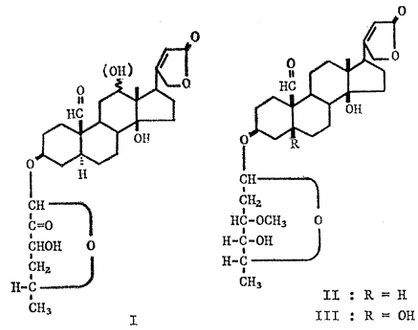

Fig. 2. Calotropin.

chromatography on silicic acid; the compound crystallized from methanol-ether as rosettes (*F*, 50 mg); melting point (mp), 195° to 202°C. The mother liquors from the crystallization of *F* were combined and chromatographed on silica gel plates with 13 percent methanol in chloroform. The band corresponding to calotropin was removed, and the remainder of the chromatogram was washed with methanol. The extracted material was combined with all the chromatographic fractions not containing the compound of R_F 0.62, to make up fraction *G* (26 g).

Fraction *F* was recrystallized from acetone to yield colorless crystals which melted at 203° to 205°C; they showed a specific rotation ($[\alpha]_D^{33}$) of +63° in methanol solution, and ultraviolet absorption maxima at 216 mμ (ε 18,100) and 310 mμ (ε 40) in ethanol solution. The literature records the following physical constants for calotropin: mp 234° to 240°C, $[\alpha]_D^{25}$ +64° (methanol), $\lambda_{max}^{ethanol}$ 217 mμ (ε 17,800), 310 mμ (ε 35.5) (*9, 10*). There was no depression of melting point on admixture with authentic calotropin (mp, 198° to 205°C), and the infrared spectra of the respective samples (KBr pellets) could be superimposed. The respective samples showed identical R_F upon thin-layer chromatography on silica gel with 13 percent methanol in chloroform, and upon paper chromatography with chloroform-formamide. Similar fractionation of the extract of leaves of *A. curassavica* from Costa Rica also yielded calotropin.

Calotropin has previously been isolated from *Calotropis procera* R. Br. (Asclepiadaceae) (*11*) and from *Pergularia extensa* (Jacq.) N.E. Br. (Asclepiadaceae) (*10*). In a study of the cardenolides of *A. curassavica* L., Tschesche *et al.* (*12*) found seven aglycones, including calotropagenin, the aglycone of calotropin. Extraction under conditions known to minimize enzymatic hydrolysis yielded the glycoside uzarin (*13*). Calotropin is thus the second glycoside to be isolated from *A. curassavica* L., although no special precautions were taken in the present study to preclude enzymic hydrolysis during the extraction procedure. It is noteworthy that Reichstein *et al.* isolated calotropin from the seeds of *P. extensa* even when the material was extracted under "fermentation" conditions (*10*).

Calotropin was very recently assigned structure I (Fig. 2) by Hassall *et al.* (*14*); it thus bears a remarkable structural similarity to apocannoside (II) and cymarin (III), which have recently been shown to cause the cytotoxic activity of extracts of *Apocynum cannabinum* L. (Apocynaceae) (*15*).

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16. This communication is part V in the series entitled "Tumor Inhibitors"; part IV is (*15*). Work supported by the National Cancer Institute (grant CA-04500-06), the American Cancer Society (T-275), and the CCNSC (contract PH43-64-551). We thank Dr. J. L. Hartwell, CCNSC, for the references on medicinal uses of *Asclepias* species; and Prof. T. Reichstein, University of Basle, for the authentic calotropin.

