyses of the composition of the brown adipose tissue of the hedgehog during hibernation (55) showed an increase in the total amount as well as percent of water prior to and during the first 3 months of dormancy, with a decrease occurring in the next 2 months. The lipid concentration seemed to vary inversely with the water, falling initially but rising at about the same time that the water concentration decreased. The total amount of lipid, however, remained relatively constant during the hibernation season, showing decreases only during the 1st and 6th months of hibernation. The protein content transiently fell in the early stages of torpidity, only to increase and level off during the latter 2/3 of the hibernation season. The relationship between the water and lipid content of the tissue led to the conclusion that to a large extent, water replaced the brown adipose tissue lipids utilized by the dormant hedgehog. A similar conclusion was reached by Hoepke and Nikolaus (163). The decrease in cell size that occurs during hibernation is apparently accounted for by the loss of fat (138).

Qualitatively similar morphological changes take place in the hibernating marmot (283). During torpidity the brown adipose tissue darkens, the average cell diameter shrinks from 30 \( \mu \) to 24 \( \mu \), and the number and size of the fat vacuoles decrease. Immediately after the hibernation season (before the animals have become very active), the brown fat has decreased to 25% of its prehibernation weight, but remains about 2% of the body weight (i.e., the rates of loss in body and brown fat weights during hibernation arc similar). During the 3-4 weeks after cessation of hibernation in the spring (mating period of the marmot), the brown adipose tissue continues to lose fat, and its weight decreases faster than does the body weight of the animal. Thus at this time the average cell diameter is 8-15 \( \mu \) and the weight of the gland is minimal (0.7-0.8% of body mass), having lost about 75% of its original weight. The nuclei in these cells do not appear to have changed morphologically, the granular cytoplasm is evident, and neither complete cell degeneration nor complete loss of lipid is seen. As in the hedgehog, the decrease in cell size is thought to reflect the loss of lipid (283).

Despite these observed variations in brown fat weight during hibernation, it appears that the amount of DNA/interscapular pad in the marmot is relatively constant from January through April (380) and similar in magnitude to that seen from the latter part of June through August when the animal is awake. On the other hand, the RNA content (per fat pad or per DNA content) varies somewhat during torpidity but is consistently higher (2-5 times) than that measured in the summer marmots (380). Although Weill et al. (380) interpreted the higher RNA
content as an indication of increased “metabolic activity” during hibernation, the high tissue levels may alternatively be explained as resulting from 1) preparation for increased protein synthesis activity after arousal and/or 2) a decreased rate of RNA degradation at the low body temperatures during dormancy. Further examination of these nucleic acid changes, with particular attention to the variations of the different RNA species, would be most informative.

The detailed work of Remillard (290), describing the compositional differences in the interscapular brown fat of female bats (M. lucifugus lucifugus), again emphasizes the loss of tissue mass during hibernation. However, in this species the water content fell in the dormant animals. Furthermore, the lipid concentration (relative to tissue wet wt) was elevated, although in terms of the amount of solid material in the interscapular pad the fat concentration was diminished during torpidity. The discrepancies between these results obtained on bats and those on the hedgehog and marmot may be related to the bat’s distinctive pattern of temperature response.

A recent study of the brown fat triglycerides of E. fuscus has indicated a shift in the fatty acid composition during the course of the year (266). Prior to the onset of hibernation the levels of palmitic and palmitoleic acids increased, followed by a decline during torpidity. On the other hand, the level of oleic acid rose significantly during hibernation of the bat, while that of linolenic acid was relatively constant. Also associated with dormancy was a greater degree of unsaturation of the triglycerides. However, Thomson et al. (364) have reported no differences in the rate of oxidation of palmitate by brown fat mitochondria from euthermic, hibernating, or aroused ground squirrels. On the other hand, they noted a slight depression of the rate of oxidation of succinate and β-hydroxybutyrate by brown fat mitochondria isolated from the torpid squirrels.

The data of Paulsrud and Dryer (266) do not confirm the findings of Wells et al. (383), who examined the composition of brown fat lipids in M. lucifugus and noted a slightly higher content of saturated fatty acids during the hibernating season. Moreover, the concentrations of lauric, palmitic, and palmitoleic acids rose during dormancy, while that of oleic acid fell. These changes were explained as the result of dietary rather than temperature influences (383), with the conclusion that there were no significant differences between hibernating and nonhibernating bats. In opposition to this, however, Paulsrud and Dryer (266) have argued that the procedure of sampling only two points (hibernating and nonhibernating) may have masked existing temporal variations.

Additionally, in contrast to the findings of Remillard (290), Wells et al. (383) also reported an increase in tissue weight as well as in the total, neutral, and polar lipid constituents of the tissue. This suggests that the nonhibernating animals may have been examined in the early stages of the hibernating season when the weight of the interscapular pad is relatively high (290). Unfortunately, the dates of capture, the length of captivity, and the maintenance conditions of the animals before death are not specified, thus making comparison and interpretation of the data somewhat difficult.

Seasonal variations in the cytochrome c content of brown fat have recently been reported for hedgehogs in which the total amount of cytochrome c (although
not the concentration per lipid-free dry wt) appears to be higher during the hibernation season (December–March) and May than during the summer months (263).

Changes in the histology and lipid composition of brown adipose tissue have been extensively studied in the ground squirrel C. lateralis (126, 342); in these, however, the large variance precluded any significant correlation between size of the brown fat pads, time of year, or length of the hibernation period. On the other hand, during the arousal of these animals rather dramatic morphological and chemical changes were observed. The capillary beds in the interscapular pads became filled with red blood cells, and the fat vacuoles largely disappeared, with a net loss of tissue glyceride and an increase in phosphatide staining (126, 342). In the brown fat of arousing C. tridecemlineatus (396) glycogen and lactate fell while pyruvate increased; in the arousing bat (E. fuscus) progressively decreasing levels of brown fat ATP have been reported (98). In the dormant hedgehog (E. europaeus) the concentration of FFA in the brown fat was three times higher than that of the normally awake animals and remained elevated during the latter phases of arousal (mean colonic temperature of 27.5°C) (206).

It should be emphasized that, with the exception of the studies on ground squirrels (126, 342, 396), hedgehog (206), and bat (98) cited above, most data concerning changes undergone by brown fat “during hibernation” are based on a comparison of measurements made before and after dormancy, and therefore the specific effects of entrance and/or arousal cannot be differentiated. Since Bailey and Davis (12) have shown that the body weight loss of the marmot during the “hibernating season” reflects losses incurred principally in the periods of transient arousal, it is conceivable that the reported effects of hibernation on the brown fat may similarly reflect only the arousal stage. The results of Grodums et al. (126) support this possibility, but certainly more detailed examination of the changes occurring in the brown adipose tissue during the arousal phase is essential before any firm conclusion can be drawn.

It is apparent, however, that the brown fat does play some role in the arousal from dormancy. Although the changes observed after arousal may be (and have been) interpreted as evidence for the storage function of this tissue, evaluation of these data in light of the high metabolic activity of brown fat (see below) points to a much more active participation of this tissue than mere storage in the awakening process.

Stressing Agents

Significant changes in cellular morphology and composition of brown fat also occur on subjection of the animals to severe stress (75, 201, 227, 276, 307, 308, 354). In rats fasted while exposed to 0–5°C for 16 hr or subjected to forced muscular exercise or to injections of formalin, the interscapular glands appeared partly involuted, edematous, and brownish red from loss of much of their lipid. Concurrent corticosterone and deoxycorticosterone had disappeared from the tissue (276). Similarly brown fat removed from rats 14–16 days after a single exposure to hypothermia (2°C colonic temperature) or 6–8 days after the last of a series of such coolings tended to be paler and reduced in mass (251).
FIG. 10. Effect of chronic cold exposure (6°C) on the sizes of the brown fat cells and the cellular lipid vacuoles [from Cameron and Smith (53)].

Such responses, however, appear to depend on an intact nerve supply to the brown fat. In cold-exposed mice (5°C) subjected to long-term starvation, the intact (innervated) interscapular lobe decreases in mass, loses most of its lipid vacuoles, and becomes considerably darker, while on the denervated side the tissue retains its lipid droplets and “light reddish-brown” appearance (316).

Neither short-term starvation nor cold exposure (0-5°C) is sufficient per se to produce the so-called “generalized stress syndrome” in the brown fat. These situations, however, do cause a decrease in the mass of the brown adipose tissue size and its lipid content (64, 268, 308, 312). Moreover, in fasted rats the ascorbic acid concentration in the brown fat is elevated (217), glycogen levels are depressed (217), and the relative concentration of total lipid palmitic acid diminishes, while oleic and linoleic concentrations appear to increase (64). This latter observation has been interpreted as indicating a preferential utilization of saturated fatty acids (64) similar to that suggested in other studies of cold effect on depot fats (205). However, Williams and Platner (388) have found no notable change in the degree of fatty acid saturation of brown fat in cold-adapted rats or hamsters.

Chronic exposure of rats to low temperatures (0-5°C for 60-90 days) leads to hypertrophy of the interscapular pads, as first demonstrated by Pač and Babincéau (260). The time course of this cold-induced hyperplasia has been studied in rats (33; Fig. 10), where during the first 6-12 hr at 6°C the interscapular multilocular cells lose their lipid vacuoles and decrease in size. By the end of 24 hr the fat droplets have reformed and the brown fat cells appear “normal.” After 24-48 hr many of the unilocular cells present in each lobe have disappeared, the gland is gorged with blood, and the tissue weight is greater than that of the controls. After 4-8 days of cold exposure these unilocular cells are largely absent, with this replacement being complete and essentially at steady state in rats exposed for 16 and 60 days or longer. The brown fat mass in the interscapular and thoracic regions in these rats increases to a greater extent than that in the cervical-axillary and renal areas (338), and by 30 days of cold exposure the relative weight of the brown adipose tissue has doubled (53, 338). The histological picture resulting from this time-dependent study is one of initial depletion of the brown fat lipids on cold exposure, with repletion of the
multilocular cells occurring at the expense of the unilocular cells whether in these areas or in depots elsewhere (53).

Trophic adaptive responses of the brown adipose tissue during cold adaptation have been shown to occur not only in the rat (19, 53, 88, 157, 193, 216, 217, 260, 261, 294, 328, 329, 338, 339, 347, 348), but also in the nonhibernating hamster (57, 62), deer mouse (292), mouse (63, 186), and musk and least shrews (58, 61). Furthermore, it appears that this response can be elicited both in old and young rodents; i.e., the amount of brown fat per body weight of 17- to 19-month-old deer mice maintained at 6°C for 6 weeks was greater than that of younger mice (1–12 months) (R. E. Smith, unpublished data). Page and Babineau (260) ascribed this cold-induced increase of the interscapular pads in rats to the three- to fourfold rise observed in fat-free dry material and water content, the total amount of lipid being unchanged. Although such an elevation of the nitrogen content has been confirmed for rats (157, 338, 348) and also noted in cold-adapted hamsters (57) and squirrel monkeys (56, 59), increased lipid levels have also been found in cold-adapted rats (157). However, no significant change in mass was observed in the squirrel monkey (56, 59), tree shrew (61), or in adult rhesus monkeys, although the brown fat in younger rhesus monkeys did respond trophically (61). Since in brown fat, the amount of phospholipid relative to the nitrogen content is not significantly altered after chronic cold exposure (348), the reported increase in nitrogen per wet weight of almost fourfold (348) suggests that the rise in nitrogen reflects an increase of mitochondrial material. This interpretation is reinforced by the finding that in homogenates of brown fat the percentage of nitrogen attributable to the mitochondrial fraction is relatively unchanged in cold-acclimated rats (32% cold vs. 36% control) although the homogenate nitrogen concentration (mg of N/g of tissue) more than doubled, as did the tissue mass (294).

Accompanying the cold-induced increase of the mass of brown fat in rats is an elevation of the tissue norepinephrine concentration (74). However, the observation that the uptake of radioactive NE (per unit tissue wt) does not appear to be altered by cold acclimation suggests that the sympathetic innervation to the brown fat developed in proportion to the tissue mass (74). Thus the elevated NE concentration was thought to reflect an increased storage per unit of nervous tissue (74).

Additionally, cold adaptation leads to an elevation of ascorbic acid in both content and concentration in the brown fat (261) and an increased lipolytic activity as well as oxidative metabolism (see below).

Low ambient temperature also affects the blood flow through the brown fat, as indicated in Table 6. Acute cold exposure of warm-acclimated rats results in a 76–135% elevation of the flow through the interscapular fat pad (192), whereas this flow in cold-acclimated rats returned to room temperature is still 7–24% higher than that of the warm-adapted rats at room temperature when calculated per unit of tissue weight (103, 192). Moreover, the flow per unit weight of brown fat in cold-acclimated rats in the cold is about 100% greater than that of the cold-exposed warm rats (192, 216) and 280–370% higher than the flow in warm rats at room temperature (192, 216). In light of the 94–310% elevation of mass of brown fat in these cold-adapted rats (157, 216, 338), the total blood flow through the inter-
TABLE 6. Effect of cold on cardiac output and blood flow through interscapular brown fat of rats

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Ratio Brown Fat Flow</th>
<th>Ratio Cardiac Output</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA cold</td>
<td>1.76</td>
<td>1.01</td>
<td>192</td>
</tr>
<tr>
<td>WA warm</td>
<td>2.35</td>
<td>1.01</td>
<td>216</td>
</tr>
<tr>
<td>CA cold</td>
<td>1.97</td>
<td>2.09</td>
<td>192</td>
</tr>
<tr>
<td>WA cold</td>
<td>2.00</td>
<td>1.11</td>
<td>216</td>
</tr>
<tr>
<td>CA warm</td>
<td>1.27</td>
<td>1.11</td>
<td>192</td>
</tr>
<tr>
<td>WA warm</td>
<td>1.07</td>
<td>1.11</td>
<td>216</td>
</tr>
<tr>
<td>CA cold</td>
<td>2.81</td>
<td>1.11</td>
<td>192</td>
</tr>
<tr>
<td>CA warm</td>
<td>3.78</td>
<td>2.13</td>
<td>192</td>
</tr>
<tr>
<td>WA warm</td>
<td>4.71</td>
<td>2.13</td>
<td>216</td>
</tr>
</tbody>
</table>

*WA = warm-acclimated, CA = cold-acclimated animals.

The interscapular gland of such animals becomes in the cold 8-20 times greater (700-1900%) than that in warm-acclimated control rats and 4-8 times greater than cold-stressed, warm-adapted rats. On the other hand, the cardiac output of the cold-adapted rats is only twice that of the warm-acclimated animals (216). Similarly, a 30-min exposure of newborn rabbits to 25°C leads to a 3.4-fold elevation of the rate of blood flow through the brown fat but only a 52% increase of the cardiac output (149, 150).

In contrast to the hyperplastic response during prolonged cold exposure, brown fat from heat-adapted hamsters (35 ± 2°C) and squirrel monkeys (36-39°C) is paler, weighs less, and has a lower protein content than does tissue from control animals (59, 62). Hence the temperature to which the animal is exposed appears to have a considerable influence on the microscopic and chemical characteristics of the brown fat as well as the effective vascularization.

The effect of altitude on mass and nitrogen content of brown fat resembles that of cold exposure. Thus the amount of brown fat per unit body weight in deer mice (Peromyscus maniculatus sonoriensis) native to 3800 m was 76% higher than that in the sea-level colony (293). When sea-level mice were translocated to 3800 m both the absolute and relative amount of brown fat rose to values comparable to that of native mice by the 3rd day (162). These masses were maintained until the 11th day, when they began to decrease, and by the 15th day after translocation the weight of the brown fat had returned to sea-level values. The time course of these changes closely paralleled the total oxygen consumption of the animals and appeared to be inversely correlated with the animal's colonic temperature. That is, 1-3 days after exposure to these hypoxic conditions, the colonic temperature fell 0.76°C, followed soon after by a rise in the metabolic rate and an increase in brown
fat mass (162, 293). The body temperature returned to control values approximately 7 days later, and thereafter both the brown fat weight and the heat production of the mice (O2 consumption) began to fall (162). Furthermore, when these translocated mice were returned to sea level their colonic temperatures were again transiently depressed, with a simultaneous elevation of the brown fat mass (162). On the basis of the similarities of the trophic responses of the brown adipose tissue to hypoxia and cold exposure it has been suggested that these responses result from body cooling regardless of the inducing agent (162, 293; cf. also 359).

