Thermogenesis of brown adipose tissue in cold-acclimated rats

SMITH, Robert E., and Jane C. Roberts. Thermogenesis of brown adipose tissue in cold-acclimated rats. Am. J. Physiol. 206(1): 143-148. 1964.—Multilocular brown adipose tissue in the rat is shown to increase in both mass and respiratory rate, in vitro, during cold acclimation. By vascular convection the resulting heat is directly applied to the thoracocervical regions of the spinal cord, the heart, and other thoracic organs. The vasculature is so arranged as to exercise a fine order of thermogenic control over the brown fat and temperature of the peripheral venous returns to the thorax, facilitated in part by a “reverse” type of countercurrent heat exchange apparently not previously described.

Evidence that brown adipose tissue is an extremely versatile, metabolically active tissue has been emphasized in recent reviews (9, 17, 25). In one of these (25) particular attention was drawn to the thermogenic potentialities of this tissue as suggested from its extreme sensitivity to environmental cold. These responses involve early transient changes in the sudanophilic character of the cells and a subsequent hypertrophy of the tissue at the expense of associated white fat stores (10-13, 21). Metabolic activities of this tissue associated with the cold response reflect influences of both neural and neurohumoral agents as well as those of other endocrine target effects (9).

Brown adipose tissue is richly vascularized, and its multilocular, highly granulated cells are replete with the full compliment of enzymes and cofactors essential to effective operation of lipid and glycogen synthesis, pentose shunt, both aerobic and anaerobic glycolysis, and also oxidative phosphorylation via the Krebs cycle and the electron transport system (see ref. 25). In the interscapular gland of the normal rat the molar quantity of ubiquinone 45 has been found to exceed the apparent cytochrome c by some 6 to 13 times (8). The abundance of cytochrome c in this tissue was established by Hook and Barron (7) together with evidence of a high respiratory activity of the slices surviving in vitro either in the presence or the absence of added substrate. Significant in relation to thermogenic properties was the additional finding that Qo, of the brown fat remained relatively high at a temperature (8 C) low enough to abolish respiration of homologous kidney slices.

On the basis of this background, it was anticipated, therefore, that metabolic heat from brown adipose tissue would be increased in response to cold, and experiments described herein were designed accordingly. In this study, however, the mere demonstration of increased thermogenesis from brown fat in response to cold (19) did not become impressive until it was recognized that the relatively small amount of heat derived from this source was in fact being applied selectively to the vascular supply of the thoracocervical spinal cord, the heart,
and related thoracic structures (20). This was shown by studies employing intravascular injections of polyvinyl acetate with results as described subsequently in this paper. Hence the present report will deal with a) thermogenesis of brown fat, b) vascular transport of the heat so evolved, and finally, c) some ideas will be introduced which appear logically to derive from the topologic relations of the brown fat to the vascular anatomy to provide therewith a sensitive mechanism of control over local thermogenesis of this tissue in vivo.

METHODS

Adult, male rats of the Long-Evans strain were maintained in individual cages under constant conditions of lighting (12-hr light cycle, with 15-w night light), temperature, and diet (food, White Diet, Simonsen Laboratories, Gilroy, Calif., and water ad libitum). At initial weights of 180–225 g the animals were divided into control and experimental groups, to be maintained respectively at 26 ± 1°C and 6 ± 1°C and used after 40–90 days at these temperatures. All animals were weighed weekly and immediately prior to sacrificing; the latter was always performed between 0830–0900 hr in the room of residence by means of a high cervical blow followed by decapitation.

Brown fat was removed from the following areas and placed immediately into beakers of iced 0.25 M sucrose: i) interscapular region, 2) superior cervical and axillary regions, 3) middorsal intrathoracic region, and 4) retroperitoneal, mediodorsal renal region. After initial practice trials it became possible routinely to remove essentially all of the brown fat from the sites indicated; not removed were two small nodules usually occurring bilaterally, high in the thoracic region near the exit of the large arteries. Also remaining was a small amount of brown fat over the arch of the aorta and the base of the pulmonary vessels. Posteriorly, trace amounts were left at the bifurcation of the abdominal aorta. Thus the total weights as reported should account for about 95% of the brown fat actually present in vivo. All tissues were blotted on filter paper and rapidly weighed prior to the preparation of slices or homogenates for metabolic studies. Measurements of oxygen consumption were made by the direct Warburg method. Nitrogen content was determined by a standard micro Kjeldahl method.