9 November 1964

Thermoregulatory and Adaptive Behavior of Brown Adipose Tissue

Abstract. *Brown adipose tissue has been shown to be a strongly thermogenic effector organ in homeothermic animals exposed to cold and in hibernators during cold-induced arousal from deep hibernation. Because of the anatomical distribution of brown fat and the utilization of vascular countercurrent heat exchange, this cold-induced thermogenic response protects the animal by contributing heat to the vital organs of the thorax, the cervical and thoracic segments of the spinal cord, and the sympathetic chain. Evidence indicates that control of thermogenic activity of brown fat is mediated by the sympathetic nervous system.*

Brown adipose tissue was first described in 1551 by Conrad Gesner (*1*), who noted its appearance in the European marmot *Muris alpinus*. Since then scholars have further demonstrated that brown fat also occurs in many other species of rodents and, sporadically, in some five other orders of mammals, including man and other primates (*2*). From its initial discovery in the marmot, brown fat has consistently appeared in all true hibernators that have been studied, but its occurrence in many nonhibernators has increased the difficulty in assigning to it a functional role (*3*). Minimal criteria would rest on the finding of some common property by which the brown fat could be shown to be essential to survival of the hibernator and at least useful to the nonhibernator during some critical phase of its life cycle.

My argument that brown adipose tissue is primarily a thermogenic effector organ appears to satisfy this common requirement, and a review of experimental demonstration of this action in both hibernators (*3–6*) and nonhibernators (*7–9*) forms the basis for the present discussion.

The salient factors to be evaluated are: (i) whether the tissue is especially capable of thermogenic activity, that is, displays intrinsic heat-producing potential; (ii) the extent to which it may show adaptive responses to changes in the thermal environment of the animal; (iii) how such heat is brought to bear most favorably in defense of the organism by anatomical location of the tissue and its vascular relations to vital structures of the body core; and (iv) by what means an on-off control of the metabolic heat evolution is achieved.

(i) *Cellular thermogenesis* is a term which denotes the capacity of living cells to produce heat through utilization of energy from various nutrients obtained from the environment. In general, the upper limits are set by the rate at which hydrogen can be oxidized to form water. More specifically, this depends upon the density of the subcellular units which perform these processes. In brown adipose tissue, something of this can be observed merely by examining these cells by phase-contrast microscopy; very striking is the appearance of the fat globules dispersed throughout the cytoplasm, giving the multilocular rather than a unilocular disposition of the stored fats as seen in the white adipose tissue. Electron microscopy of the multilocular cells reveals the fat globules to be closely surrounded by granules containing the oxidative machinery (mitochondria) of the cell (*10*). Surrounding these cells is an extraordinary matrix of capillaries, denoting abundant capacity for transporting materials to and from the cells, as well as presenting a heat-transfer system par excellence. In lieu of any demonstrable work function for these cells, their entire metabolic yield may thus be available for heat evolution. In recent reviews of the biochemical properties of the tissue, Johansson (*3*) and Smith and Hoijer (*11*) have emphasized the great capacities of the cell for anaerobic as well as aerobic respiration, the presence of a well-developed pentose shunt activity, and, in particular, the abundance of the cytochromes (*12*) required in terminal electron transfer mainly associated with ultimate oxidation of hydrogen.