**HORMONAL INFLUENCE**

The seasonal variations in morphology and composition of brown fat, especially in animals that hibernate, having been viewed as manifestations of endocrine intermediation (217), have led naturally to diverse studies of relevant hormonal effects on the brown adipose tissue (Table 7).

**Adrenal Corticosteroids and ACTH**

That brown fat reacts in acute cold somewhat like the adrenal cortex in stressed animals (75, 227, 308, 354, 365) formed the basis of earlier interest in the relationship between these organs.

Removal of the adrenals leads to a progressive loss of glycogen (370) and lipid from the brown fat (111, 217, 218, 240), with an increase in the water content such that the tissue weight remains unchanged (217, 218). Administration of cortisone to adrenalectomized rats is followed by an increase in the fat content and weight of the brown fat (217, 218, 240), this augmentation being as great as that seen after cortisone treatment in intact rats (217, 218). On the other hand, deoxycorticosterone acetate does not affect the brown fat weight in either intact or adrenalectomized rats, but does increase lipid stores at the expense of water content.

Short-term administration of cortisone to intact rats in moderate amounts (215, 217, 218), as well as cortisone administration to pregnant rats (305), tends to increase the weight of the brown fat; however, similar treatment in mice and hamsters leads to an initial increase in mass of the tissue, followed by a decrease (10, 240, 322). Likewise, large doses of cortisone or its administration over prolonged periods of time result in atrophy of the brown fat (8, 299).

As might be expected, the changes in lipid content occurring after hypophysectomy are similar to those seen after removal of the adrenals (111, 240). Moreover, daily injections of ACTH or cortisone (111, 240) or chorionic gonadotrophin (297) in hypophysectomized animals prevent lipid depletion, with the brown fat retaining its normal appearance. In addition, administration of ACTH or a crude lyophilized anterior pituitary extract stimulates lipid accumulation in the brown fat of intact rats (13, 34, 217, 218, 362); however, no such lipogenic effect is obtained with growth hormone (34, 262), nor does growth hormone interact with the "lipid-accumulation" activity of ACTH when administered to the same rats (34).
Since the elevated lipid content of brown fat in ACTH-treated rats is paralleled by increases in water and nonfat dry material, no change in the lipid concentration of the tissue is apparent (111).

These trophic responses of brown fat in glucocorticoid- and ACTH-treated animals appear also in treatment of animals after adrenalectomy and hypophysectomy, respectively; in substance, these effects implicate the adrenal cortex in the regulatory control of brown fat lipid storage. Since ACTH does not evoke lipid deposition in brown fat of adrenalectomized animals nor induce any such effect in vitro, its action on lipids of brown fat is presumably exerted via the adrenal cortex.

In contrast to this apparent lipogenic activity of ACTH and glucocorticoids in vivo are the in vitro studies showing that in white fat both ACTH (194, 256) and dexamethasone (104) stimulate lipolysis and FFA release, but that in brown fat dexamethasone elicits no such response (104); here also ACTH is effective only with abnormally high dosages (194, 256).

In view of such discrepancies it has been suggested that the enhanced lipid deposition in the brown fat of ACTH- or glucocorticoid-treated animals may be an indirect effect, secondary to elevated glucose levels made available to the brown fat from other tissue sources (104, 240). Pertinent here is the report that cortisone administration to fasted rats is followed by dose-independent elevations of the brown fat glycogen (101) and lipid levels (240). Moreover, dexamethasone addition does not detectably influence the uptake or metabolism of glucose by brown fat minces, although it markedly depresses these activities in white fat (104).

These observations thus support the contention that the brown fat lipid deposition seen after in vivo administration reflects the action of the glucocorticoids on the carbohydrate metabolism in other tissues in the body and that the increased accumulation of glycogen seen in cortisone-treated rats represents an initial stage in lipid synthesis. This view is consistent with the findings that the cortisone-induced stimulation of brown fat lipid content is lower than normal after fasting or adrenalectomy (101, 240). That is, the lipogenic action of the glucocorticoids becomes pronounced under conditions where glycogen levels have increased and lipid stores are somewhat depleted (240).

Interestingly, short-term administration of cortisone or ACTH significantly increases the blood flow through the interscapular brown fat of rats (215). That this effect of ACTH is also due to the action of ACTH on the adrenal cortex is suggested by the finding that this hormone elicits no such response in adrenalectomized rats (215). On the other hand, from the observation that intravenous infusion of ACTH into newborn rabbits induced a rise in temperature over the brown fat, it has been inferred that ACTH has a direct action on this tissue (151). However, both the unphysiological dose and the failure here to measure the glucocorticoid response to the ACTH infusion may challenge this interpretation of the results.

It appears, however, that the increased ACTH and glucocorticoid release after cold exposure may influence the effectiveness of the brown fat as a thermogenic organ by affecting 1) the amount of potential substrate (i.e., lipid), 2) the oxygen supply to the tissue, and 3) the convective heat loss from the tissue.
TABLE 7. Effect of cold exposure and hormones on brown fat composition and metabolism*

<table>
<thead>
<tr>
<th></th>
<th>Chronic Cold</th>
<th>ACTH</th>
<th>Thyroid</th>
<th>Glucocorticoids</th>
<th>Insulin</th>
<th>Glucagon</th>
<th>Catecholamines</th>
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<tbody>
<tr>
<td>Lipid content</td>
<td>↑ (348)</td>
<td>↑ (13, 34, 217, 218, 262)</td>
<td>↑ (217, 218, 262)</td>
<td>↑ (217, 218, 240, 262, 322)</td>
<td>↑ (217, 239)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O content</td>
<td>↑ (260, 348)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen content</td>
<td>0(13, 101, 217)</td>
<td></td>
<td>0(217, 219)</td>
<td>0(101)</td>
<td></td>
<td>↑ (5, 101, 109, 217, 219, 315, 346*, 371, 386)</td>
<td></td>
</tr>
<tr>
<td>O2 consumption</td>
<td>↑ (57b, 59b, 186b, 292b, 338*)</td>
<td>↑ (198*, 151)</td>
<td>↑ (186b)</td>
<td>0(186)</td>
<td>↑ (15*, 190*, 309*)</td>
<td>↑ (198*)</td>
<td></td>
</tr>
<tr>
<td>Blood flow</td>
<td>↑ (103, 192, 216)</td>
<td>↑ (215)</td>
<td>↑ (215)</td>
<td>↑ (151)</td>
<td></td>
<td>↑ (103, 150, 151, 192, 216)</td>
<td></td>
</tr>
<tr>
<td>Triglyceride lipolysis</td>
<td>(198*)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ (133, 135, 346*)</td>
<td></td>
</tr>
<tr>
<td>FFA release</td>
<td>(198*)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ (82*, 105*, 193*, 198*, 287*, 346*, 386*)</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>↓ (343*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose → glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction</td>
<td>↑ (157, 204, 265, 343, 348)</td>
<td>0 (104)</td>
<td>↑ (306, 346)</td>
<td>↓ (346)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose → gynicola-FA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose → glyceride-glycol</td>
<td>↑ (157, 204, 343, 348)</td>
<td>0 (104)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose → CO₂</td>
<td>↑ (343)</td>
<td></td>
<td>↑ (306, 346)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses are references; those referring to in vitro studies using 95-100% O₂ as gas phase are designated by superscript a, those using air as gas phase by superscript b, and those involving in vivo or tissue culture work with no superscript; ↑ = increase, ↓ = decrease, 0 = no change. FA = fatty acids, FFA = free fatty acids. † Increase only with high, unphysiological doses.
If indeed the lipogenic activity of cortisone is secondary to its promotion of elevated blood glucose levels and subsequent synthesis of glycogen in brown fat, one might expect insulin to act synergistically with the glucocorticoids in effecting brown fat lipogenesis. Such an interaction on fatty acid synthesis has been demonstrated in the perfused rat liver (7). Moreover, Engel and Scott (101) have shown that adrenalectomy significantly depresses the stimulatory effect of insulin plus glucose on glycogen deposition in the interscapular fat pad of the rat. Similarly, in the intact rat, although administration of cortisone plus glucose does not significantly affect glycogen storage in the brown fat, addition of insulin to this treatment results in a striking elevation of the glycogen deposits (101).

Insulin administered alone to rats being refed after a fast (101, 217, 219, 371) markedly enhances glycogen content in the brown fat, but it apparently has no such effect when given over an extended period of time (217, 219). Additionally, insulin does not affect the glycogen content of brown fat in normal or alloxan-diabetic rats when administered jointly with epinephrine (371). However, when added to monolayer cultures of brown fat cells, insulin stimulates glycogen deposition (5, 315) and increases the number and size of the fat droplets (239). Moreover, despite a report of inconsistent metabolic responses of brown fat to in vitro incubation with insulin (16), other studies indicate that on incubation of brown fat slices with insulin and glucose-U-14C, there is: 1) stimulation of both the uptake of glucose (309, 346) and synthesis of glycogen (346), 2) enhanced conversion of glucose to lipid (fatty acids as well as glyceride-glycerol) by as much as 5- to 20-fold (309, 346), and 3) increased \( Q_{O_2} \) and lactate production of the brown fat (309).

Thyroid Hormone

The influence of the thyroid gland on brown adipose tissue appears particularly relevant to the thermogenic function of this tissue in cold-exposed animals in view of the well-documented requirements of the hormone for survival in the cold (32, 102, 223, 224, 306).

Unlike insulin, thyroid administered to rats by feeding over a period of several weeks induces hypertrophy of the brown fat involving augmented lipid levels with little change in the water or nonfat dry material content (217, 218). Conversely, hypothyroidism (effected by treatment with thiouracil) is attended by lipid depletion of brown fat (111, 233), although rats that are both hypothyroid and castrated appear to have more glycogen in their brown fat than do controls (108).

The mechanism by which the thyroid gland elicits this lipogenic response of brown fat has been considered with various attempts to differentiate between an enhanced conversion of glycogen to lipid and a stimulation of lipid mobilization from peripheral deposits. Lachance (217) examined both glycogen and lipid levels in the brown fat of the rat, noting elevations in the latter 48 hr after injection of thyroxine with no concomitant increase in glycogen content. These results and the observation that thyroxine treatment depletes the depot (white) fat argue in favor
of an augmented lipid mobilization. However, Lachance also found that by 24 hr after thyroxine injection the lipid stores had not yet increased, but the glycogen content was 60% higher, albeit this change was not statistically significant (217).

Since these variables were not analyzed before the 24-hr postinjection time, one cannot be certain that the glycogen level did not peak earlier, with an accelerated lipid synthesis preventing detection of any such glycogen accumulation at the periods examined. That such may be the case is suggested by an earlier study on fasted, thyrotoxic rats, where recovery feeding was followed by an accumulation of brown fat glycogen that peaked at 5 hr. The maximum weight of brown fat, however, was not reached until 48–72 hr, and at this time glycogen was rapidly disappearing from the tissue (370).

On the other hand, the observation that abundant lipid reserves appear to be essential for the thyroid-induced hypertrophy of brown fat in adrenalectomized rats (217, 218) supports the possibility that the associated lipid accumulation depends on mobilization from peripheral stores and further suggests that the impediment of the thyroxine response in adrenalectomized rats is due to lack of mobilizable fat rather than direct mediation of the thyroxine effect through the adrenals. Certainly the cortisone- and thyroxine-induced hypertrophies of brown fat differ with respect to the content of water and nonfat dry material (see above) and the response of the white fat depots; i.e., cortisone administration does not deplete the fat depots as does thyroxine (217, 218). Furthermore, that the lipogenic response to joint administration of the two hormones either to intact or adrenalectomized rats is both enhanced and almost additive supports the view that this lipogenesis is evoked independently by the two hormones. However, the increased adrenal weights seen after thyroxine treatment indicate that this hormone does undoubtedy affect cortisone release (217, 218).

In any event it is apparent that the hypertrophic response of brown fat to experimental hyperthyroidism or thyroxine injection differs somewhat from that induced by cold; i.e., in cold the brown fat responds with hyperplasia and an increased accumulation of lipid, water, and nonfat dry material. Moreover, it has been shown recently that the brown fat of cold-exposed hypothyroid mice still increases its mass (186), thus implying that factors other than thyroxine are involved. Additionally, the elevated Q_{02} of brown fat associated with chronic cold exposure has not been consistently observed after thyroid feeding or thyroxine injection (186, 217).

On the other hand, thyroxine administration appears to affect the metabolic response of the tissue to NE (186). That is, although continuous exposure to cold or injection of NE into euthyroid mice enhances the Q_{02} of surviving slices of brown fat, no stimulation is observed when using hypothyroid mice (186). These results are consistent with the suggestion (211) that in adipose tissue, thyroid hormones may regulate the activity (amount) of adenyl cyclase, the enzyme system through which the thermogenic effect of NE appears to be mediated (see below). It may be, therefore, that the magnitude of the thermogenic response of the brown fat in cold-exposed mammals is at least partially dependent on the action of thyroid hormones.
FUNCTION OF BROWN ADIPOSE TISSUE

Prior to the demonstration that brown adipose tissue was an important thermogenic organ (324, 329, 335–338) much speculation and controversy existed as to the function of this tissue, particularly in respect to hibernation. Since these theories have been extensively reviewed by Johanssen (199) and Smalley and Dryer (325), emphasis is given here only to those early theories relating to roles of the brown fat during the induction and maintenance of hibernation.

Induction of Hibernation

The suggestion that brown fat may actively participate in the initiation of hibernation is based primarily on observations that extracts of this tissue from hibernators have a metabolic depressant effect when administered to nonhibernating animals. Wendt (384, 385) injected extracts from hibernating hedgehog brown fat into rats and noted a reduced metabolic rate, blood pressure, and temperature at low dosages and death in larger dosages. These results, however, were in contradiction to those of Kroll (212), who had observed no depressant effect of brown fat extracts from hibernating hamsters and hedgehogs.

Hook’s (167) injection into rats of crude peanut oil extracts of the brown adipose tissue of hibernating ground squirrels and woodchucks was also followed by depressed oxygen consumptions. Preparations from omental fat and kidney, however, had no effect on the oxygen utilization in vivo, whereas administration of peanut oil alone increased this rate. Similar depressant effects were reported by Zirm (399, 400) and attributed to the yellow-green fluorescent material he isolated from brown fat of hibernating hedgehogs. Failure of extracts from other tissues (liver, lungs, spleen, and kidney) of the hibernating hedgehog, as well as brown fat from the nonhibernating animal, led Zirm to conclude that the brown adipose tissue in dormant hibernators contained some specific substance(s) capable of reducing the body temperature and, further, that this substance was important in the initiation of hibernation. Taken in support of his conclusions were his observations that brown fat from the hibernating hedgehog implanted into mice caused a depression of the body temperature, this decrease being proportionately related to the size of the implant. Zirm’s work, however, has come under criticism by Langer-Schierer and Langer (222), who contend that the effects observed by Zirm may have been caused by some nonspecific factor characteristic of tissues of the reticulo-endothelial system (257) or some material stored in the brown fat during hibernation.

Recent attempts to confirm this metabolic inhibitory effect have not been successful (e.g., 29). Subcutaneous implants of ground squirrel brown fat fragments as well as homogenates did not depress the body temperature in the recipient mice nor affect their heat production when cold-stressed (248). But this lack of response may be attributable to the fact that the implants did not become vascularized, although they also were not encapsulated (248). Similarly, Smalley was unable to
observe any body temperature depression in mice receiving implants of brown fat from bats (323). Furthermore, following Zirm's extraction procedure, Smalley made preparations of bat brown fat that were ineffective when injected into mice and moreover did not depress the respiration in vitro of kidney and liver of mice or brown fat and liver slices of bats. However, Smalley's extracts did display a yellow-green fluorescence under ultraviolet illumination, but, contrary to the results reported by Zirm, this fluorescent material was also present in extracts from bat liver, pectoral muscle, spleen, and brain as well as bovine neck muscle, although it was absent in white fat preparations from bats. Both paper chromatographic and polarographic analyses indicated that the substance responsible for the fluorescent properties of the extracts was a riboflavin phosphate (323). Thus, although exact duplication of Zirm's experiment has not been attempted, studies quite similar to his have produced results that do not support his view that brown fat contains a hibernation-inducing substance. In fact, rats injected with brown fat extracts from hibernating and summer hedgehogs and subsequently exposed to \(-10\, ^\circ C\) for 3 hr maintained higher body temperatures (36.5°C) than did the controls (34.0°C) (130, 131), possibly indicating a protective effect of the tissue against body temperature depression.