For respiratory measurements on cervical gland slice, 0.2–0.3 g of tissue were used/5 ml Warburg flask to give a nitrogen content of about 1.8 mg Kjeldahl nitrogen for control tissues and 3.8 mg N for the tissues from the acclimated animals. The reaction medium in the flask contained: 25 μM tris buffer, pH 7.4; 15 μM PO4 as KH2PO4 buffer, pH 7.4; 5 μM MgCl2; 2 μM disodium ATP; 10 μM KCl; 37.5 μM glucose; and sufficient 0.25 M sucrose to give a final volume of 1 ml. The center wells were charged with 0.05 ml 10% KOH and 1 cm2 corrugated filter paper inserted. All flasks were flushed with pure oxygen for exactly 6 min followed by 6 min equilibration at 37°C before the first reading was made; measurements were taken for 20–30 min.

For QO2 measurements of slices from the interscapular gland, 30–70 mg wet tissue were used per flask, giving a Kjeldahl nitrogen equivalent of 0.2–0.9 mg N/flask for the controls and 0.6–1.7 mg N/flask for tissues from the cold-adapted animals. The medium and other conditions were the same as for the respiratory measurements except that the KCl concentration was 30 μM and 10 μM EDTA was added. The Kryptofix was replaced by 10 mM MnCl2.

TABLE 1. Changes in relative size and composition of brown adipose tissue from various loci in normal and cold-acclimated rats

<table>
<thead>
<tr>
<th>Tissue Loci</th>
<th>Body Wt. g</th>
<th>Gland Wt. g</th>
<th>mg N/g Gland</th>
<th>mg N/g Body Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Interscapular</td>
<td>436</td>
<td>0.726 0.07</td>
<td>6.92</td>
<td>4.72</td>
</tr>
<tr>
<td>Cervical-axillary</td>
<td>451</td>
<td>0.944 0.04</td>
<td>7.93</td>
<td>5.51</td>
</tr>
<tr>
<td>Thorax</td>
<td>458</td>
<td>0.488 0.03</td>
<td>7.83</td>
<td>3.48</td>
</tr>
<tr>
<td>Renal</td>
<td>458</td>
<td>0.805 0.06</td>
<td>6.53</td>
<td>5.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>a'</th>
<th>b'</th>
<th>c'</th>
<th>d'</th>
<th>e'</th>
<th>n'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interscapular</td>
<td>361</td>
<td>1.140 0.07</td>
<td>15.44</td>
<td>17.60</td>
<td>3.10</td>
<td>14</td>
</tr>
<tr>
<td>Cervical-axillary</td>
<td>370</td>
<td>1.120 0.05</td>
<td>12.94</td>
<td>14.31</td>
<td>3.05</td>
<td>7</td>
</tr>
<tr>
<td>Thorax</td>
<td>372</td>
<td>0.652 0.02</td>
<td>19.85</td>
<td>9.26</td>
<td>1.84</td>
<td>8</td>
</tr>
<tr>
<td>Renal</td>
<td>376</td>
<td>0.975 0.07</td>
<td>14.10</td>
<td>14.47</td>
<td>7.77</td>
<td>9</td>
</tr>
</tbody>
</table>

\( P < 0.001 \) for \( (e'-e'), (d'-d), (a'-a) \); \( P < 0.05 \) for renal \( (a', c') \).

* Standard error of the mean.

**FIG. 1.** Plot of interscapular gland weight versus body weight for control and cold-acclimated rats. Regression line obtained by method of least squares. At 400 g, cold versus control, \( t = 6.333, P < 0.001. \)
BROWN FAT: THERMOGENESIS IN COLD

**Fig. 2.** Plot of total mass of brown fat obtained versus body weight for control and cold-acclimated rats. Regression line obtained by method of least squares. At 400 g, cold versus control, t = 3.885, P < 0.01.

To prepare homogenates of the brown fat from all four regions, the weighed tissue was first chopped with scissors and added to 4-20 volumes of iced 0.25 M sucrose. This was homogenized by 30 complete strokes in a Teflon-glass homogenizer with the pestle driven at 750 rev/mm. The resulting homogenate was filtered through a single layer of cheese cloth. In order to evaluate cold-induced changes in respiration of these homogenates, it was necessary a priori to adjust the amount of nitrogen per flask as nearly as possible to a common level since the apparent $Q_{O_2}$ (N) tended to increase with the amount of N per flask. The suspension contained 0.6-2.0 mg Kjeldahl nitrogen/ml, and for $Q_{O_2}$ measurements 0.5 ml of this was added to each 5-ml Warburg flask along with 0.5 ml of a reaction medium containing: 10 μM α-ketoglutarate; 2 μM disodium ATP; 5 μM MgCl₂; 10 μM KF; 25 μM tris buffer, pH 7.4; 15 μM PO₄ as KH₂PO₄ buffer, pH 7.4; 25 μM glucose, and 25 KU units of hexokinase; KOH was placed in the center wells as before. Subsequent treatment and measurements were the same as those used for slice.