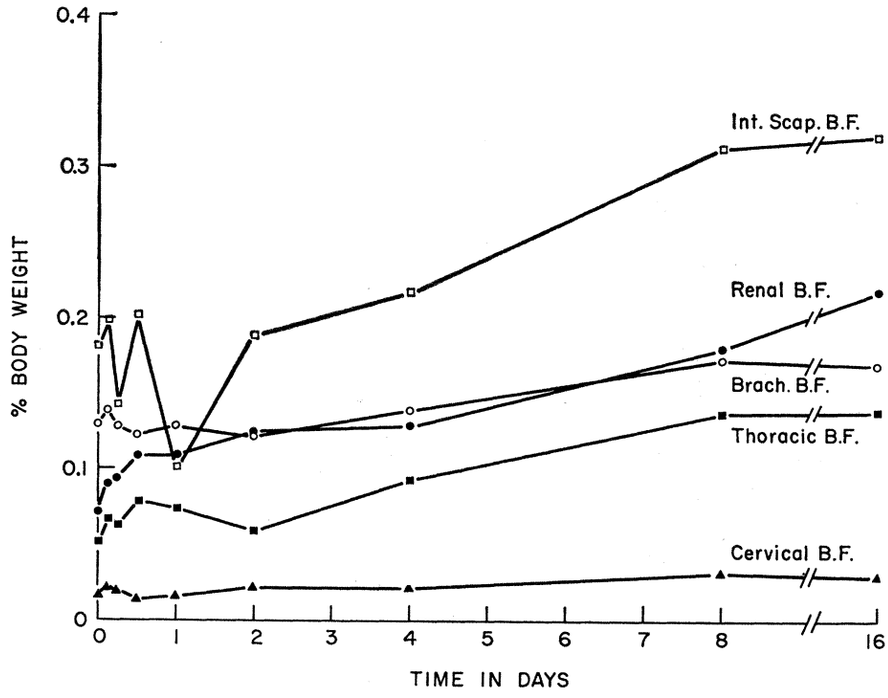


Fig. 1. Changes in masses of brown adipose tissue (B.F.) from rats exposed to 6°C for various times shown in relation to the percentage body weight.

Significantly, the temperature sensitivity of brown adipose tissue is sufficiently low to allow respiration at temperatures lower than that of other organs (*13*). From these and my own related data (*7, 9, 14*), it is concluded that there is, indeed, a high thermogenic potential in brown adipose tissue, which is tapped during periods of cold-stress.

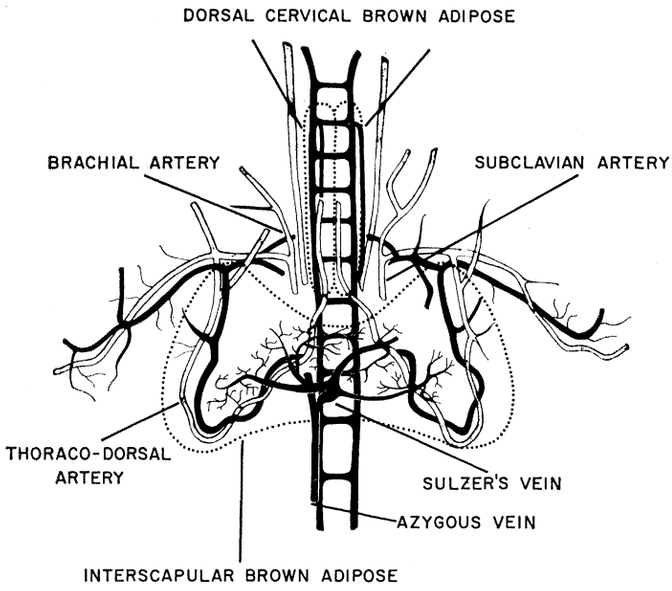
(ii) *Adaptive response to cold.* Exposure of the rat to cold elicits immediate thermogenesis from brown fat regions, which entails rapid utilization of endogenous fat within the cell and general dissolution of the white fat cells normally associated with areas of brown fat cells in the normal, unacclimated animal (*14, 15*). After more prolonged exposure to cold, the brown fat in the rat becomes hypertrophied through hyperplastic development, and oxygen consumption by the tissue also rises, so that increases in total heat evolution rise as the product of these two concurrent changes (Table 1). On the basis of data from experiments in vitro, this rise may approach a factor of six or more over the heat production of the total brown fat of a normal rat. The time dependence of the hyperplastic changes in the respective sites of brown fat during exposure to cold (6°C) are illustrated in Fig. 1, which shows that the most rapid growth of the tissue occurs generally within the time interval 96 to 192 hours. By radioautographic studies with H^3-labeled thymidine, I have shown that this period is preceded at 48 to 96 hours by a peak of DNA synthesis in the reticuloendothelial precursors of the brown fat cells (*15*). No mitotic figures have been seen in the differentiated cells.