**Maintenance of Dormancy**

The well-documented observation that the weight of the brown fat is highest at the beginning of the hibernating season and lowest at the end and shortly after the successive period of breeding, as well as the reported loss of lipid during the hibernation season (see above), has lent support to those suggesting that brown fat serves as a metabolic storage organ (220, 222). However, the weight of the brown fat during the hibernation season, as well as that in starved animals, is depressed relatively less than is the body weight and white fat depots (75, 136, 152–155, 283, 323, 372, 373), indicating that the fat in the brown adipose tissue is not readily available even during starvation and thus may not be in equilibrium with the other fat stores of the body. Moreover, as previously discussed, the decrease in mass of brown fat tissue during the season of hibernation may reflect changes uniquely associated with arousal.

On the other hand, in the brown fat of hibernating *C. tridecemlineatus* the turnover of catecholamines is 33% of that in active squirrels, whereas the rates in heart and brain decrease to 25% and 6%, respectively, of control values (96). Since these animals are capable of thermoregulation even at the reduced temperatures of hibernation (235), it seems likely that the metabolic activity of the brown fat, as mediated through the sympathetic system (see below), is involved in preventing the body temperature from falling as low as the ambient temperature (96). Klar (203) noted that the redox potential of the brown fat from hibernating hedgehogs was considerably more negative than that from the winter- or summer-awake animals and interpreted this as an indication of increased brown fat activity during hibernation.

Thus the available data tender support to the concept that the brown fat is a
specialized system and does not act as a general storage depot, but rather releases its lipid as required for the high-level thermogenic activity manifested during arousal and other periods of thermal stress.

**Arousal from Dormancy**

Evidence that the brown adipose tissue in hibernators may contribute significantly to heat production during arousal was first suggested by Smith and Hoijer in 1962 (337) and demonstrated in 1963 by Smith and Hock (335, 336) with thermocouples inserted into the several available sites of brown fat in hibernating marmots; these sites included superior cervical, axillary, and interscapular brown fat as well as relatively neutral areas not immediately subtended by brown fat (i.e., in a middorsal site in the T4–L2 area and a midventral site near the xiphoid process). During arousal (at 7°C) the actual temperatures as well as their rates of increase were higher in the brown fat than in the neutral locations. Removal of the marmot into a −12°C environment 90 min after the initiation of arousal resulted in an accelerated rise of the brown fat temperature in the axillary region and a transient but prolonged fall in the interscapular (not a major site of brown adipose tissue in the marmot) and superior cervical areas, wherein the temperatures began again to rise rapidly some 40 min after transfer. These observations, predicated on the earlier studies on thermogenesis of brown fat in the rat (328, 329, 337), led Smith and Hock to conclude that metabolic heat production is indeed a major function of brown fat in arousing hibernators (336).

A similar conclusion was reached by Smalley and Dryer (324) on the basis of temperature measurements of brown fat during the arousal of the bat E. fuscus, wherein the brown fat temperature exceeded that of other thoracic areas, including the heart, by about 3°C. These results have since been confirmed by Hayward et al. (148), who have shown that within 2 min of initial disturbance the brown fat temperature of the torpid bat surpasses that of the heart and remains about 1°C higher throughout arousal.

On the other hand, Lyman and Taylor (236) found brown fat temperatures to be lower than heart temperatures in several arousing rodents (woodchuck, hamster, dormouse). It has also been reported that the brown fat temperatures in arousing ground squirrels (C. lateralisis) do not exceed that of the heart until the rectal temperature nears 16°C (148). Since a massive shift of the circulation toward the posterior area occurs at this point, the elevation of the brown fat temperature over that of the heart during the final stages of hibernation was explained in terms of the “cooling effect” from peripheral blood returning to the warm cardiac muscle (148).

However, these measurements of heart temperatures in the woodchuck and ground squirrel were obtained with thermocouples in the aortic arch rather than in the heart muscle itself (148, 236). The observation that approximately 21% of the total brown fat in C. lateralisis (0.95% of body wt) surrounds the aortic arch suggests that the thermal contribution of this brown fat to the temperature of the blood in this vessel may be significant (172).
Although a thermogenic function for brown fat during arousal from torpidity has been adduced from the temperature recordings and the increased blood flow through the tissue (51), there has been some question as to the quantitative contribution of the heat produced by the brown adipose tissue in relation to the animal's total heat production.

In an attempt to estimate this contribution, Joel (193) has calculated the amount of lipid (triglyceride) that would be necessary to supply enough heat to warm the ground squirrel (C. tridecemlineatus) over a 30-C range (assuming a specific heat of the tissues of 1 kcal/deg per kg body wt). In comparing the computed value of 0.62 g with the actual amount of lipid that disappeared from the brown fat during arousal (0.95 ± 0.24 g), it was noted that complete oxidation of this lipid would contribute more than the necessary calories for the 30-C temperature rise and also require enough oxygen to account for the total oxygen consumption of the animal. Therefore, the conclusion that all this lipid is metabolized by the brown fat itself is tantamount to stating that the oxygen consumption of this tissue accounts for the total oxygen utilization of the ground squirrel during arousal, a conclusion that, as pointed out by Joel, is completely untenable. However, finding in C. tridecemlineatus that the brown fat contains about 20% of the total cytochrome c and about 60% of the amount in the anterior region of the animal, Joel et al. (198) have maintained that this tissue is capable of oxidizing an important fraction of the disappearing lipid. In fact, assuming a direct correlation between the amount of cytochrome c and the respiratory metabolism and also making use of the observation that the posterior circulation does not open up until approximately 1 hr after the initiation of arousal (at colonic temperature near 15 C), one can calculate that the brown fat metabolism during arousal of C. tridecemlineatus may be as high as [(60% × 60 min) + (20% × 30 min)]/90 min = 46.7% of the total.

Joel further contends that the brown fat supplies sizable amounts of oxidizable substrates for use by other tissues and thus contributes indirectly to the animal's thermogenesis (193). This contribution, however, can be wholly assessed only in light of the loss of lipid as well as glycogen and other substrates from various regions of the body (white fat, liver, and muscle in particular).

Using the in vitro $Q_{O_2}$ of brown fat to estimate the amount of heat that could be generated by the tissue, Ball (14) has compared this to the heat theoretically needed to rewarm the anterior portion of the ground squirrel and finds that the brown fat could supply 61% of this heat during the first 2 hr of arousal. However, as with Joel's calculation, this value represents at best the potential contribution of the tissue, since both are based on a maximum estimate of the tissue metabolism and a minimum estimate of the squirrel's total heat production, the heat loss to the environment during rewarming being neglected.

Along somewhat similar lines Hayward and Ball (146) have approximated in the bat (E. fuscus) the brown fat metabolism during arousal based on the product of the metabolic rate of the tissue in vitro and the duration of arousal. Comparison of this product with the actual amount of oxygen consumed by the bats during arousal indicated that the brown fat utilized 5.7% of the total oxygen consumption. The amount of heat represented by this percentage is considered by Hayward and
Ball to be too low to account for the thermogram pattern of heat distribution they obtained when the brown adipose tissue was approaching 22 C. The thermogram, a measure of the intensity of infrared radiation and thus of the surface temperature of the area, pictured the brown fat region as being the “hottest” area of the body. Because of these apparent discrepancies, Hayward and Ball concluded that the respiratory rates of brown fat as measured in vitro, although being high in relation to that of heart and liver, did not accurately reflect the in vivo metabolism and hence that the conditions needed to obtain maximum rates in vitro had not been achieved (146).

This possibility notwithstanding, it should be emphasized that the value of 5.7 % represents the thermal contribution of brown fat averaged over the entire arousal period, and it appears likely that the proportional heat contribution of the tissue might be greater early in the arousal period, i.e., before the opening of the posterior circulation and the onset of shivering.

Consideration of the limitations involved in the estimate discussed above led to an examination of the thermal contribution of brown fat as a function of arousal time in the ground squirrel C. lateralis (172). The assumption was made that the oxygen consumption rates obtained in vitro in the presence of catecholamines reflect the brown fat metabolism in vivo during arousal and, further, that during arousal the time course of the oxygen uptake of this tissue parallels that of the brown fat temperature. Reasoning from this, Horwitz et al. (172) have derived equations describing the oxygen consumption of the tissue as a function of time of arousal. Comparison of the calculated amount of oxygen consumed by the tissue over various time intervals with the measured heat production of the animal permitted estimation of the heat attributable to the brown fat, which ranged from 10–15 % initially to 5–7 % as the squirrels approached normothermic body temperatures.

However, since these values are directly dependent on the in vitro Q_10, the suggestion by Hayward and Ball (146) that the respiratory rates thus far obtained in vitro are lower than those occurring in vivo must be considered. In support of this possibility is the observation that although catecholamine addition stimulates in vitro respiration of brown fat from newborn rabbits by a factor of 1.7–2.3 (82), a 6.5-fold increase of the in vivo oxygen consumption of brown fat follows norepinephrine infusion (150). Hence, it has been argued that if the magnitude of these differences between the in vitro and in vivo stimulatory effect of catecholamines on rabbit brown fat metabolism is indicative of that occurring in other species, the calculated thermal contributions of the brown fat in arousing C. lateralis would be elevated to 40 % initially and to 20 % near the time when the posterior circulation would be opened (172).

Also related to this question of the importance of brown fat during arousal are the observations on arousing curarized animals. Hayward and Lyman (147) have reported that the time required for the brown fat temperature to increase from 15 to 35 C in E. fuscus is not altered by curarization. On the other hand, after curare treatment arousal of the hamster and dormouse proceeds at 40 % (hamster) and 20 % (dormouse) the normal rate (147). It thus appears that the contribution of shivering, as well as brown fat thermogenesis, in arousal varies with the species.
In this regard removal of about 50% of this tissue in the hedgehog has led to the death of the animals in hibernation; i.e., the dormant animals were unable to re-warm when exposed to extreme cold (399). Loss of 50% of the brown fat in marmots also caused a decreased resistance to cold that could be related to the amount of remaining brown fat (369), although Dubois (99) found that marmots thus deprived of the interscapular pad could still arouse from torpidity. Dubois (99) also demonstrated that an intact subclavian circulation was necessary for arousal. Recent work has also indicated that removal or major destruction of the brown fat in bats (324) and ground squirrels (87) does not prevent the animals from arousing from hibernation. However, Smalley (323) has reported that during the initial stages of rewarming the time required for bats without interscapular lobes to raise the temperature of the anterior musculature 5 C was 1.6 times that in the intact bats (31 min as opposed to 19). On this basis he has argued that the heat production of brown adipose tissue is significant, albeit not essential for arousal. Caution is needed, however, in interpreting results of brown fat extirpation, for in most animals removal of all deposits of this tissue is virtually impossible.

Unfortunately the data required to determine accurately the proportion of heat attributable to the brown fat (i.e., measurement in vivo of tissue oxygen consumption during arousal) are not yet available, due primarily to technical difficulties. On the basis of present data, however, it would appear that during the first part of the arousal period the brown fat metabolism could account for at least 10–15% (172) and possibly over 50% (14, 193) of the total heat produced. The thermogenic importance of brown fat depends not only on the amount of heat produced by the tissue, but also on the distribution of this heat. In view of the topology and vascularity of the brown adipose tissue (see above), it would appear that even the minimum estimate of a 10% heat contribution might be considered significant in terms of the localized application of this heat to the thoracocervical region of the spinal cord and vital processes in the thoracic area.

**Adult Nonhibernators**

Although hypertrophy of brown fat in cold-acclimated rats was first observed by Pagé and Babineau (260), it did not acquire significance until it could be associated with a thermogenic potential (328, 329, 337). At that time, however, it was noted that brown fat of cold-adapted rats undergoes a trophic response involving not only an increase in mass (53, 157, 260, 338), but also an elevation of the endogenous respiratory rate such that total oxygen consumption of the brown fat is 2–6 times that of the untreated rat (328, 338). Subsequent studies (cf. Table 8) have indicated that a similar metabolic stimulation occurs in cold-adapted mice (186), squirrel monkeys (56), true shrews (61), tree shrews (61), deer mice (292), and hamsters (57). On the other hand, such an effect has not been observed by Joel (193), who found no differences in the endogenous respiratory rates of brown fat fragments from cold-exposed rats.

As well as not confirming such a cold-induced metabolic increase in the brown fat in vitro, Joel (193) has also questioned the importance of this tissue as a “sig-
TABLE 8. Effect of cold acclimation on rate of brown fat oxygen consumption*

<table>
<thead>
<tr>
<th>Species</th>
<th>Control</th>
<th>Cold</th>
<th>Control</th>
<th>Cold</th>
<th>QO2 Tissue</th>
<th>QO2 Cold</th>
<th>Brown Fat Site</th>
<th>Preparation</th>
<th>Substrate</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Laboratory rat (Rattus)</td>
<td>52.8</td>
<td>66.9</td>
<td>42.0</td>
<td>94.2</td>
<td>1.27</td>
<td>2.24</td>
<td>Is</td>
<td>Slice</td>
<td>Glucose</td>
<td>338</td>
</tr>
<tr>
<td>Deer mouse (Peromyscus)</td>
<td>88.6</td>
<td>134.6</td>
<td>1.5</td>
<td>Is Slice</td>
<td>None</td>
<td>186</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hamster (Cricetus)</td>
<td>32.3‡</td>
<td>64.5‡</td>
<td>2.00‡</td>
<td>Mw α-G-P</td>
<td>57</td>
<td></td>
<td></td>
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<tr>
<td>Least shrew (Cryptotis)</td>
<td>110</td>
<td>629</td>
<td>5.65</td>
<td>HOM Succinate</td>
<td>61</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Musk shrew (Eumys)</td>
<td>420</td>
<td>1306</td>
<td>3.11</td>
<td>HOM α-G-P</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree shrew (Tupina)</td>
<td>33‡</td>
<td>46‡</td>
<td>1.11‡</td>
<td>HOM Succinate</td>
<td>61</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Raccoon monkey (Manace)</td>
<td>566</td>
<td>302</td>
<td>0.04‡</td>
<td>HOM Succinate</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squirrel monkey (Saimiri)</td>
<td>131.3</td>
<td>193.5</td>
<td>1.41‡</td>
<td>Ax HOM α-G-P</td>
<td>59,59</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Abbreviations used: QO2 N = ml O2/mg N per hr; QO2 tissue = ml O2/100 mg wet wt per hr; HOM = homogenate; Mw = mitochondria (washed); α-k-G = α-ketoglutarate; α-G-P = α-glycerophosphate; β-OH = β-hydroxybutrate; Is = interscapular; C = cervical; Ax = axillary; Ir = interrenal; Th = thorax; P = pooled.
† Not significantly different from 1.0 (i.e., P > 0.05). ‡ Values given as ml O2/mg protein per hr. § Smith, unpublished data.

significant thermogenic source in the rat. He has argued that although the mass of brown fat may double during long-term cold exposure, it reaches a level of only 1% of the body weight (338). This notwithstanding, it has been argued that the topological and vascular characteristics of the brown fat in these animals (cf. ANATOMY AND TOPOLOGY) are such as to channel the heat from this tissue directly to the thoracocervical spinal cord and the vital organs of the thorax (329, 338).