Details of the regional vascular supplies to the respective areas of the brown fat were studied both by dissection of latex-injected animals and by direct observation of polyvinyl acetate casts of the circulatory system. The latter were prepared by injection of blue and red polymer intravascularly into a series of freshly anaesthetized rats and other rodents and a subsequent ablation of the tissues with caustic potash (KOH) solution.

**RESULTS**

**Metabolic heat production.** During cold acclimation the total mass of brown fat in the rat increases absolutely to about 1.25 times that found in the control rat (Table 1). However, per gram body weight this increase is approximately 1.6 times that of the controls, due to the smaller body weights of the cold-exposed animals. While the latter changes are highly significant by the t test, a clearer illustration of them is provided from the regressions of tissue mass versus body weight for the respective sites involved (Fig. 1); these show, in all but the thoracic site, parallel slopes with ordinates strongly displaced upward in the cold-exposed group. However, in such plots the disparity in both tissue and body weights between the cold-exposed and control animals of similar ages is greatly emphasized, as shown clearly by the regressions of aggregate brown fat recovered versus body mass (Fig. 2). As there is essentially no overlap of the variates along either coordinate, it was judged best simply to test statistically the difference in total brown fat as the difference between the regression lines at an arbitrary midpoint of 400 g body weight. As this difference was evidently significant ($P < 0.01$), the conclusions stated above were accepted.

There is also a significant ($P < 0.001$) increase in the Kjeldahl nitrogen per gram of brown adipose tissue (Table 1, c) indicating a change in composition during acclimation in the direction of an increased concentration of metabolically active, nitrogen-containing components of the cells. Further evidence of cellular changes has been obtained by means of histological sections and radioautographs made during the period of cold acclimation (see 21; Cameron and Smith, in preparation).

Cold-induced changes also occur in oxygen consumption of slice (Table 2) and homogenates (Table 3) of brown fat from each of the respective sites. Computed on the basis of nitrogen content, the $Q_{O_2}$ of interscapular tissue slice is not altered by the cold treatment; whereas taken per milligram wet weight, the increase in $Q_{O_2}$ approaches significance. On the other hand, brown fat slices from the pooled cervical and axillary sites of the cold group show a significant increase in $Q_{O_2}$ (N) over that of the controls, and based on estimates from Table 1 this difference would also obtain on a wet weight basis. Significantly here, these latter sites contain very little white adipose tissue, unlike the interscapular pads which in normal rats are usually quite fat.

**Table 2.** $O_2$ consumption in vitro of brown adipose tissue slice from various loci in normal and cold-acclimated rats

<table>
<thead>
<tr>
<th>Loci</th>
<th>Interscapular</th>
<th>Cervical and Axillary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Q_{O_2}$, mg N</td>
<td>$Q_{O_2}$, mg tissue</td>
</tr>
<tr>
<td>Control, 26 C</td>
<td>59.8 ± 0.42</td>
<td>25.8 ± 0.14</td>
</tr>
<tr>
<td>Cold, 6 C</td>
<td>66.8 ± 0.94</td>
<td>47.9 ± 0.62</td>
</tr>
</tbody>
</table>

* Estimated from gland composition data Table 1.  † Standard error of mean.
masses (g) of brown adipose tissues from the various adipose tissue locations in normal and cold-acclimated rats.

TABLE 3. Oxygen utilization of homogenates in vitro and mass (g) of brown adipose tissue from normal and cold-acclimated rats

<table>
<thead>
<tr>
<th>Tissue Loci</th>
<th>$Q_{O_2}$ (N)</th>
<th>Tissue Wt. (Wet)</th>
<th>$Q_{O_2}$ (Wet)</th>
<th>Total $Q_{O_2}$ (Wet) c</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 30°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interscapular</td>
<td>(145) †</td>
<td>0.728</td>
<td>1,097</td>
<td>1,825</td>
<td>15/15</td>
</tr>
<tr>
<td>Cervical-axillary</td>
<td>(118) †</td>
<td>0.994</td>
<td>666</td>
<td>672</td>
<td>8/7</td>
</tr>
<tr>
<td>Thorax</td>
<td>(303)</td>
<td>0.485</td>
<td>3,396</td>
<td>4,081</td>
<td>10/9</td>
</tr>
<tr>
<td>Interrenal</td>
<td>(121)</td>
<td>0.805</td>
<td>924</td>
<td>827</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Cold Acclimated, 6°C