(iii) *Topology of brown fat and vascular transfer of heat.* Brown fat (see *2, 3, 16*) occurs in organized patches or pads at several discrete loci and is particularly well displayed in certain hibernators and in such rodents as the rat. Over the interscapular region of the rat is a butterfly-shaped, bilobed body with its lateral extensions passing ventrolaterally beneath the inferior margins of the scapulae, along the route of the thoraco-dorsal vessels, to join at the axilla a further accretion of brown fat over the confluence of the axillary and brachial vasculature. Dorsomedially, between the deep epiaxial muscles and cephalad of spinous process of T_2, appears a bilateral pair of pads which I have designated the superior cervical location. Within the thorax, brown fat overlays partially the thoracic aorta and especially the thoracic veins as these receive the intercostals and the thoracic drainages from the venous complex of the inner vertebral sinuses of the spinal cord. Caudally along the aorta, the brown fat extends to the level of the kidneys where it spreads into a sheet engulfing usually the suprarenal bodies, the renal pedicles, and iliac returns, with variable extensions onto the femo-

Table 1. Oxygen utilization by homogenates in vitro and masses (*8*) of brown adipose tissues from various loci in normal and cold-acclimated (approximately 60 days at 6°C) adult male Long-Evans rats (*9*).

Tissue locus	Control (26°C)				Cold acclimated (6°C)					P
	$q_{O_2}(N)$* (*a*)	Wet wt of tissue (mg) (*b*)	q_{O_2}(wet)† (*c*)	Total O_2 ($b \times c$) (*d*)	No. of animals n/n'	$q_{O_2}(N)$ (*a'*)	Wet wt of tissue (*b'*)	q_{O_2}(wet) (*c'*)	Total O_2 ($b' \times c'$) (*d'*)	($d' - d$)
Interscapular	145 ± 13‡	0.728 ± .07	1037 ± 179	755	15/15	277 ± 13	1.140 ± 0.07	4268 ± 223	4865	0.001
Cervical-axillary	118 ± 23	.994 ± .04	666 ± 145	662	8/7	167 ± 23	1.120 ± .05	2090 ± 262	2341	.01
Thorax	303 ± 37	.485 ± .04	2322 ± 219	1126	10/9	444 ± 27	0.650 ± .06	6431 ± 391	4180	.001
Interrenal	121 ± 32	.895 ± .06	924 ± 211	827	10/10	249 ± 40	.975 ± .07	3091 ± 539	3014	.01

* Microliters of O_2 consumed per milligram of nitrogen per hour (μl O_2 mg^{-1} N hr^{-1}). Nitrogen determined by microkjeldahl method. † μl O_2 mg^{-1} wet wt hr^{-1}. ‡ Mean ± standard error.



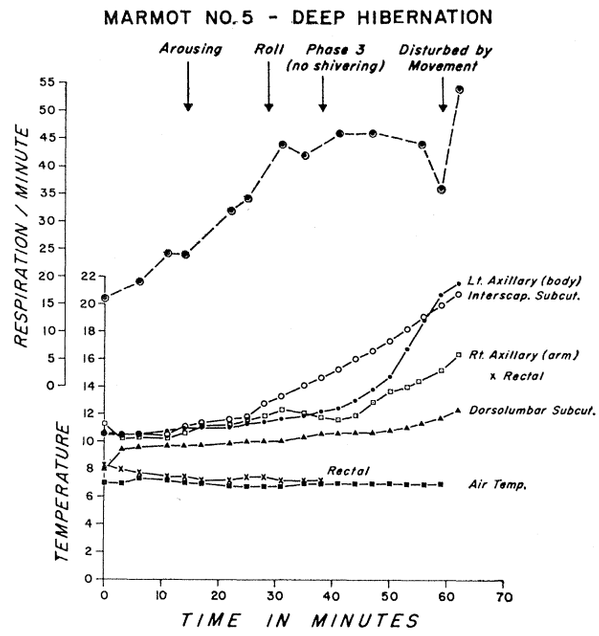


Fig. 2 (left). Dorsal aspect of vascular supply to cervical and interscapular brown fat deposits, as observed in polyvinyl vascular replicas. [Courtesy *Am. J. Physiol.*] Fig. 3 (right). Temperatures *in situ* of axillary and interscapular brown fat locations of a hibernating marmot during unseasonal arousal when the temperature of the air was near that during hibernation (*5*). The temperatures of the brown fat increase before and more rapidly than the rectal and subcutaneous temperatures (°C).