Support for such thermogenesis in vivo was obtained with cold-acclimated rats by comparing, with chronic indwelling thermocouples, the concurrent temperatures of the interscapular brown fat pads and blood in the right and left precaval veins, each at the level at which, on the left side, the confluence with the azygos occurs (333). As the major venous returns from the interscapular brown fat enter the left precaval in the rat, thermogenesis from these areas should reflect in a positive temperature differential between left and right precaval veins. On remov-
ing the rat from its environment of 6 C to one of -8 C, the temperature on both sides rose (Fig. 11), but the left side remained consistently above the level of the right by a highly significant increment of 0.56-1.34 C during each of three such exposures. Conversely, when the animal was removed from -8 C to a temperature of 22 C, the right side rose appreciably more than the left, while the bilateral difference was largely suppressed, albeit not abolished.

In their early studies Donhoffer et al. (92, 93) implanted thermocouples in warm-adapted rats and reported that reduction of the ambient temperature from 30-31 C to 18-20 C was immediately followed by a decreased oxygen consumption and lower temperatures in the colon, brain, and interscapular fat. However, soon thereafter (lag times of 1-15 min were observed, depending on the nature and level of anesthetic), the brown fat temperature and the total VO_2 had begun to increase while the colonic and brain temperatures ceased to fall. On transfer of the rats to a thermoneutral temperature brown fat temperature rapidly decreased in parallel with the rate of oxygen consumption (92, 93). More recently Szelenyi has reported that the temperature rise of the brown fat occurring in warm-acclimated rats exposed to cold is accompanied by a fall in the oxygen tension (Po2) of the tissue (358).

Similarly, in cold-exposed, warm-adapted adult guinea pigs and nonhibernating ground squirrels, thermometric recordings indicate that the brown fat is fully capable of thermoregulatory heat production (92-94). Moreover, the data of
Donhoffer and Szelenyi (94) suggest that lower ambient temperatures are needed to elicit maximum thermogenic activity from this tissue in cold-adapted as compared with warm-adapted rats.

The assertion that brown fat thermogenesis is significant in adult nonhibernators is further supported by temperature differences observed between blood entering and leaving the brown fat in rats cold exposed for 3–4 weeks (5 C) and returned to 26 C for 2 weeks (187). In these experiments thermocouples were placed on the interscapular gland, the left thoracodorsal artery, the deep central vein draining the tissue, and the inner and outer surfaces of the skin overlying the fat pad. Immediately after transfer of these preparations into a chamber at 4 C, the temperature of the brown fat began to rise, followed shortly by an increase in venous temperature. Thus the initial difference between the venous and arterial temperatures \[ -0.5 \text{ C} \leq (T_{\text{venous}} - T_{\text{arterial}}) <0 \text{ C} \] rose rapidly to an average steady state of +1.0 C, while the brown fat became almost 6 C warmer than the outer skin surface. In terms of caloric output these thermal differentials were equivalent to a heat production of 102 ± 7 cal/hr from the interscapular pad. Taking this output to be representative of brown fat in other areas of the body, the heat yield from the total brown fat mass (1.1 % of body wt) was extrapolated to a value of 347 ± 13 cal/hr (187).

Although this latter value was only 8.2 % of the total heat production (shivering as well as nonshivering) of these cold-stressed rats, these in vivo measurements of the blood temperatures emphasize the importance of the brown fat as a thermogenic vascular heater distributing heat to the cervical and thoracic regions.

It should be noted, however, that the significance of thermogenesis in the brown fat of adult nonhibernators seems to vary with the species. Thus, in a recent comparative study by Chaffee et al. (61), the cold-induced trophic responses of brown fat from several species appeared to decrease the higher their phylogenetic position. That is, cold-acclimated shrews appeared to be rodentlike in the response of their brown fat, whereas the effect of low ambient temperature was markedly reduced in the protoprimate, Tupaia, and no cold-induced brown fat hyperplasia or increase in oxidative potential was observed in the adult rhesus monkey although some response was noted in younger monkeys (61). These responses, however, should be considered also in respect to paleoclimatic conditions of origin, degree of aging, nutrition, and ambient temperature during earlier development (cf. 188).

Newborn mammals of many species, including humans, are capable of increasing their heat production in response to a cold stimulus (e.g., 39, 41, 247, 249, 250) without a concomitant increase in electrical activity of the musculature (e.g., 39, 41, 246). Thus in the newborn, nonshivering thermogenesis has been attributed to the relatively abundant brown fat. Its distribution in the newborn mouse along the azygos vein, dorsal aorta, and other vessels feeding the periphery, as well as its metabolic and trophic responses to cold in the adult, was adduced to support the concept that brown fat might exercise thermal control of the neonate by thermo-
genically “jacketing” the peripheral vascular returns and also providing heat for the spinal cord (331).

After the initial disclosures of Silverman et al. (318) on thermogenesis of brown fat in infants, the first conclusive evidence relating brown fat to neonatal thermoregulation was reported by Dawkins and Hull (80–82), who noted that in cold-exposed rabbits (12 hr old) the temperature of the interscapular brown fat always exceeded that of the colon. They also showed that on reduction of the ambient temperature from 35 C (thermoneutral temperature) to 25 C, the \( \text{V}_\text{O}_2 \) almost tripled; and although temperatures of the colon, interscapular brown fat, and subcutaneous lumbar musculature initially fell, the temperature of brown fat rose to control levels within 10 min while the temperature of the colon and musculature continued to decrease. A steady state was reached, 20–30 min after cold exposure, in which the temperature over the area of the brown fat was 1.3 C higher than the colonic temperature. Hypoxia, which abolished the increased oxygen consumption also eliminated these thermal differences, with the temperatures in all three measured areas falling sharply. Moreover, a return to air-breathing was immediately followed by increases in total oxygen utilization and brown fat temperature, whereas elevation of the colonic temperature lagged behind that of the interscapular area by approximately 10–15 min.

Accompanying this calorigenic response of the brown fat was the marked stimulation of blood flow through the tissue, the venous outflow increasing more than 300 % (from .42 to 1.39 ml/min) during mild cold exposure (149, 150). This augmented flow was not notably affected by hypoxia (149, 150).

Similarly, on initiation of NE infusion (2 \( \mu \text{g/kg of body wt per min} \)) the \( \text{V}_\text{O}_2 \) and brown fat temperature rose, followed by an increase in the colonic temperature (80–82) and an elevation of the venous outflow of the brown fat from .40 ml/min to 1.66 ml/min (149, 150).

The quantitative significance of the thermal contribution of the brown fat in newborn rabbits is indicated by the decreased in vivo effect of cold exposure and NE after excision of the tissue. After removal of 59–85 % of the interscapular and cervical brown fat, the animal’s heat production in response to cold and NE was reduced by 82 % and 80 %, respectively, whereas reductions of 32 % and 20 % were observed in rabbits where the scapulae were removed but the brown fat left intact (178, 180). Moreover, the magnitude of the elevation in oxygen consumption evoked by the NE was directly related to the amount of intact brown fat remaining (180). In subsequent studies data have indicated that the rabbit’s metabolic response to cold and NE is correlated with the fat content of the tissue (182).

A more critical evaluation of the quantitative importance of the brown fat metabolism in the newborn is afforded by the measurements of the rate of tissue oxygen uptake in vivo. During NE infusion (2 \( \mu \text{g/kg of body wt per min for 10 min} \)) in 3- to 7-day-old rabbits, the oxygen consumption of the animal almost doubled and the oxygen uptake of the brown fat increased by a factor of 6.4 (150). Significantly, the increased amount of oxygen used by the brown fat during infusion accounted for over 73 % of the excess oxygen consumed by the rabbit (15), thus indicating that this tissue is the major site of heat production in this response.
Although the brown adipose tissue in rabbits begins to be replaced by unilocular (white) fat from the 7th day after birth, this replacement is not complete until approximately 3 months of age (82). It is therefore not surprising that, as in the newborn rabbit, a correlation between the increased oxygen consumption of the intact animal and the brown fat temperature has been demonstrated in the 12- to 14-day-old rabbit exposed to cold (93).

Similarly, the extensive studies of Brück and Wünnenberg (43, 46) have shown that cold exposure as well as NE administration results in nonshivering heat production in newborn guinea pigs, accompanied by a simultaneous increase in blood flow and temperature of brown fat although the colonic temperature falls. Moreover, blockage of this induced chemical thermogenesis by alderlin [2-isopropylamino-1-(2 naptho)-ethanol hydrochloride] eliminates the temperature differences between the interscapular brown fat and colon, and it reduces, but does not abolish, the augmented blood flow through the brown fat (43, 46). As the guinea pig ages, the chemical thermogenic response to cold stress diminishes and is largely superseded by shivering within 2-3 weeks after birth (40, 44, 45, 397). These events are associated with a gradual replacement of the multilocular by unilocular fat cells to the extent that cold exposure of the 25- to 42-day-old animal fails to elicit any increase of temperature in the interscapular fat pad (46, 47, 397).

That brown fat also plays a role in the response of human infants to cold is suggested by the minimal change in temperature of the skin over the nape of the neck [under which sheets of multilocular fat are normally present (3, 35, 141, 283)] during moderate cold exposure of the neonate (214, 318). Moreover, Dawkins and Scopes (84) have noted that the elevated VO₂ of newborn infants during mild cooling is associated with increased plasma glycerol levels but no change in plasma FFA. These data have been adduced as reflecting brown fat thermogenesis, based on the fact that, subsequent to triglyceride lipolysis in fat slices, the ratio of release of FFA to glycerol from brown fat is considerably lower than that from white fat (82).

Further indirect evidence for the stimulation of brown fat in response to cold in the human neonate has been supplied by Heim and Kellermayer (154, 155), who have compared histologically the brown adipose tissue from necropsies of 80 infants. Significantly, the tissue studied from neonates nursed at 34–35 C (thermo-neutral temperature) was replete with fat, whereas 83% of the brown fat prepared from infants conventionally swaddled and nursed at room temperature (23–27 C) exhibited fat depletion. The fact that considerably more heat was produced in the last group than in the former (155) suggested that the observed histological differences resulted from fat mobilization induced by the thermal stress of the environment.

In contrast to the nonshivering thermogenesis manifested by many neonatal species (see above), no such response can be elicited in miniature pigs (K. Brück, personal communication). Instead, a linear relationship between electrical activity of muscle and cold-induced oxygen uptake has been noted; moreover, no metabolic increase occurs on NE administration. The observation that these pigs exhibit neither brown fat nor appreciable nonshivering thermogenesis (K. Brück,
FIG. 12. Effect of NE (iv) on the temperature of the interscapular brown fat and central venous drainage in rats cold acclimated for 4 weeks and returned to 26°C for 2 weeks. ΔT = change of temperature in brown fat minus change in colon temperature (○) or change in venous temperature minus change in colon temperature (●) (Y. Imai, unpublished data).

personal communication) is wholly consistent with the proposed function of this tissue.

Thus, the significance of brown fat to the nonshivering thermogenic response of the newborn mammal lies both in the amount of heat produced as well as in its localized application. Moreover, depletion of the fat vacuoles in the brown fat of newborn rabbits does not occur during starvation (152, 153, 176), which suggests that stimulation of brown fat metabolism is primarily a specific response to thermal stimuli rather than part of a general response to stress.

CATECHOLAMINES AND THE SYMPATHETIC NERVOUS SYSTEM

Analyses of the effects of the catecholamines on brown fat assume particular importance in light of their accepted role in potentiating the calorigenic response to cold (e.g., 173, 174) and the high NE content as well as turnover in the brown fat tissue (27, 79, 96, 317, 349, 381, 393). Additionally, the NE turnover in brown fat is significantly increased during cold stress of both warm- and cold-adapted rats (74).

It is thus significant that intravenous infusion of NE, epinephrine, or isoproterenol (2 µg/kg of body wt per min for 10 min) is followed by a 2- to 5-fold stimulation of the venous outflow from the brown fat of newborn rabbits (149–151) and guinea pigs (43, 46), a temperature increase of 1.0–2.1°C over the rabbit brown fat area, but a rise of only 0.6–1.0°C in the colonic temperature (82, 151). Similarly, NE infusion increases the brown fat blood flow 2–6.5 times in warm-acclimated rats and 0–6.8 times in cold-adapted rats (103, 216). Additionally, NE injection into cold-adapted rats is followed by temperature differences between the blood entering and leaving the interscapular gland ranging from 0.6 to 2.0°C (187). In these animals the response of the brown fat, as measured by the changes either in tissue or blood temperature, became asymptotic with increasing NE dose (Fig. 12). Moreover, intravenous infusion, as well as in vitro incubation of the tissue with catecholamines, augments the brown fat O₂ consumption by factors of 6.5 (149, 130) and 1.7–5.0 (82, 146, 193), respectively.

Examination of the action of catecholamines on lipid metabolism of brown
fat has led to varying results from different laboratories (Table 7). Although infusion of epinephrine into adult rats stimulated lipolysis, no such response was observed in either the 10-day-old animals (133) or after in vitro incubation with brown fat slices (256).

Similar discrepancies appear with respect to the in vitro stimulation of FFA release from brown fat of rabbits (71, 72, 82), ground squirrels (193) and rats (135, 194, 256). As suggested by Joel (193), these differences may be largely dependent on the use of an oxygen-rich atmosphere, a condition that appears to be essential for adequate in vitro maintenance of this highly respiring tissue (193); for in those experiments where no catecholamine stimulation could be demonstrated, air, rather than 95–100 % oxygen, was used as the gas phase (Table 7).

As occurs in white adipose tissue, the catecholamine-induced enhancement of triglyceride breakdown emerges as a probable mechanism by which these hormones mediate their thermogenic activity. It appears, however, that unlike their action on white fat, the catecholamines may not facilitate lipid synthesis in brown fat; and in fact, Steiner and Cahill (346) have reported in vitro inhibition of brown fat lipogenesis by NE. After incubation of brown fat fragments with NE, no change was noted in the conversion of glucose-U-14C to glyceride-glycerol but recovery of label in glycogen, fatty acids, and CO₂ was decreased (346). NE addition also suppressed fatty acid synthesis and CO₂ production from acetate-2-14C. Steiner and Cahill (346) have proposed that this apparent antilipogenic activity of NE results from inhibition of acetyl-CoA synthetase by the elevated fatty acid levels, a phenomenon that has been demonstrated in brown fat homogenates (344).

Although the brown fat glycogen and lipid accumulation observed after severance of the adrenergic nerve supply of the interscapular pad of mice (142, 316) was also interpreted as an antilipogenic activity of the catecholamines, it now appears that this response reflects the reduced lipid mobilization (142, 179, 181, 316) rather than accelerated synthesis (143). Similarly, recent work utilizing the technique of immunosympathectomy has indicated that impaired adrenergic innervation of the brown fat (about 90 % atrophy) does affect the net balance between lipogenesis and lipolysis in the tissue (89, 347, 348). That is, although chronic cold exposure of immunosympathectomized rats was still followed by high levels of urinary NE (304) and brown fat hypertrophy (89, 348), the relative decrease in lipid content seen in the normal animals was greater than that noted in the treated ones (347, 348). In view of the aforementioned in vitro studies, these results have been interpreted as most probably reflecting an impairment of lipid mobilization (347, 348). Indeed, a depression of both lipolysis and lipogenesis has been observed in the denervated brown fat of rats that were fasted and refed (69, 127).

Despite this evidence for the inhibition of lipogenesis by the catecholamines, there is no doubt that these agents are able to evoke a thermogenic response from brown fat. The fact that the systemic levels of NE required for lipolysis in murine brown fat approach the lethal dose (317) has suggested that release of NE from nerve endings rather than circulating catecholamines is the likely physiological stimulus for brown fat lipolysis (317). Certainly, this tissue is generously innervated by adrenergic fibers (see above) and enjoys as rich a concentration of NE as does...
TABLE 9. Norepinephrine content of various tissues*

<table>
<thead>
<tr>
<th>Animal</th>
<th>BAT</th>
<th>WAT</th>
<th>Heart</th>
<th>Spleen</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>.49</td>
<td>1.40 ± .05</td>
<td>.04 ± .01</td>
<td>.97 ± .03</td>
<td>381</td>
</tr>
<tr>
<td>Mouse</td>
<td>.49 ± .05</td>
<td>.06 ± .01</td>
<td>.66 ± .05</td>
<td>.46 ± .10</td>
<td>349</td>
</tr>
<tr>
<td>Ground squirrel (C. tridecedineatus)</td>
<td>.77 ± .04</td>
<td>.73 ± .02</td>
<td>.68 ± .05</td>
<td>.46 ± .10</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>.98 ± .08</td>
<td>1.29 ± .10</td>
<td>.349</td>
<td>.349</td>
<td>96</td>
</tr>
</tbody>
</table>

* Values = µg NE/g tissue wet wt; BAT = brown fat; WAT = white fat.