<table>
<thead>
<tr>
<th>Tissue Loci</th>
<th>$Q_{O_2}$ ′ (N)</th>
<th>Tissue Wt. (Wet)</th>
<th>$Q_{O_2}$ ′ (Wet)</th>
<th>Total $Q_{O_2}$ ′ (Wet) d</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interscapular</td>
<td>(277)</td>
<td>1.140</td>
<td>4,688</td>
<td>4,965</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cervical-axillary</td>
<td>(167)</td>
<td>1.120</td>
<td>2,990</td>
<td>3,111</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Thorax</td>
<td>(444)</td>
<td>0.950</td>
<td>6,431</td>
<td>7,380</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interrenal</td>
<td>(249)</td>
<td>0.970</td>
<td>3,981</td>
<td>4,171</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Approx. 60 days at 6°C. † $\mu$L $O_2$/mg N hr; $Q_{O_2}$ (wet) expressed as $\mu$L $O_2$/g wet wt. hr. ‡ Standard error of the mean.

Homogenates from all brown fat loci show marked increases in $Q_{O_2}$ (N) and $Q_{O_2}$ (wet) in cold-acclimated animals (Table 3); this, coupled with the increase in tissue mass, gives a total oxygen consumption of brown fat of 3-6 times that in the control animals. From these data it may be concluded that the brown fat increases its total heat production in response to cold exposure. For an estimate of the amount of this heat, the observed (in vitro) oxygen consumption of the interscapular pad was converted into calories (Table 4); and as the indicated values rise from 3.4 to 24 cal/hr, a heat level of at least 7 times the control value could evidently be evoked by this pad in response to cold.

To test by approximation our theory (vide infra) that this tissue serves as a thermogenic vascular heater, the above values were applied to some data on blood flow (Hannon, personal communication) through the interscapular gland of the cold-acclimated rat, whereupon it appears (Table 4) that the temperature of blood emerging from this tissue could be metabolically increased by as much as 0.6°C before it reaches the thorax.

Vascular relationships affecting heat transfer. Considered a priori, the absolutely small amount of heat produced by brown fat tissue could be of no intrinsic significance relative to total body heat production unless this heat were in some way brought to bear upon a relatively isolated heat sink of small size and perhaps of high sensitivity to temperature. Of organs vital to survival in cold, the thoracic and cervical regions of the body might be satisfied by direct conductance or vascular convection of heat from such generative loci. Also, given the knowledge of a possible thermogenic role for brown adipose tissue, we were able to attach functional significance to: a) some suggestions earlier made by Polimanti (14), namely that the brown fat constituted an insulative overlay within the thoracic cavity and b) Dubois' (3) finding that the hibernating marmot, upon ligation of its subclavian vessels, was rendered incapable of arousal. Likewise, the finding by Sulzer in 1774 (26) of a direct venous connection between interscapular brown fat and the azygous vein could be readily adduced as indicative of a direct convective heat transfer to the heart.

Thus a further study of the topologic relationships and the vascular connections of brown fat with vital structures was undertaken by use of the latex and polyvinyl resins. As applied to the rat these preparations clearly demonstrate the circulatory supply to the cervical and interscapular brown fat. Thus from each, a well-defined venous return proceeds from the brown fat tissue into the inner vertebral sinus at levels of spinal segment T8 and above (Fig. 3). Additionally, the interscapular pad, after giving off its superior rami to the spinal segments between T6 and T8, drops abruptly by a large vein through the left transverse vertebral process of T1 and join the azygous vein at a point some 5 mm from its confluence with the left precaval vein. This vein evidently corresponds to the 4th thoracic vein (5), though we have in some places referred to it as "Sulzer's vein," after its discoverer. Veins from the smaller deep mid-dorsal pair of pads empty bilaterally into the inner vertebral sinuses cephalad at C6 or C7 and caudad at C6 or C7; the latter point corresponds approximately with the origins of the vertebral vein. Bilaterally from the interscapular brown fat lobes, as these extend under the posterior margin of the scapulae, venous returns forming the thoracodorsal vein join the axillary and hence the brachial and subclavian veins. This course is largely imbedded in brown fat.

As illustrated in Fig. 4 the arterial supplies to the cervical and interscapular pads are bilateral and each lies in close apposition to the corresponding veins; the unpaired central venous returns from these pads, however, are not associated with corresponding arteries. Further, the bilateral arterial supply to the interscapular pad continues through this tissue and extends forward...
FIG. 3. Drawing (lateral view) from polyvinyl replicas of adult rat circulatory system showing principal features of vascular drainage from brown fat pads of interscapular and dorsal cervical regions.

into the region of the paired dorsal cervical pads; this latter extension, however, is evidently unaccompanied by a corresponding vein.