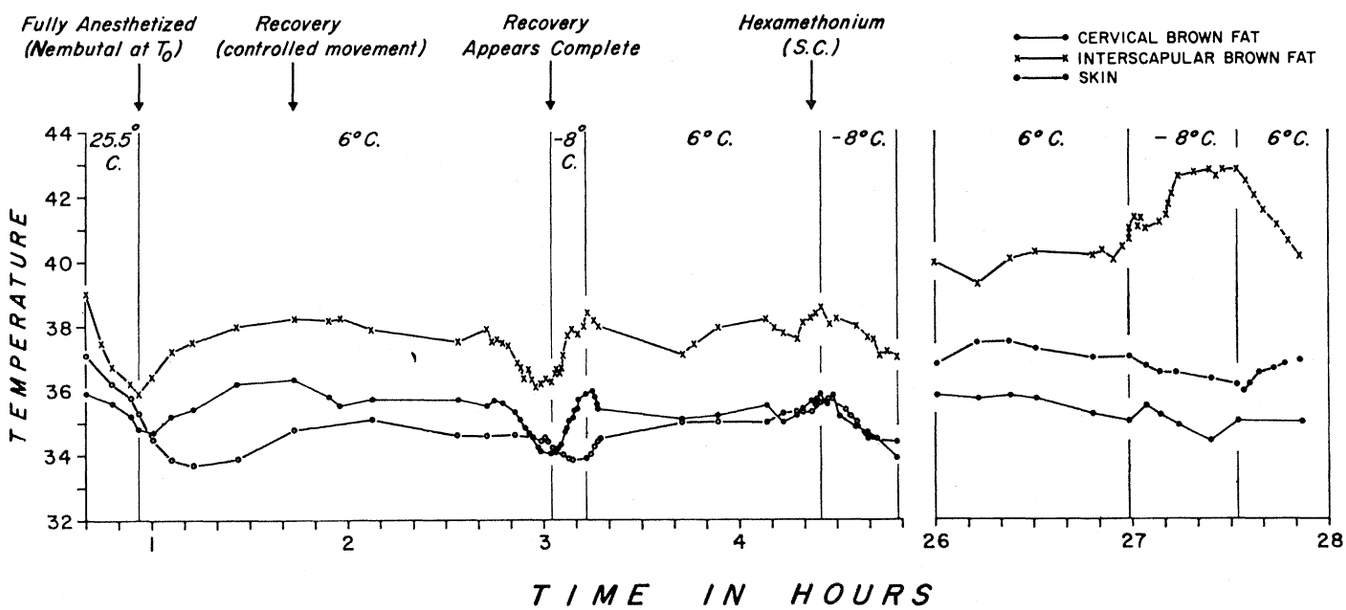


Fig. 4. Stimulation of thermogenesis in vivo of interscapular and cervical brown fat pads by transient exposure of a cold acclimated rat (132 days at 6°C) to a subzero environment (−8°C). Also shown is the inhibitory effect of subcutaneous hexamethonium upon this response. Open circles, subcutaneous skin temperatures.

ral bifurcations of the aorta and inferior vena cava.

The relations of the brown adipose tissue to the course of the vascular supplies have provided important clues to the functional role of this tissue in defending the animal against cold. Clearly there is intimate contact between brown fat deposits and returning venous drainages from the periphery of the animal. Polimanti (*17*) noted this in the marmot as early as 1913, but in lieu of knowledge of the thermogenic potential of brown fat he concluded that this tissue must serve as an insulator against cooling by blood returning from the periphery. In the light of current data, however, one reasonably concludes that in the topological relation of brown fat to these venous returns we have an active thermal jacket ("electric blanket") which can apply its local metabolic heat directly to the blood stream as this returns to the thorax; the vital structures are thus protected from undue cooling. Presumably this arrangement also has a moderating effect upon the countercurrent heat exchange by which the more peripheral arterio-venous channels conserve heat (*18*).