FIG. 13. Effect of stimulating the nerve bundle innervating the interscapular brown fat of a cold-acclimated rat on changes in temperature (ΔT) of the arterial blood entering the fat pad (thoracodorsal artery) and the venous blood (central vein) draining the area (Y. Imai, unpublished data).

As previously mentioned, denervation severely inhibits lipid mobilization from brown fat even during stress (68, 179, 181, 316), and 24 hr postoperative, the norepinephrine has disappeared from the brown fat (27, 317). However, although the lipid mobilization is affected 3-6 hr after denervation, NE levels are still normal at that time, suggesting that an intact nerve supply is essential for effecting the release of the catecholamines (316, 317). Denervation also influences the receptivity of the brown fat to catecholamines, the tissue being less sensitive to exogenously added epinephrine (143).

Consistent with these denervation effects on lipid metabolism of brown fat is the observed rise in free fatty acid content of the medium after in vitro stimulation of the nerve fibers supplying the interscapular gland of adult rabbits (79). Moreover, stimulation of the cervical sympathetic nerve in newborn rabbits was followed by a temperature rise of 0.2-0.6 C in the cervical brown fat (179, 181). Similarly, in the rat (Fig. 13) stimulation of the nerve bundle innervating the interscapular brown fat elicited a thermogenic response from the tissue, as indicated by the elevation of the temperature of the venous blood draining the fat pad, while that of the entering arterial blood increased only slightly (Y. Imai, unpublished data). Thus, these data strongly suggest sympathetically mediated thermogenesis of this tissue.

Whether such sympathetic regulation is exerted directly on the fat cells or in-
directly through vascular channels has been a point of controversy. The observation that the nerve fibers in the tissue terminate on the fat cells as well as capillaries (33, 142, 255, 316) was not confirmed by the earlier work of Wirsen (390, 391), who found no adrenergic fibers on the cells or capillaries, but only in the arterial walls down to the precapillary level. Recent technical improvements, however, have allowed detection of a fine network of adrenergic terminals enveloping the individual brown fat cells (392, 393). Moreover, the response of the isolated nerve-fat preparations to electrical stimulation (72) suggests that this effect is not mediated through the vascular system. Further support for this view is the finding that although the $\beta$-adrenergic blocking agent alderlin completely abolishes the thermogenic response of the brown fat in cold-exposed guinea pigs, a cold-induced increase of blood perfusion through the tissue still occurs, albeit this increase is reduced by the pharmacological agent (46).

THERMOGENESIS AND REGULATION

Brown adipose tissue, once identified with thermoregulation, brought cognizance of a new metabolic heating potential and awareness of its capacity for directing heat flux by vascular convection specifically in relation to the thorax and the cervicothoracic region of the spinal cord (329, 338). Implications thereto were examined: 1) anatomically, by dissection of the venous drainage of the cervical and interscapular fat pads and study of corroded polyvinyl casts of the vascular system (329); and 2) physiologically, by showing that the blood in the unilateral azygos of cold-acclimated, chronically prepared and cold-stimulated rats raised the temperature of the left precaval vein at the azygos junction to about 0.6–1.0 C above that measured at the same level on the right side (Fig. 11). The difference in temperature was attributed mainly to the thermal contribution of the interscapular brown fat.

Contemporaneously, it was being shown in dogs (lightly anesthetized with Pernocton) that cooling of the spinal cord by perfusion of the subarachnoid space or via the canal by thermodes extending between L7 and C3 rapidly induced shivering with an abrupt rise in $\dot{V}O_2$ (286, 319). This response was maximized to about 3.5 times basal by coincident cooling specifically of the spinal cord and esophagus with the animal in an ambient temperature of 30 C, which alone wholly damped the rise of $\dot{V}O_2$ in response to spinal canal cooling; spinal cooling with reduction of ambient temperature to 5 C resulted in a $\dot{V}O_2$ of about 2 times basal. From these findings it appeared that, in addition to the hypothalamic and skin thermoreceptors, there were also thermal sensory elements within the spinal cord.

Along similar lines, but more in relation to brown fat, Wünnenberg and Brück's (48, 395) localization of the spinal thermal sensorium at C6-T1 in the neonatal guinea pig is a most valuable demonstration. Thus, to the extent that brown fat may contribute to the warming of the sensorium, shivering would be inhibited. Notably, the cold-acclimating rat achieves nonshivering thermogenesis coincident in time with the asymptotic phase of the cold-induced hyperplasia of
brown fat (53). Moreover, an active suppression of shivering by local heating of the
cord would explain the fact that during arousal of the hibernator, shivering does
not become prominent until the abrupt period of blood mixing that signals the
rapid warming phase of the caudal regions (e.g., 172, 336).

With the introduction of the cervical spinal thermal monitor, several degrees
of freedom in control are made available. Perhaps the most simple integration
would be one employing negative feedback on the warming mechanisms, respec-
tively those of shivering and the sympathetic NE-activated visceral and brown fat
heat production. Hence, rising temperature in the spinal canal would be directly
associated with central inhibition of shivering.

Vascular Heat Exchange

Vascular channels governing thermal distribution by convection constitute
the main lines of heat transport. Equally important are the devices of A-V ex-
change of heat by direct transmural conduction. Net transfer of heat is then de-
termined by the instantaneous transmural temperature gradient, which in turn
depends on both the $\Delta T$ and the local transmural conductance as well as the re-
spective volume flow in each channel.

**Countercurrent exchange.** For the case of countercurrent flow in parallel vessels,
e.g., as with an artery and vein, heat moving down the transmural gradient will
tend to be returned toward its source; this amounts to a negative feedback and is a
well-known (302, 303) means exploited widely in mammals to conserve (at times
by several hundredfold) their body heat against a cold environment.

As brown adipose tissue in many mammals overlays the main vascular chan-
nels communicating with the periphery, its effect on the countercurrent equilibria
during cold exposure has been considered (329, 332, 334). In principle, a thermo-
genic overlay in the case of a cold environment has the net effect of warming both
the venous and arterial bloods but the venous more than the arterial; this invokes
the assumption that the temperature of the returning venous blood will be lowered
by heat lost to the environment. Moreover, if the brown fat exceeds both arterial
and venous temperatures, the effect will be to steepen the intravascular gradients
and more sharply define the body core.

In such areas as the axillae, the arteriovenous vasculature passes through the
overlying brown fat and, beyond developing countercurrent equilibria, may also
particularly nullify, by local thermogenesis, the existing peripheral arteriovenous
differences in temperature. In Figure 2 these adjustable null points (334) are indi-
cated along the internal intercostals as these course countercurrently under the
costal strips of brown fat, the veins returning varying amounts of arterial heat to
the azygos; this vein itself appears to be a primary equalizing area, as it also may
undergo direct countercurrent exchange with the thoracic aorta. Notably, in
neonatal infants (3) and hibernators, the internal mammarys are usually covered
with brown fat, thus giving potential control over the null points.

**Thermomultiplier and positive feedback.** With the interscapular brown fat in the
DORSAL CERVICAL BROWN ADIPOSE

BRACHIAL ARTERY

SUBCLAVIAN ARTERY

AZYGOUS VEIN

FIG. 14. Diagram drawn from corroded plastic replica of vasculature of interscapular and cervical brown fat lobes in the rat, as observed from dorsal aspect; forward direction at top. Dotted lines outline schematically the general perimeters of the fat lobes; note how juxtaposition of thoracodorsal arteries and veins give opportunity for transmural thermal exchange as in Fig. 15 (from Am. J. Physiol.).

THORACO-DORSAL ARTERY

INTERSCAPULAR BROWN ADIPOSE

FIG. 15. Model for optional positive feedback, illustrating thermomultiplier principle, diagramed to be analogous to one side and the center of the vascular circulatory replica shown in Fig. 14. Afferent arterial flow to the interscapular (or cervical) brown fat in the rat $F_1$ is shown to be in countercurrent with $F_2$, the venous return (analog to thoracodorsal vessels of Fig. 14). When brown fat is activated $T_a > T_r$, whence $T_a > T_2$ and $T_3$ is further amplified and so on; thermogenic control can be exerted by vasomotor dilatation of the unpaired venous shunt to $(F_1 - F_2)$, which may dump the heat load into the heat sink, i.e., the area of the thoracocervical spinal cord and the thoracic structures.

rat, the heat exchange via the bilateral thoracodorsal vasculature (Fig. 14) might, in a warm environment, tend to behave as a countercurrent negative feedback. In cold, however, when the tissue is under active metabolism, the bilateral countercurrent veins impart heat to the arteries, tending thus to accelerate the rates of
local metabolism. In principle, this is both a heat-conserving device and, optionally, also a positive feedback wherein by a thermomultiplier effect a very rapid rise in temperature of the brown fat may be developed (332–334, 338; Fig. 15).

As this system is employed as a positive feedback device, it becomes intrinsically unstable, for which both precise and sensitive controls are indicated. Analysis of the model (Fig. 15) has shown this system to be highly sensitive to flow rates, in that rapid changes are induced by small variations of the vascular supply; a mass transfer control is also provided by the vascular dumping via the one or more large unpaired central venous shunts, which drain into the inner vertebralplexuses, and subsequently by the vertebrals, thoracic and intercostal veins to the azygos, and then into the thoracic structures, which, with the brain and spinal cord, constitute a major heat sink.

In a number of functional thermoregulatory respects, the spinal cord resembles the liver in that both are primary heat sinks, are placed in series connection with an extrinsic heat source, and for each there is an unpaired venous shunt to the heart and the thoracic systems. Both of these venous returns are uniquely subject to cyclic pumping action driven by the respiratory movement. In the instance of the large and lesser suprathoracic veins in the rat, one may directly visualize this action externally on retraction of the interscapular pad at its hilus.

The relatively fixed volume of these vertebral sinuses is therefore capable of being perfused by flow between the respective soft organ systems that bound this bony cavity. In a low-pressure system of this nature, and one that is open to both the ambient atmosphere and the pleural cavity, convective transport of blood and heat could be considerably influenced both by intrathoracic pressure changes and by forces exerted at the periphery. Bowsher (36) has pointed out in particular the profound importance of cyclic respiratory pressures in driving an ebb-and-flow venous exchange between the thorax and these vertebral plexuses; this also occurs with the abdominal cavity.

**Febrile State**

In view of the quantitatively significant contribution of brown fat to the total heat production of the cold-stressed newborn, it is interesting to postulate that the fever spikes observed in children may, in some cases, primarily reflect alteration of brown fat activity. One might propose that such alterations may result from a direct effect of the pathogenic agent on the brown adipose tissue since it is well known that a number of viruses [rabies (5, 6, 23, 351, 352), polio (9), Coxsackie (06, 07, 125)] find this tissue a suitable substratum. Alternatively, it is also possible that some pathogens may precipitate a febrile state by affecting the sympathetic nervous system such that thermogenesis in brown fat is activated.

Since at the present time there has been no attempt to correlate brown fat metabolism with various thermogenic syndromes, such a relationship remains within the realm of speculation.
METABOLISM

General Metabolic Characteristics

As is true for any tissue, the heat produced by the brown fat is the consequence of its integrative metabolic activity. In terms of capacity, it is certainly significant that the cells of brown fat possess a high concentration of mitochondria in which the cristae are numerous and densely packed (Fig. 6). This characteristic correlates well with the high cytochrome content (193) and activity (192, 364) in the tissue and indicates the oxidative potential of the brown fat. Indeed, although the reported rates of oxygen consumption of brown fat slices are quite variable (Table 10), they are about 2.3 times higher than those of liver measured at 35-37°C under similar incubation conditions. Addition of glucose alone to brown fat slices does not markedly stimulate the endogenous respiratory rate (37, 146, 168, 197). However, exogenous succinate stimulates the metabolic rate of slices by factors of 2.6 (168) to 10 (172). Pyruvate, lactate, β-hydroxybutyrate, citrate, and α-ketoglutarate are also metabolized more rapidly than glucose by ground squirrel slices, but not to the extent of succinate (168).

Enzymatic analyses of the brown fat indicate that the activities of hexokinase, glutamate oxaloacetate transaminase, and glutamate dehydrogenase are low relative to that of the succinate, isocitrate, and malate dehydrogenases as well as fumarase (389). These observations are thus consistent with the relatively low rate of glucose utilization (glycolytic activity) of this tissue as compared to metabolic

<table>
<thead>
<tr>
<th>Species</th>
<th>QO₂</th>
<th>QO₂ Brown Fat</th>
<th>Substrate</th>
<th>Rel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>207</td>
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<td>None</td>
<td>Glucose</td>
<td>197</td>
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<td>338</td>
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<tr>
<td>102</td>
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<td>217</td>
</tr>
<tr>
<td>6.9</td>
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<td>41.9</td>
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<tr>
<td>41.1</td>
<td></td>
<td>Glucose</td>
<td>Glucose</td>
<td>37</td>
</tr>
<tr>
<td>Laboratory mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88.6</td>
<td></td>
<td>None</td>
<td>Glucose</td>
<td>186</td>
</tr>
<tr>
<td>Ground squirrel (C. tridecem-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lineatus)</td>
<td></td>
<td>162-440</td>
<td>Glucose</td>
<td>168</td>
</tr>
<tr>
<td>Bats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taphozous sp.</td>
<td>78</td>
<td>2.33</td>
<td>Glucose</td>
<td>121</td>
</tr>
<tr>
<td>E. fuscus</td>
<td>392</td>
<td></td>
<td>Glucose</td>
<td>146</td>
</tr>
<tr>
<td>Newborn rabbits</td>
<td>95</td>
<td>2.33</td>
<td>Glucose</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td></td>
<td>Glucose</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td></td>
<td>α-Ketoglutarate</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td></td>
<td>α-Glycerophosphate</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td></td>
<td>Pyruvate + malate</td>
<td>231</td>
</tr>
</tbody>
</table>

* QO₂ = μl O₂/100 mg tissue per hr.
rates involving the TCA substrates (see above). Additionally, the activity of mitochondria-linked enzymes is greater in the brown fat than in the liver of ground squirrels, although the converse situation applies to certain nonmitochondrial enzymes as catalase, glucose 6-phosphatase, and acid phosphatase (364).

In view of the association between the thermogenic response of cold-exposed neonates and the lipid content of the brown fat (3, 153, 155, 182), it is noteworthy that tissue homogenates, free cells, and isolated mitochondria are capable of oxidizing fatty acids as well as \( \alpha \)-glycerophosphate (15, 57, 59–61, 98, 165, 166, 208, 209, 232, 266, 345, 389). In fact, it appears that the rates of oxygen uptake obtained when \( \alpha \)-glycerophosphate is added to brown fat tissue slices (57) or isolated mitochondria (59, 231) are greater than those seen with most other substrates, fatty acids being somewhat exceptional (389).

At present, there appears to be some question as to the site of fatty acid activation. Williamson et al. (389) have reported that the NE stimulation of respiration of hamster brown fat cells, as well as that induced by exogenous oleate, is carnitine-independent, implying that the site of fatty acid activation is intramitochondrial. On the other hand, homogenates of brown fat from rats do exhibit carnitine-dependent fatty acid oxidation (15, 209). Whether or not these contradictory results reflect species variations and/or differences in the in vitro preparations is not at all clear.

The in vitro respiratory rates obtained with exogenous substrate may still be lower estimates of the potential metabolism in vivo. Whereas reported rates of oxygen consumption of tissue slices from newborn rabbits have ranged from 95–139 \( \mu L O_2/100 \text{mg of tissue per hr} \) [endogenous rates (82, 231)] to 600 \( \mu L O_2/100 \text{mg of tissue per hr} \) [substrate + NE (231)], the in situ “resting rate” was reported to be 558 \( \mu L O_2/100 \text{mg of tissue per hr} \) and that under NE stimulation averaged 3600 \( \mu L O_2/100 \text{mg of tissue per hr} \) (150).