DISCUSSION

The significance of this vascular geometry appears most extraordinary when viewed in terms of the thermogenic role assigned to the brown fat. If one accepts the latter, then it follows that brown fat is returning metabolically warmed blood to the thorax via its venous drainage. One immediate consequence of this is to bathe en route the thoracic and cervical spinal cord with warmed blood and secondly to supply heat directly to the heart. Likewise, through the overlay of brown fat along the middorsal line and intercostal margins, the respective venous returns, including azygous, are also contributing blood which in a cold external environment is at least conductively warmed to a temperature above that which would otherwise attend the venous returns from most of the thoracic wall. The sympathetic chain, almost completely covered by brown fat, is also subject to such heating. This principle of heating the cool peripheral blood by passage through “metabolic warming blankets” is also applicable to the confluences at the axillary as well as the posterior venous returns and clearly would serve in thermal protection of the central body core against the peripheral cooling of a cold environment.

A second principle also derives from the combination of vascular geometry and a peripheral thermogenic source, as described above for the interscapular and cervical brown fat pads. One may note in these a dual venous return system wherein artery and vein are closely juxtaposed while passing largely through an overlay of brown fat. Applying now the principle of countercurrent heat exchange (reversed as it were from that adduced by Scholander (16) for the peripheral appendages), it is obvious that temperature in these paired vessels will tend to be higher in venous than in the arterial blood, and thus heat will flow from the efferent venous blood to the afferent arterial stream. In consequence the tissue will tend to become still warmer; and the more it is
warmed, the higher will be the rise in metabolism and likewise the intrinsic heat production. By means of the central venous drainage a shunting mechanism is available which keeps this system from going “critical” and utilizes the body core as a heat sink, thus providing natural control over the feedback system without necessarily shutting down the arterial input. Further studies on this circulatory arrangement and its control mechanisms will be reported separately; however, it is quite obvious that this vascular relationship constitutes a primary means by which metabolic heat from sites of brown fat can be maximized and also rapidly modulated.

As a final requirement to functional thermoregulation, the heat producing mechanism must itself be subject to rapid “on-off” control. That the latter is mediated by sympathetic nervous supply is strongly suggested, particularly in the interscapular region, by results of unilateral denervation (see ref. 6.) by which the ipsilateral fat pad is rendered relatively insensitive to nutritional exchange of lipids (1) and also becomes rapidly depleted of its noradrenaline stores (18). By electrical stimulation of an excised, interscapular nerve-fat preparation Correll (2) has been able to induce significant increases in the tissue esterase activity. More directly our own studies (see 21) have shown thermogenesis of the interscapular pad in the intact, conscious rat to be blocked completely by hexamethonium and also by topical action of xylocaine to the bilateral nerve supply.

CONCLUSIONS

The demonstration of increased mass of interscapular brown fat in response to cold here confirms the earlier findings of Page and Babineau (13) and extends these to show that the increase in mass is common to all of the major sites of brown fat and can lead in sum to a near doubling of total tissue mass under the conditions imposed. Concurrent rises in oxygen consumption of the brown fat, in vitro, bring the total heat production (mass X QO₂) from this tissue to the order of seven times that of control values. As applied to the interscapular brown fat this heat is enough to raise the temperature of its venous drainage by perhaps 1°C or more. The vascular topology favors convection transfer of this heat to cervical cord and thorax where functional integrity is critical to the cold defenses of the homeothermic animal. Elsewhere brown fat overlies the peripheral vascular routes into the thorax in such a way as to provide direct thermal jacketing of all major returns from areas normally subject to environmental surface cooling. This local heating could affect both neuronal and vascular function, in view of the anatomical coincidence of their structural ramifications.

A special attribute of the vascular relationship to a thermal source at or near the periphery derives from application of the principle of countercurrent heat exchange, but in this case to a system in which the venous outflow from the area heats the arterial input and the attending rise in heat production is literally bled off via a lone central vein directly to heat sinks comprised of structures essential to survival in cold. This is believed to be a new finding of potentially general importance to thermoregulation in the mammal and possibly elsewhere as well.

Evidence adduced both from the literature and from unpublished work in this laboratory strongly suggests that the on-off effector system for the thermogenesis of brown fat is mediated by the sympathetic nervous system.

For preparing the circulatory replicas noted under Methods the authors are pleased to acknowledge the technical contributions of Irene Rask; they are also indebted to Dr. R. J. Hock of the University of California White Mountain Research Station for making available captive specimens of a number of wild species of rodents for this study. The injected materials were obtained from Ward’s of California.

REFERENCES