It is fairly obvious in either case that between cooled periphery and thorax there must be null points where arterial and venous temperatures are equal and heat transfer approaches zero. Such points (see *18*) would appear to be at the extreme periphery of the appendage and proximal, near a point where the brown fat begins to overlay the returning venous supply. At the latter point the temperature of the venous return would begin to exceed that of the outgoing arterial supply. The physiological significance of the dual null point could be in providing a means whereby the intensity of the peripheral cooling could be matched by changing the locus of these null points relative both to each other and to the body core. The proximal null point would be shifted outward, the greater the intensity of metabolic heat production by the brown fat; conversely the peripheral null point would shift proximally in response to increased cooling at the periphery. A third null point would also appear to lie somewhere along the pulmonary circulatory loop. While these concepts remain speculative pending experimental demonstration, they provide guide lines for direct investigation of the dynamics of heat exchange between body core and "shell."

Besides these series arrangements between peripheral inputs and brown fat overlays, another very specialized relation exists between the brown fat pads of the interscapular and superior cervical regions and the associated vasculature. As described elsewhere (*8, 9*), these locations are supplied bilaterally by arterial channels which are closely juxtaposed with corresponding venous returns (Fig. 2). Hence countercurrent heat exchange may readily occur along these routes. However, the significant development here is the fact that, because brown fat is potentially highly thermogenic, the venous returns, being higher in temperature than the arteries, will tend to transfer heat to the arterial supply with resulting conservation and even amplification of the heat being produced at these tissue sites. A positive feedback system is thus incipiently present by virtue of both local metabolic heat production and the Arrhenius effect (direct effect of temperature on reaction rates). Without some form of damping, such a system would normally become unstable and lead to rises in temperature which would be self-limiting at the point of tissue damage. However, a most efficient damping device is provided for each of these locations by an alternate venous drainage, unattended by an artery, which feeds directly into the inner vertebral sinuses of the cervical and thoracic segments and thence via the unpaired vertebral and thoracic veins into the great veins entering the heart (*9*).

The physiological efficacy of this system has been studied by means of direct thermal measurements both during the cold-induced arousal of the hibernating marmot (*5*), (see Fig. 3) and in cold-acclimated rats (Fig. 4). This arrangement not only permits very rapid adjustment of the on-off thermogenic responses, but also favors the capacities for transient injections of large quantities of heat into vital structures such as the spinal cord and heart.

(iv) *Regulation of brown-fat thermogenesis* is evidently mediated by the sympathetic nervous system, at least in respect of the activity of the interscapular and cervical pads of the rat. It is established that nutritional status of the interscapular gland is dependent on an intact nervous supply (*19, 20*), and that norepinephrine normally present in the gland disappears after denervation (*20*); also it has been shown that brown fat shows marked enhancement of lipase following direct stimulation of its intact nerve supply in vitro (*21*). My experiments have clearly shown by direct thermometry that thermogenesis of brown fat in the unanesthetized, intact, cold-acclimated rat is readily blocked by injection of hexamethonium (Fig. 4) or by local application of xylocaine to the bilateral nerve supply to the interscapular brown fat (*22*).

Of probable significance in this respect is the anatomical disposition of brown fat over the nerve trunks of the axillary-thoracodorsal course and also along the dorsomedial course of the thoracic sympathetic chain; thus, direct conductive heating could be available to these neuronal elements, as well as the local heating effects suggested earlier in respect to convective heating of the cervical and thoracic segments of the spinal cord. From these, of course, emerge all of the major neuronal controls of the thoracic organs (*14*).

Of singular interest is the relative abundance of brown fat in the neonatal infant and also in newborn rodents (*14, 15*). In view of the well-known thermolability of these species in the neonatal stages, I suggest (*23*) that brown fat may contribute heavily to the heat balance and thermoregulation during this phase of development.

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