Not only does brown fat possess a high metabolic capacity, but the response of ground squirrel tissue slices to low incubation temperatures suggests that cellular respiration may be less sensitive to temperature than that of other tissues of the body (168, 193). That is, the \( Q_{O_2} \) of brown fat slices measured at 8 \( ^\circ \text{C} \) was 36% of the 38-\( ^\circ \text{C} \) value whereas kidney retained only 15% of its 38-\( ^\circ \text{C} \) respiratory rate (168). These findings have been confirmed by Joel (193), who observed that ground squirrel brown fat at 8 \( ^\circ \text{C} \) respired at 31% of the 37-\( ^\circ \text{C} \) rate, although liver and diaphragm rates fell to 20% and kidney and white fat to 8–9%. Similar results have been obtained for the \( Q_{O_2} \) (with \( \alpha \)-ketoglutarate) of homogenates of rat brown fat (295). However, the temperature sensitivities of fatty acid oxidation by rat brown fat appear much greater (98) than do those for the \( Q_{O_2} \). As indicated in Table 11, the conversion rate of labeled palmitate or oleate as well as succinate to

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1 Bruce and Wiebers (38) also compared the respiratory rate of brown fat slices with that of other tissues from the bat at 35 and 16 \( ^\circ \text{C} \). However, they incubated the tissues in Krebs-bicarbonate buffer (pH 7.4), which they placed in the Warburg flasks and gassed with 100% oxygen. Since this procedure drives off the CO\(_2\) and changes the pH (to a test in our laboratory, the pH under these conditions rose above 8.5 at both temperatures), their results are considered highly unphysiological and incapable of being compared to those of other investigators.

2 This was done to ensure that the incubation medium was standardized for all tissues.
TABLE 11. Temperature sensitivities of brown fat metabolism*

<table>
<thead>
<tr>
<th>Specie/Preparation</th>
<th>Metabolic Process</th>
<th>5-10 °C Rate (5% of 37-40 °C Rate)</th>
<th>5 °C Rate (5% of 37-40 °C Rate)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground squirrel†</td>
<td>O₂, (succinate)</td>
<td>36</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>(slice)</td>
<td>O₂, (succinate)</td>
<td>31</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>Control rat</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>36</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>(homogenate)</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>38</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>Cold-acclimated rat</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>38</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>(homogenate)</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>38</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>Torpid bat‡</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>38</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>(homogenate)</td>
<td>Q₁₀ (α-ketoglutarate)</td>
<td>38</td>
<td>295</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Bruce and Wiebers (38) not included (see text).
† C. tridecemlineatus.
‡ E. fuscus.

14CO₂ at an incubation temperature of 5 °C is less than 10% the rate observed at 40 °C. On the other hand, these processes are not as temperature labile in preparations from torpid bats (E. fuscus). Corresponding with this greater retention of activity at low temperatures by the bat homogenates are the lower energies of activation observed in these preparations as compared to those from the rat (98). Moreover, the actual rates of fatty acid oxidation are greater in the bat tissue homogenates than are those from rat brown fat (98). Thus, as pointed out by Hook and Barron (168), at the reduced body temperatures prevailing during the various phases of hibernation, the brown fat cells appear to retain a significant proportion of their respiratory activity.

Metabolic Basis and Control of Brown Fat Thermogenesis

Before discussing the various aspects relating to the control of brown fat heat production, we would like to emphasize the distinction between the thermogenesis of brown fat in response, respectively, to chronic and acute cold exposures. As
previously indicated, in animals chronically subjected to low temperatures the brown fat undergoes trophic changes that include increases in 1) mass, 2) effective vascularity, 3) mitochondrial nitrogen content, and 4) endogenous respiratory rate. These modifications, which undoubtedly reflect the influence of a number of factors (e.g., thyroxine, catecholamines, etc.), contribute to an elevation of the metabolic potential of the tissue, and one may consider the brown fat as possessing a new metabolic base line in the cold-acclimated animal.

In contrast to this new steady state of brown fat metabolism (heat production) observed in cold-adapted animals is the response seen when the animal (warm- or cold-acclimated) is acutely cold-stressed. Under such conditions, the brown fat thermogenesis in situ is also increased (92, 93, 150, 187). However, the fact that stimulation of the sympathetic nerves innervating the brown fat, as well as intravenous infusion of NE, elicits an elevation in brown fat temperature suggests that this thermogenic response is mediated primarily through the sympathetic nervous system with NE being the probable transmitting agent.

It appears, therefore, that whereas the increased thermogenesis of brown fat seen during cold stress is mediated by release of NE from the sympathetic nerve endings, the elevated heat production of brown fat observed after chronic cold exposure reflects a somewhat different interaction of neurohumoral influences. In this regard, Steiner and Cahill (346) found that incubation of NE with slices of brown fat from control rats failed to stimulate the incorporation of 14C-glucose into the tissue lipids; however, tissue slices from cold-exposed rats exhibited a greater conversion of 14C-glucose into the brown fat lipid than did tissue from control animals (343). Thus, conclusions based on data obtained in studies of the effect of cold acclimation cannot be extrapolated a priori to explain the mechanism by which NE or acute cold stress stimulates the heat production by the brown fat.

Chronic cold exposure. In an attempt to account for the increased brown fat metabolism in cold-acclimated animals, it has been suggested that there is greater utilization of “inefficient” pathways and/or pathways that involve increased ATP turnover.

The fatty acid synthesis-oxidation cycle is one such “ATPase cycle” and is based on the continual breakdown of triglycerides to fatty acids, to acetyl CoA, and resynthesis back to fatty acids (cf. Fig. 16, scheme II). As originally proposed (238), this model postulated that in cold acclimated animals a shift might occur in carbohydrate metabolism such that glucose would be converted to triglyceride via acetyl CoA rather than being directly oxidized by the Embden-Meyerhof and TCA-cycle systems. The thermodynamic argument advanced was that direct oxidation of 12 moles of glucose to CO2 and H2O generated 456 moles of ATP and 3000-5000 kcal of heat; however, conversion of this glucose to tripalmitate with subsequent hydrolysis of the triglyceride and oxidation of the fatty acids via the TCA cycle would yield an additional 400 kcal of heat as well as providing 22 moles of ADP and 3 moles AMP for rephosphorylation. It was therefore suggested that lipogenesis might “partially underlie the process of non-shivering thermogenesis in the cold-acclimated rat” (238). Moreover, it was also noted that recycling of acetyl
FIG. 16. Diagram of mechanisms proposed as basis for the cold- or NE-induced increase of brown fat metabolism. Scheme I represents the triglyceride reesterification cycle (17) whereby triglycerides are hydrolyzed to glycerol and fatty acids and then reesterified. Scheme II summarizes the fatty acid synthesis cycle (237) wherein the fatty acids are oxidized to acetyl CoA and then resynthesized back to fatty acids. Also depicted is the suggested uncoupling action.

CoA to fatty acids (i.e., without oxidation of the acetyl CoA) could also be a "major thermogenic pathway" (238).

It should be emphasized, however, that since the cell is not a closed system only the "substrates" (and not the cofactors) are regenerated. Thus, in evaluating the energy balance of these reactions, one must include the cost of supplying the reduced NADP for resynthesis of the fatty acids as well as the energy produced as the NADH and FADH₂ resulting from β-oxidation pass through the electron transport chain. It is true, however, that participation of glucose in the cycle is less efficient than direct oxidation, due to the additional chemical reactions. Hence, calculation of the energy balance of the cycle in terms of input and output as well as the internal cycle of reactions themselves indicates that approximately 160 kcal (calculated on the basis that hydrolysis of 1 mole ATP yields 7000 cal) are evolved as heat with each turn of the cycle (13.3 kcal/mole of glucose).

The evidence supporting the importance of the operation of this cycle in terms of the thermogenesis of the brown fat is not conclusive. The results of Patkin and Masoro (265), who administered ¹⁴C-glucose to rats, indicated that not only was there no increase, but there was actually a depression of glucose conversion to fatty acids in the interscapular pads of the cold-acclimated animals. On the other hand, increased ¹⁴C-glucose incorporation into the brown fat lipids of cold-adapted rats has been reported by Shields (313) and Saucier (300).

As pointed out by Steiner and Cahill (343), however, these in vivo studies did not take into account possible differences in the glucose pool size and turnover rates
or incorporation into glyceride-glycerol, any of which could appreciably influence the amount of label incorporated into the fatty acids. Thus, these investigators injected glucose-U-14C into rats on the 9th day of cold exposure and examined the 14C activity in the fatty acids, glycogen, and glyceride-glycerol of brown fat as well as the blood glucose levels. Their results indicated an elevated conversion of glucose into the neutral lipids of the brown fat as both fatty acids and glyceride-glycerol. The increased lipogenesis and lipid turnover seen after cold exposure in situ were consistent with their observations on tissue fragments incubated in vitro with glucose-U-14C (343), although in the latter experiments more 14C was recovered in the fatty acid fraction than in the glyceride-glycerol. Similar increases in glucose-14C conversion (in vivo) into neutral lipid fatty acids, glyceral, and phospholipid have been observed by Steiner et al. (348) in rats exposed to 5 C for 12 weeks, thus eliminating the earlier objection that the trend seen then (343) may have reflected transient changes in the brown fat during the 9-day period of cold exposure.

Somewhat different results have been observed by Himms-Hagen (157) after injection of glucose-U-14C into control and cold-acclimated (33–74 days at 2–4 C) rats. Although the amount of label present in the brown fat lipids of the cold-adapted rats was 10–50 times greater than that in the control, 71% of this label was recovered in the glycerol moiety of the triglycerides, with only 27% found in the fatty acids and 2% in the glycerophosphate of the phospholipids. These data were interpreted as being incompatible with simply an increased fatty acid synthesis-oxidation cycle, but were seen as evidence supporting the model of triglyceride reesterification (see below).

Conversely, the work of Baumber and Denyes (19) clearly indicates an increased in vitro incorporation of 14C from acetate-1-14C into the brown fat lipids of cold-adapted (3–8 weeks) hamsters. These results, however, should not be considered proof of accelerated fatty acid synthesis, for again no assay of the tissue acetate pool before and after cold exposure was made.

The triglyceride hydrolysis-reesterification cycle, which was initially offered to explain the effect of various hormones on the oxygen uptake of white adipose tissue, visualizes the triglyceride fraction of the cell as being continually hydrolyzed to glycerol and fatty acids and then reesterified (17; cf. Fig. 16, scheme I). For each mole of triglyceride participating in this cycle, 4 moles of ATP are hydrolyzed (ca. 28 kcal) and the equivalent of 7 moles of ADP made available for rephosphorylation. Thus, this mechanism not only generates heat but also increases the proportion of ATP to ADP, thereby providing a theoretical basis for stimulation of the oxygen consumption of the tissue. As indicated below, however, most of the evidence adduced in support of the importance of these reactions in cold acclimation remains somewhat equivocal.

As previously indicated, incubation of labeled glucose with brown fat fragments from cold-exposed rats (9 days) resulted in a greater incorporation of glucose into the fatty acids as compared with the glycerol portion of the triglyceride (343).
On the other hand, the in vivo experiments of Himms-Hagen (157) cited above suggest that the triglyceride cycle is intensified in the brown fat of the cold acclimated rat. In this study, however, no measurement of the precursor pool (i.e., α-glycerophosphate) was made, although the fact that cold affected the specific activity of the glycerol moiety of the phospholipids less than that of the triglycerides was taken as evidence that possible changes in the α-glycerophosphate pool could not explain the results.

Obviously this latter assumption is not justified if the phospholipid content of the brown fat increases substantially more than the triglyceride level after cold exposure. The only study to date reporting measurements of the neutral and phospholipids of brown fat before and after chronic cold treatment (12 weeks) is that of Steiner et al. (348). They found in the lipid content (primarily triglyceride) a decrease from 98% of the total tissue lipid to 88% in the cold rats and an increase of the phospholipids from 2.3% in the controls to 11.6% in the cold treated animals. Similarly, it has been reported that the oxygen consumption per wet weight of brown fat is 4.1 times higher in homogenates when obtained from cold-adapted rather than from control rats (338). Assuming that the oxygen uptake of the tissue is directly proportional to the functional mitochondrial units (density) and, further, that the phospholipid of the tissue resides primarily in these particulates, these results suggest a possible four- to fivefold elevation of the phospholipid concentration. Consistent with this possibility is the fact that although the nitrogen concentration (mg N/g wet wt) of brown fat from cold-acclimated rats was seen to increase more than twofold, the percentage of homogenate nitrogen found in the mitochondrial fraction did not differ significantly from that of the controls (294) (i.e., mitochondrial nitrogen also increased twofold). Since the cold-acclimated rats of Himms-Hagen (157) showed no increase in total lipid concentration and the phospholipid content of the tissue in non-cold-exposed rats is only 2% of the total lipid while that of the triglycerides approaches 85–98% (Table 5), for simplicity one can view the triglyceride concentration as being relatively unaltered after cold acclimation. Hence, on the basis of these considerations and the observation that the brown fat mass of the cold-adapted rats used by Himms-Hagen increased approximately four times, the total radioactivity present in the triglyceride glycerol of the cold animals can be calculated as $9.1^4 \times 4 = 36.4$ times greater than that of the controls with the amount of label in the phospholipid glycerol being $22.4–56.0^4$ times higher in the cold-exposed rats.

Thus, since the phospholipid content of brown fat appears to increase more than that of the triglycerides during chronic cold exposure, it is felt that in lieu of direct measurement of the concentration of the various lipid fractions and/or the precursor pools, the activity of the triglyceride cycle cannot be properly evaluated from the specific activity of the selected components.

On the other hand, all of these incorporation studies suggest that in these cold-acclimated animals there may indeed be a greater rate of lipid synthesis in the

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4 Specific activity—cold/specific activity—control.

5 Assuming a twofold increase of phospholipid concentration (338), radioactivity = $2.8^4 \times 2 = 22.4$, whereas the value increases to 56.0 assuming a fivefold elevation (348).
brown fat. However, even if such an increase existed, it could be considered as part of the general elevation of tissue metabolism resulting from the greater mass (and therefore greater enzyme content) and altered composition, for there is no conclusive evidence that the activity of either of the two cycles discussed is selectively stimulated during chronic cold exposure.

**Acute cold exposure.** In view of the evidence for the mediation of NE in the cold-induced thermogenic response of brown fat (in vivo), it is interesting that this sensitivity is also reflected in vitro as NE elevates the respiratory rate of tissue slices (146, 193, 231) and isolated cells (232, 273, 389).

The mechanism by which NE stimulates brown fat metabolism appears analogous to the situation in white fat wherein the conversion of ATP to 3', 5'-AMP (as enzymatically catalyzed by adenyl cyclase) is increased (52, 356). The evidence favoring this derives from the observations that 1) catecholamines have no apparent effect on either brown fat homogenates (15) or isolated mitochondria (232), a finding consistent with the concept that adenylyl cyclase is located in the plasma membrane (355); 2) theophylline, which inhibits destruction of cyclic AMP, mimics the stimulatory effect of catecholamines on the respiratory rate of brown fat slices (232) and free cells (287, 288); and 3) the dibutyryl derivative of cyclic AMP also enhances the $Q_{O_2}$ as well as lipolysis of isolated brown fat cells (287, 389). It would appear, therefore, that NE acts by stimulating production of cyclic 3', 5'-AMP, which in turn activates a lipase catalyzing the hydrolysis of triglycerides to FFA and glycerol. Moreover, studies with isolated brown fat cells of hamsters suggest that the ATP utilized for the NE-induced fatty acid activation may derive primarily from GTP generated by substrate level phosphorylation in the TCA cycle (389).

In terms of current theory (i.e., that electron transport limited respiration is controlled by the ratio of ADP to ATP) explanations of the respiratory effects elicited by NE have hinged on mechanisms involving either an increased ATP turnover or a dissociation of the "classical" ADP control of respiration. Notably here, the cellular ATP:ADP ratio has been shown to decrease initially on NE addition to isolated hamster brown fat cells (232, 389), while the increase in respiratory rate follows after a short lag period (389). These observations are qualitatively consistent with either an NE-induced uncoupling or an increased ATP turnover. However, some caution is needed in interpreting such data based on cellular content, for they may not accurately reflect small changes in specific cellular compartments. That is, it would appear that it is not necessarily the cellular ATP:ADP ratio that bears directly on the regulation of ADP-controlled respiration, but rather the intramitochondrial ATP:ADP levels and/or the extra-mitochondrial ATP:intra-mitochondrial ADP.

Both the fatty acid resynthesis and the triglyceride reesterification cycles have been proposed as possible ATPase systems, since in theory each could be activated by the NE-induced acceleration of lipolysis (see above).

The only evidence to date bearing directly on the fatty acid resynthesis model comes from the work of Steiner and Cahill (346). Incubating NE with brown fat slices, they found that although NE stimulated lipolysis, it did not affect the con-
version of $^{14}$C-glucose to labeled glyceride-glycerol and in fact depressed the $^{14}$C recovery into fatty acids. Hence the significance of this cycle in terms of the NE elevation of brown fat $Q_{O_2}$ has yet to be established.

Results interpreted as supporting the operation of the triglyceride cycle have been reported for the brown fat of cold-exposed, 2-day-old rabbits in which there occurred a sixfold elevation of glucose-$U^{14}$C conversion to glycerol with no change in incorporation into fatty acids (204); however, since the precursor pool ($\alpha$-glycerophosphate) was not measured, interpretation of the data remains speculative. Pertinent here is the recent demonstration by Kornacker and Ball (15, 209) that the epinephrine-induced respiratory stimulation of brown fat fragments is not accompanied by an increased uptake of labeled glucose or glycerol into the glyceride-glycerol fraction. Moreover, no increased incorporation of palmitate-$U^{14}$C into triglycerides was associated with the elevation of the respiratory rate of homogenates by cofactor additions. Conversely, the incorporation of labeled palmitate into triglyceric fatty acid was increased when $\alpha$-glycerophosphate, as well as the cofactors, was added to the homogenates; however, the amount of FFA reesterified only approached 30% of that converted to $CO_2$ (15, 209). Additionally, Lindberg et al. (232) have reported that the amount of tritiated oleate recovered in the di- and triglyceride components of brown fat cells incubated with NE is less than 25% of the label recovered in water (i.e., oxidized) and accounts for less than 1% of the concomitant increase of respiration (232). Thus, although it is possible that only a small pool of the total triglycerides is rapidly hydrolyzed and reesterified (15), it appears more likely that, unlike the situation in white adipose tissue, the stimulatory effect of NE on brown fat respiration is not explained by increased utilization of ATP for reesterification of FFA (15, 232).

Recently, Prusiner et al. (273) have suggested that ATP turnover could be increased by the cyclic conversion of fatty acid and its CoA derivative. There is at present, however, no evidence that the operation of this pathway could account for the NE-induced respiratory increase.

The possibility that brown fat mitochondria may not be under respiratory control has received considerable attention, first as a general property of the tissue and lately as a possible consequence of NE stimulation of lipolysis. Low P/O values for brown fat mitochondria from rats were initially reported by Lepkovsky et al. (228), and in more recent studies oxidative phosphorylation of these mitochondria has been reexamined with somewhat conflicting results (4, 95, 128, 159, 165, 166, 196, 231, 267, 340, 364).

In both control and cold-acclimated rats, incubation of brown fat mitochondria with succinate and $\alpha$-glycerophosphate yielded P/O ratios that were essentially zero, while values approaching unity were obtained with $\alpha$-ketoglutarate (340). In fact the only evidence for the presence of phosphorylation coupled to the electron transport system was the inhibition of ATPase activity by oligomycin (340).

In these experiments attempts were made to counteract the possible uncoupling effect of fatty acids by homogenizing the tissue in the presence of bovine serum albumin (BSA, 6 mg/ml). Neither this procedure nor the addition to the re-
action flask of glutathione (10^{-3}M) or BSA (6 or 15 mg/ml) was successful in establishing respiratory control or in elevating the P/O ratios. Thus it was suggested that although substrate level phosphorylation could occur in these brown fat mitochondria, “classical” (as in liver) electron transport-coupled phosphorylation might be absent (340).

Similar results were also reported for brown fat mitochondria isolated by several methods from newborn rats (231), rabbits (231), ground squirrels (364), and hamsters (364). Although a variety of substrates was tested, phosphorylation was obtained only with glutamate and α-ketoglutarate plus malonate (231). Even with these substances, however, the P/O ratio was less than unity, again suggesting substrate-level phosphorylation. As with adult rats (340), addition of albumin did not affect the P/O ratio nor were the mitochondria sensitive to DNP (231). Furthermore, when brown fat and liver tissues from newborn rabbits (231) or adult rats (159) were fractionated together, the resulting mitochondria yielded P/O ratios that could be explained by a mixture of tightly coupled liver particulates and "uncoupled" brown fat mitochondria. These results, therefore, indicated that if some uncoupling agent were released during isolation of the mitochondria, only the brown fat particulates and not those of the liver were affected.

Consistent with these reports of poor phosphorylation are electron-microscopic data (2, 231) in which negatively stained mitochondrial preparations fail to reveal the “elementary particles” postulated as the site of the coupling factors for ATP formation (280). On the other hand, these elementary particles have been described in brown fat mitochondria isolated from newborn guinea pigs (166). Additionally, mitochondria from newborn and 20-day-old guinea pigs exhibited both respiratory control and oxidative phosphorylation with several Krebs cycle intermediates and short-chain fatty acid-carnitine esters. This respiration could be inhibited by oligomycin and stimulated by DNP (166). However, mitochondria from newborn guinea pigs, in contrast to those from 20-day-old animals, failed to show oxidative phosphorylation or respiratory control with decyl- and palmitylcarnitine (165, 166). Furthermore, Hohorst and coworkers have also obtained respiratory control and high P/O ratios after adding ATP or GTP to isolated brown fat mitochondria from adult cold-adapted rats as well as newborn rabbits (165).

In all these experiments, Hohorst's and those from other laboratories, oxidative phosphorylation of brown fat mitochondria (4, 128, 159, 196) has required the presence of BSA, suggesting that fatty acid uncoupling may explain the conflicting results in the literature. Pertinent here is a study of the effect of BSA on brown fat mitochondrial energetics (159). Since fatty acid uncoupling of liver mitochondria can be prevented by addition of BSA during the isolation procedure (30, 226), attempts to obtain coupled brown fat mitochondria were made by adding albumin (up to 25 mg/ml) to the initial homogenization medium, as well as at different steps of the preparatory procedure. None of these methods, however, yielded mitochondria showing significant oxidative phosphorylation. Thus, unlike liver mitochondria, those of brown fat exhibited significant oxidative phosphorylation only when the BSA (18 mg/ml) was present in the reaction flask. However,
recent work in several laboratories has suggested that in the absence of BSA, but with ATP and carnitine present, isolated brown fat mitochondria may show respiratory control (95, 160).

From these in vitro studies, therefore, it is apparent that brown fat mitochondria do indeed possess an energy-conservation mechanism coupled to electron transport. The demonstration that these particulates isolated from rat brown fat can accumulate significant amounts of Ca++ (161) substantiates the presence of such a mechanism, although a recent study reported the lack of Ca++ uptake by mitochondria from ground squirrels (364). The ineffectiveness of BSA additions at various stages of the isolation procedure as well as the liver-brown fat “hybrid” experiments (see above) indicate that if fatty acids released during the preparation procedure are responsible for the low P/O ratios observed in brown fat mitochondria incubated without BSA, these mitochondria must be more sensitive than those from liver since the liver particulates were unaffected.

It therefore appears that although brown fat mitochondria may resemble liver and heart mitochondria in terms of the permeability restrictions for TCA intermediates (389), the electron transport-coupled, energy-conservation mechanism observed in isolated brown fat mitochondria differs somewhat from that seen in liver particulates. However, recent work in our laboratory indicates that these differences may not reflect an in vivo uncoupling of the brown fat mitochondria.

In a series of experiments in which cold-acclimated rats were acutely cold-stressed, injection of DNP (10 μmoles) was followed by an increase in the interscapular brown fat temperature (Fig. 17). Notably, this increase typically preceded that of the liver and colon. Similarly, in experiments at room temperature (23–24 C), DNP treatment either prior to or directly after NE administration resulted in a thermogenic response of the brown fat that was greater than that obtained with either agent alone (169–171). Since DNP has no direct cardiovascular effects (123), these results were taken as evidence of a DNP-induced respiratory stimulation of the brown fat via the classically defined pattern of uncoupling of oxidative phosphorylation. In agreement with this interpretation are the results from a series of in vitro experiments in which: 1) DNP stimulated the QO2 of brown fat fragments, 2) both DNP and dicumarol increased the respiratory rate of brown fat homogenates; and 3) the QO2 of the homogenates was stimulated by addition of an acceptor system (170, 171).

Thus, in view of the calorigenic response of the brown fat to injected DNP, it was concluded that brown fat mitochondrial respiration in vivo is coupled to oxidative phosphorylation (169–171). Similarly, in situ experiments with hamsters (274) as well as in vitro studies using isolated brown fat cells from both rats (105, 287) and hamsters (232, 274) support this conclusion, at least under conditions where the tissue (cells) is not under catecholamine stimulation.

Additionally, the observed synergistic effect of DNP with NE (see above) implies that the NE-induced brown fat thermogenesis cannot be fully explained by stimulation of an ATPase cycle (170, 171). That is, if NE were simply increasing the amount of ADP available for rephosphorylation, one would not expect a greater thermogenic effect after DNP treatment. However, this apparent synergism
FIG. 17. Effect of DNP (iv) on temperatures of the colon, liver, and interscapular brown fat of cold-acclimated rats acutely exposed to a severe (A) and mild (B) cold stress [170, 171; from Can. J. Physiol. Pharmacol.].

does not preclude the possibility that NE might physiologically uncouple the brown fat mitochondria by virtue of increased fatty acids within the cells (105, 196, 232, 273, 288, 389).

One mechanism by which NE might indirectly uncouple has been suggested by Fain and Reed (105, 288). Utilizing brown fat cells isolated from rats fasted 18-hr, they reported that catecholamine stimulation of respiration appears to be dependent on K⁺. That is, although omission of K⁺ from the incubation medium did not affect the fatty acid release by NE or theophylline, no concomitant respiratory increase was noted. In the presence of K⁺, however, NE induced a stimulation of the rate of oxygen consumption as did theophylline, when added with K⁺, Rb⁺, or Cs⁺. Moreover, addition of valinomycin, which increases K⁺ accumulation by isolated mitochondria, also elevated the rate of oxygen utilization of the brown fat cells incubated with K⁺. Hence, Fain and Reed have proposed that the NE effect
results from increased utilization of energy for K\(^+\) uptake. They also suggest that the fatty acids may interact with the mitochondrial membrane such that K\(^+\) leaks out of the mitochondria and thus the expenditure of an energetic intermediary for the compensatory uptake of K\(^+\) is increased (105).

Unfortunately, no direct measurement of the NE effect on the actual movement of K\(^+\) was made. Furthermore, the apparent dependency of the respiratory stimulation on the presence of K\(^+\) need not be interpreted as a fatty acid-induced leakage of K\(^+\) from the mitochondria. Alternatively, K\(^+\) may be necessary as a counterion for permeability of the anionic substrate across the mitochondrial membrane. This possibility is supported by the recent report that rat liver mitochondria are impermeable to all the Krebs cycle intermediates when Ca\(^{++}\) is being accumulated, but not so when K\(^+\) is the transported cation (202). It may be, therefore, that the requirement for K\(^+\) is not a manifestation of a NE-uncoupling action, but rather an essential for the transport of substrate into the mitochondria.

That NE can induce uncoupling, however, has been adduced from experiments conducted in Lindberg's laboratory (232, 273) in which NE was added to brown fat cells isolated from hamsters starved 48 hr. Such preparations were responsive to uncoupling agents and appeared to be extremely sensitive to NE, the respiratory rates of the cells being stimulated 10-fold or more by the catecholamine. However, at maximal NE-induced respiration, uncoupling agents no longer elicited any increase in the rate of oxygen consumption (232, 273). This observation, as well as the fact that oleate addition mimicked the effects of NE, led to the suggestion that NE might be uncoupling the mitochondria in these isolated cells (232).

The results from these in vitro studies, however, differ considerably from those obtained in our laboratory (170, 171) when NE was administered intravenously into cold-acclimated rats in a dose sufficient to elicit a maximum thermogenic response from the brown fat (defined as the maximum temperature increase of the brown fat minus the corresponding change in core temperature). In contrast to the effects noted by Lindberg and colleagues (232, 273), treatment with DNP at the peak of the response to such a maximum NE dose further elevated the tissue temperature (Fig. 18). Additionally, although administration of a second dose of DNP
was not followed by a change in the brown fat temperature, injection of another
dose of NE again effected a thermogenic response from the tissue. Moreover, the
response to this latter dose was just as great as that obtained before treatment with
DNP (Fig. 18), implying that the NE-induced thermogenesis does not reflect any
significant degree of uncoupling (170, 171).

The apparent contradiction between the results obtained in our studies and
those from Lindberg's laboratory are probably related to basic differences between
the in vivo and in vitro systems. The observation that at maximal NE stimulation
uncoupling agents no longer increase the respiratory rates of the isolated cell
preparations (see above) could indicate either 1) that cellular respiration is maxi-
mal as a result of some component(s) of the electron transport chain (rather than
ADP) being limiting or 2) that the NE is indeed effecting an uncoupling action.
Since this does not appear to be the case when a "maximum" NE dose is adminis-
tered in vivo to the cold-acclimated rat, the obvious question raised is the extent
to which the in vitro system accurately reflects the situation in situ. Thus, one might
argue that the magnitude of the NE-induced response of the isolated cells, having
been released from any normal constraints of tissue structural integrity or neuro-
humoral control, may exceed that which could occur under physiological condi-
tions. The fact that the NE stimulation of the respiratory rates of these cell prepa-
lations is much greater than has been observed in vivo (150) or with tissue fragments
(146, 193, 231) might be construed as supporting such a possibility. It therefore
may be that in the experiments with isolated cells, NE doses used do effectively
uncouple the brown fat mitochondria. However, the in vivo evidence gathered to
date suggests that such an effect may not be the primary physiological mechanism
by which the brown fat thermogenesis is mediated.

Similarly, the fact that the magnitude of the thermogenic response to a pulse
of NE does not appear to be diminished after DNP treatment in vivo (see above)
also implies that the effect of NE cannot be explained simply by a stimulation of
an ATPase cycle. This interpretation is strengthened by the previously mentioned
synergistic response noted after joint treatment with DNP and NE. This latter re-
sponse is most plausibly explained as resulting from increased release of substrate,
which can be oxidized more rapidly after DNP administration. Moreover, in
experiments conducted at 23–24 C, treatment with DNP does not appreciably alter
the temperature of the brown fat in the cold-acclimated rat, although the tempera-
tures of the liver and colon are slightly elevated (170, 171).

On the basis of these data, therefore, it has been suggested that the thermo-
genic effect of NE on the brown fat reflects an increased availability of substrate
(170, 171). That is, under conditions where the brown fat is not stimulated (by
cold stress and/or NE release), the oxygen consumption (heat production) of the
tissue is limited by substrate rather than ADP.

It should be noted that this proposal appears not to be supported by studies
with isolated cells (389; Chance and Fain, unpublished data). In these prepara-
tions, addition of NE to the medium decreased the fluorescence attributable to the
pyridine nucleotides (389), increased the fluorescence due to the flavin pigments
(389), and decreased the absorbance of cytochrome b (389; Chance and Fain,
unpublished data). These data, which were interpreted as indicating an oxidation of the pyridine nucleotides and cytochrome b and reduction of the flavins, led Williamson et al. (389) to suggest that the endogenous respiration of the isolated cells is limited by phosphate acceptor and that NE may induce release of phosphate acceptor control.

Somewhat similar results have been reported in hamsters in which the temperature of the brown fat and the pyridine nucleotide fluorescence on the surface of the tissue were measured (274). In arousing hamsters, as well as those that had been housed at room temperature, injection of NE was followed by an increased brown fat temperature and a transient decrease of the fluorescence of the order of 22%. This decrease was attributed to oxidation of the pyridine nucleotides and was interpreted as indicating that the respiratory chain was not substrate-limited (274). However, examination of the published temperature record for the arousing hamster reveals that the NE was infused at a brown fat temperature near 24 C. Since previous studies of brown fat thermogenesis during arousal have indicated that the brown fat temperature continues to rise rapidly until it approaches 30 C (146, 148, 172), it seems reasonable to assume that the brown fat in the arousing hamster in the study of Prusiner et al. (274) was still being stimulated by the sympathetic system at the time of NE infusion. Similarly, although no colonic temperatures are reported for the experiments with “warm-acclimated” hamsters, at the time of NE administration the brown fat temperature was 28–29 C. This strongly suggests that these animals were hypothermic and that the brown fat may have been under some degree of sympathetic stimulation. Unfortunately, the published temperature records illustrate less than a 2 min period before the NE infusion and it is therefore difficult to evaluate the thermal state of the animals. If indeed the brown fat were under the influence of endogenous NE, one might not expect the tissue to be substrate-limited.

It should be apparent, however, that even though the initial NE-induced increase of brown fat heat production in vivo may be explained by increased substrate availability, in order for the high metabolic rate to be sustained, there must be a concomitantly increased ATP-turnover and/or an uncoupling. At the present time, however, it must be concluded that the basis for sustaining the high metabolic rate has not yet been defined.

SUMMARY AND CONCLUSIONS

Multilocular brown adipose tissue (hibernating gland), described grossly by Gesner in 1551, became explicitly identified in 1961 as a thermogenic effector organ in mammals.

Within the class Mammalia, brown fat occurs widely in seven orders and is especially prominent in the newborn, ranging initially in the human infant from about 2 to 6% of total body weight. Retention of relative mass with age is greatest in the hibernators and generally least in those species indigenous to the tropics.

Appearing in early fetal stages, the tissue develops from mesenchymal anlagen
in close conjunction with the blood-vascular system and thereby acquires a profuse complement of unmyelinated neuronal components of sympathetic origin.

At birth, or as early as 28 weeks in the human fetus, the multilocular cells of the brown adipose tissue are well differentiated and disposed into characteristic topology relating to the vertebral axis, especially around the cervicothoracic spinal cord and extending middorsally from the mediastinum caudally along the azygos vein and abdominal aorta variously to the renal and inguinal level. Cephalad, the brown fat extends from the aortic arch generally along the systemic vasculature. These vessels are complemented bilaterally with dorsal, cervical, interscapular, and axillary lobes. Ventrally in the thorax, especially in hibernators, brown fat extends along the main course of the internal mammarys. In effect, the vasculature from the entire periphery is at some point invested with brown adipose tissue. In consequence, thoracic structures and cervicothoracic segments are well protected from thermal deficits.

The cells of brown adipose tissue carry an extraordinary potential for metabolic power, both absolutely and relative to other cell types. Thus, structurally, the central nucleus is surrounded by cytoplasm carrying large numbers of mitochondria, these frequently in juxtaposition with the numerous fat vacuoles. Electron micrographs show the mitochondria internally to be heavily lamellated with cristae.

In normal rats exposed to cold, these cells are activated, and within 3-6 hr the fat vacuoles have virtually disappeared, to be restored however within 24 hr. The loci of these cells are highly vascularized and richly innervated; clearly the intracellular geometry is also disposed to maximize, for both mitochondria and fat vacuoles, the surface-to-volume ratios essential in effecting metabolic exchange at optimal rates. Notably, the relative oxidative power of the cells, as based on cytochrome oxidase, is reported to be higher than that of heart muscle.

Changes in cellular composition of the brown fat with age develop mainly through the displacement of the multilocular by unilocular white fat. These transitions are affected by the nutritional and environmental conditions; e.g., in cold exposure wherein the multilocular cells tend to remain or perhaps continue to differentiate de novo from reticuloendothelial precursors. Whether white fat cells arise from differentiation of a stem cell common to both brown and white fat cells is undecided. Notably, no mitotic events have yet been visualized in "mature" brown fat cells.

Somewhat anomalous are the brown fat areas of the neonatal hamster in which the Smalleys have noted only unilocular cells at birth; this form, however, is subsequently displaced by multilocular cells within a few days postpartum.

Age-dependent changes occur mainly in composition of cell populations, wherein unilocular cells become dominant. However, the continuing capacities for reestablishment of the multilocular population in aged nonhibernators, e.g., Swiss mice and Peromyscus, have been observed in unpublished studies in our laboratory. These data support the argument that the level of brown fat relative to body mass may be a species-specific ratio that does not exceed a given maximum irrespective of the contour of the cold challenge.
Observed during cold exposure are compositional changes associated with hyperplasia entailing increases in the total nitrogen and phospholipid content of the brown fat. This has been attributed to the attending rise in the cumulative mitochondrial frequency, introduced in the brown fat cells both by their concurrent displacement of the unilocular cell population as well as the apparent differential increase of mitochondrial material per brown fat cell. These cold-induced changes thus additionally involve net enhancement of the electron carriers, including especially the cytochromes and coenzyme Q. A question seldom entertained in such studies is the extent to which the treatment has simply changed the cell population, a development we have observed in rats and mice within 2 weeks of cold exposure. Hence the "warm-adapted" animal is, in respect to brown fat, not a true control to the cold-treated animal, since in the latter (e.g., in rats) the cell population has shifted during cold exposure from one of predominantly unilocular cells into one almost purely multilocular.

In varying degrees, a similar compositional problem is introduced in the seasonal cycles of brown adipose tissue, which has been repeatedly studied in diverse species of hibernators for more than a century. The cyclic changes in mass of the brown adipose tissue are fairly similar among the hibernating species observed in this respect. In general, the maximal mass is reached just prior to the entrance into hibernation, and it gradually decreases during hibernation; as the cessation of hibernation in spring signals the annual mating season, the brown fat further recedes so that not until early summer does the nutritional reestablishment begin, with a marked storage period developing usually in late August and September. Notably the major storage fat is in retroperitoneal, omental, and other typical depots of white fat, all of which undergo maximal enlargement and virtually fill the abdominal cavity. The pattern of fat storage among diverse hibernators varies in relation to the presence and duration of subcycles of dormancy and the activities during the transient arousals. Among the cytological changes associated with brown fat during hibernation are decreases in the size of the brown fat cells, which in the hedgehog have been reduced by spring from 40 to some 20 µ in diameter. However, assuming the vascular bed remains the same, the smaller cells acquire an increased ratio of capillary surface to cell surface, which materially improves the transfer coefficients for metabolic exchanges.

One of the more classical characteristics of brown adipose tissue, reported in some detail by Hammar in 1895 and since confirmed by a number of studies, is its relative immunity from inanition; this tissue maintains its mass at the expense of unilocular depot fat. In similar studies Cramer observed that in prolonged starvation the exitus was reached when the brown fat finally had been irreversibly reduced to a reddish mass of vascular residua.

In response to chronic exposure to cold, rats initiate in the brown fat a syndrome of dissolution of the unilocular component and induction of cytogenesis de novo that, within 96–192 hr, results in a hyperplastic increase of about 1.5 times in tissue mass. During this process the nitrogen of the tissue rises by about the same magnitude as the total mass; this is probably attributable primarily to the mitochondrial fraction, which also rises in concert with the enhanced \( Q_{10} \) of the tissue.
Total metabolism of the brown fat is thus increased by the factors of mass and respiration to more than fivefold; the vascularity also increases, attended by a comparable rise in blood flow.

Notably, exposure of the animal to a hyperthermic environment is conducive to essentially the reverse of the response of brown fat to cold. Thus heat induces reduction in the vascularity, a loss of protein, and a diminished total mass of the tissue.

Functionally, brown adipose tissue as a primary thermogenic effector organ has been most extensively studied in terms of its role during: 1) cold-induced arousal of the hibernating mammal; 2) acute cold exposure of both warm- and cold-adapted adult mammals of various species; and 3) in cold-stressed neonatal mammals, including newborn human infants.

Arousal of the hibernator is a physiological syndrome that may be induced through any of several sensory modalities, including either those of heat or deep cold. Essential to the full development of the arousal are the midbrain and inferior levels, including in the marmot the spinal segments to or below C₄, and in some species, probably to T₁. Aside from the spinal innervation to the respiratory musculature, segments C₄₋₅ provide for activation of the brown adipose tissue in conjunction with the sympathetic innervations via the middle and inferior/thoracic (stellate) ganglia.

As a thermogenic effector organ, the brown adipose tissue, tends to vie with the heart, exchanging heat with the thoracocervical segments of the spinal cord and, via the carotid and vertebral arteries, probably drives the brain through the lag phase of its warming. During this phase, the active rise of temperature in the cervicothoracic and cephalic regions precedes that of the colonic temperature; however, as Dubois showed in 1896 in marmots, the temperature of the liver rises at a rate comparable to that of the thoracic and cephalic (brain and oral) structures, while the axillary region rises most rapidly.

Certainly a sardonic prank of nature was perpetrated on Raphael Dubois, who, having excised the interscapular “glande hivernal” from the hibernating marmot, found no great delay in time of arousal. Moreover, on occlusion of the subclavian flow of the hibernating marmot, he found the animal unable to arouse; his conclusion was reasonable, viz., metabolic heat was generated by heart, liver, and respiratory musculature. As oxidation of fat in situ was not then in vogue, no specific function was given to fatty tissue; additionally, the interscapular area is not a major site of brown fat in the marmot, approximately 50% of the total brown fat being found in the axillae.

A more recent and successful effort at excising the brown fat has been made on the hibernating bat, with the result that time for arousal has been significantly prolonged. Conversely, curarized hibernating bats are reported to be capable of arousal despite the loss of the usual heat from motor activity. Here it appears that possibly a near equilibrium of energetic potential exists, i.e., one by brown fat and visceral metabolism, the other by visceral and muscular activity, neither being without sympathetic metabolic thermogenic effects on viscera, muscle, and white fat depots. It is of interest also that the bat, during arousal, can generate con-
sistenty a higher temperature in the brown adipose tissue than appears in the heart. With squirrels this order of organ temperatures appears to depend somewhat on the conditions and time of arousal.

Relating to the thermogenesis of arousal, various estimates have been made of the percentage of the total heat produced during arousal that can be assigned to the brown adipose tissue. Several estimates have compared the metabolic potential of the brown fat with the theoretical caloric requirement for raising the temperature of the cold animal approximately 30°C. These values of 50–60% represent maximum estimates, however, since body heat losses were ignored. On the other hand, minimum estimates of the thermal contribution of brown fat (obtained by comparing the rate of oxygen consumption of the tissue in vitro with that measured for the arousing animal) range from 10–15% initially to 5–7% as the cold-induced arousal nears completion. Thus, although the data essential for accurate quantitation of the thermogenic contribution of the brown fat during arousal are not yet available, present information suggests that in the arousing hibernator, this tissue accounts for at least 10–15% and possibly as much as 40–60% of the total energy expenditure.

The evidence for brown fat thermogenesis in adult nonhibernators derives primarily from thermometric recordings in the guinea pig and rat. These data indicate that in the cold-stressed animal: 1) the brown fat temperature rises in concert with the oxygen consumption of the animal, but the temperatures of the colon, liver, and brain lag behind; and 2) the temperature of the venous blood draining the brown fat also rises such that the A-V differences may be as great as 1°C.

Additionally, although the brown fat mass is only 1% of the body weight of cold-acclimated rats, thermogenesis of this tissue has been estimated at about 8% of the total metabolism manifested during acute cold exposure. Moreover, since this total metabolism included shivering as well as nonshivering components, the proportion of brown fat metabolism to nonshivering thermogenesis becomes even greater.

The proportional thermal contribution of brown fat is significantly greater in neonates than in adults. In newborn rabbits the brown adipose tissue amounts initially to 6–8% of the body mass and under conditions of a mildly cooling environment can be made to account for some 73% of the total respiratory cost. Although this brown fat initially represents a major site of heat production, it soon gives way to increasing dependence on the shivering mechanism.

Brown fat relative to other tissues is of small mass, but conversely, when activated, it develops higher heat content by its intrinsic metabolic power and the corresponding rise in temperature. Thus calories are available to all other tissues of lower temperature; however, not all receive this heat because the flux is carried rather exclusively to specific sites by vascular convection. The critical point is that these sites are themselves of small mass and of shapes, presumably befitting their specialized functions.

Among such structures are the spinal cord and paravertebral ganglia, the adrenal bodies, the axillae, the base of the heart, and the aortic arch. Thus, in
the acutely cold-exposed animal the central core is protected partially by virtue of some closely applied heating by brown fat, which, amounting to 5% or less of the total body weight, is serving to transport heat by specific vascular convection.

Warming of the spinal cord of newborn guinea pigs at C5-T1 tends to suppress shivering; the afferent pathways are apparently somewhere near the ventral spinothalamic tracts. This establishes a new thermosensor station and suggests a negative feedback control loop. Further, it appears likely that by this mechanism, the suppression of shivering may occur during the early phases of arousal of hibernators. Moreover, in cold-acclimated rats the same reflex would tend to synchronize the time of cold-induced hyperplasia of brown fat with that of the onset of nonshivering thermogenesis.

Vascular heat exchange, recognized as a primary heat transport mechanism, has been similarly examined in terms of transmural conductance, which in A-V countercurrent exchange may effect extraordinary thermal economies by negative feedback. Metabolically active tissues served by paired A-V vasculature are suggested to exhibit a tendency toward heat accumulation by a thermomultiplier effect. This is illustrated by the interscapular brown fat pad, where vasmotor controls are available together with a venous shunt capable of discharging warm blood into the inner vertebral sinuses of the spinal cord and the thoracic structures. In this relation the spinal cord is in series-parallel with the venous flow from interscapular-cervical brown fat to the large thoracic veins. This series relationship takes full advantage of the cyclic thoracic pressure in effecting ebb and flow both into the cord as well as the more usually considered relationship to the abdominal vasculature.

The high relative mass of brown fat in human newborns and young children suggests that at these ages, the thermogenic instabilities related directly to pathogenesis (with such symptoms as febrile spikes) may involve stimulation of brown adipose tissue in diverse ways.

As demonstrated by ultrastructural studies, brown adipose tissue is well suited as a thermogenic source by virtue of its high mitochondrial density. Moreover, this high metabolic potential is further enhanced by chronic cold exposure through induction of hyperplasia and an associated differential elevation of mitochondrial material.

The physiological mediator by which brown fat metabolism is stimulated is most probably norepinephrine released through the sympathetic innervation to the tissue. Studies to date indicate that NE so acts by stimulating production of 3',5'-AMP (via adenyl cyclase), which in turn activates a lipase catalyzing triglyceride hydrolysis. Both the released fatty acids and glycerol (after its conversion to α-glycerophosphate) can be readily oxidized in vitro by the brown fat, and it appears that either or both may serve as the fuel during NE stimulation of brown fat metabolism.

The biochemical mechanism by which NE can induce a sustained elevation of the rate of brown fat oxygen consumption is not yet completely understood. It does appear, however, that this effect cannot be explained by such "ATPases" as the triglyceride reesterification or fatty acid synthesis-oxidation cycles. Addi-
tionally, although initial studies suggested that brown fat mitochondria might be incapable of oxidative phosphorylation, it is now apparent that these particulates do have an electron transport-coupled oxidative phosphorylating system and, further, that this system is functional in vivo at least under conditions where the brown fat metabolism is not stimulated. However, it is not clear whether, as has been proposed, NE indirectly uncouples brown fat mitochondria by elevating the intracellular levels of fatty acids. As an alternative to this possibility, it has been suggested that the NE-induced stimulation of brown fat heat production may be explained by increased substrate availability and that the high metabolic rate may be sustained by a concomitant elevation of ATP utilization necessary to satisfy the needs of the actively respiring cells. Unfortunately, at the present time the mechanism by which NE acts physiologically is not yet well defined.

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