The Effects of Environmental Temperature on the Anatomical Organization of Adipose Tissues

By F. Al-Hilli * and E.A. Wright **

THE anatomical distribution of white and brown adipose tissue in the mouse was described. It was not possible to determine the amount of the white fat but the brown fat was estimated in five areas; interscapular, axillary, cervical, thoracic and abdominal. The microscopic organization of interscapular fat of group of mice maintained at 33°C, 21°C and 8°C was also described. Brown and white adipose tissue was found to convert to and from each other due to changes in the fat loading of adipocytes.

IN MOST mammals, there are two distinct types of adipose tissue which differ in their colour, distribution, vascularity, structure and metabolism (1). One type is the familiar yellow or white adipose tissue (WAT) which forms the bulk of body fat; the other has a characteristic brown colour and a lobular appearance and is called brown adipose tissue (BAT). The anatomical and microscopic organization of these types especially the BAT have been studied in a variety of animals (2). Studies however, on the organization of these tissues in the mouse particularly under different environmental temperatures have received little attention.

MATERIALS AND METHODS

Inbred colony of strain A, albino mice of both sexes aged 6 months maintained at 33°C (hot group), 21°C (control group) and 8°C (cold group) (9, 9 and 8 mice respectively) from the age of 25 days were used. The details of these experimental conditions were described previously (3). The mice were killed by deep ether anaesthesia and carefully dissected under the dissecting microscope to determine the distribution of WAT and BAT. The dissected specimens were weighed to the nearest 0.05 mg, fixed in 10% formol saline, processed for histological examination (5 um thickness and stained with Haematoxylin and Eosin and Reticulin stain.

Two additional groups of mice aged 3 months maintained at 8°C and 33°C from the age of 25 days were also used. From the first group (2 mice) which were maintained at 8°C a biopsy was taken from the interscapular fat; the animals were allowed to recover from the surgical anaesthesia (about 2 hours) and then transferred to the hot room (33°C). Similar biopsy was taken from the second group (2 mice) which were maintained at 33°C and then transferred to the cold room (8°C). After 4 weeks, the mice of both groups were killed and the remaining interscapular fat was examined histologically.

RESULTS

A. Gross Anatomy : The morphological organization and distribution of WAT and BAT was confirmed histologically in all cases.

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The WAT was mainly subcutaneous along the dorsal midline, around the base of the tail and thighs and in the anterior abdominal wall especially at its lateral borders. It also filled the axillary fossae, present in the ventral side of the neck and was interspersed between the lobules of the parotid gland and the major vessels of the neck. In the abdomen it was found in the retroperitoneum, mesentery and in the mesometrium in the females and as testicular fat bodies in the males. WAT was not however, limited to these areas. It was demonstrated in small amounts almost anywhere where there was areolar connective tissue. It was thus difficult to estimate the amount of this type of fat in the mouse.

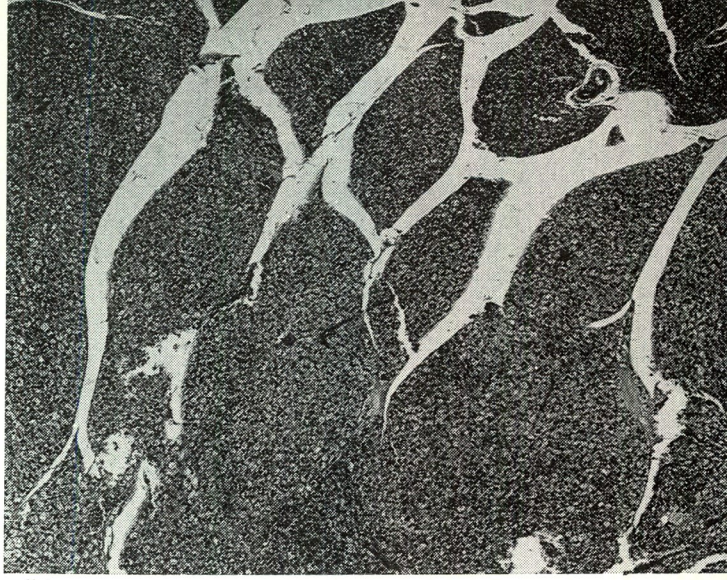
BAT occured in the form of glistening lobules, often in relation to blood vessels and mixed with WAT in the loose connective tissue. Its colour varied according to the environmental condition. Thus it was brown in the cold (8°C), light brown in the control (21°C) and yellowish in the hot (33°C) group.

The presence of BAT was particularly estimated in 5 areas.

1. Interscapular BAT : This formed the largest deposits of BAT in the mouse. There were two symmetrical elongated masses on either side of the dorsal midline which filled the depression between the muscles over the scapulae. These masses were clearly separated from each other and secured to the underlying semispinalis capitis muscles by thin vascular connective tissue. The anterior upper thirds of these masses were covered by the rhomboideus major muscles and the anterior lower two thirds by skin.

The mean weights of the interscapular BAT were 12.25 gm, 4.5

(a)



(b)

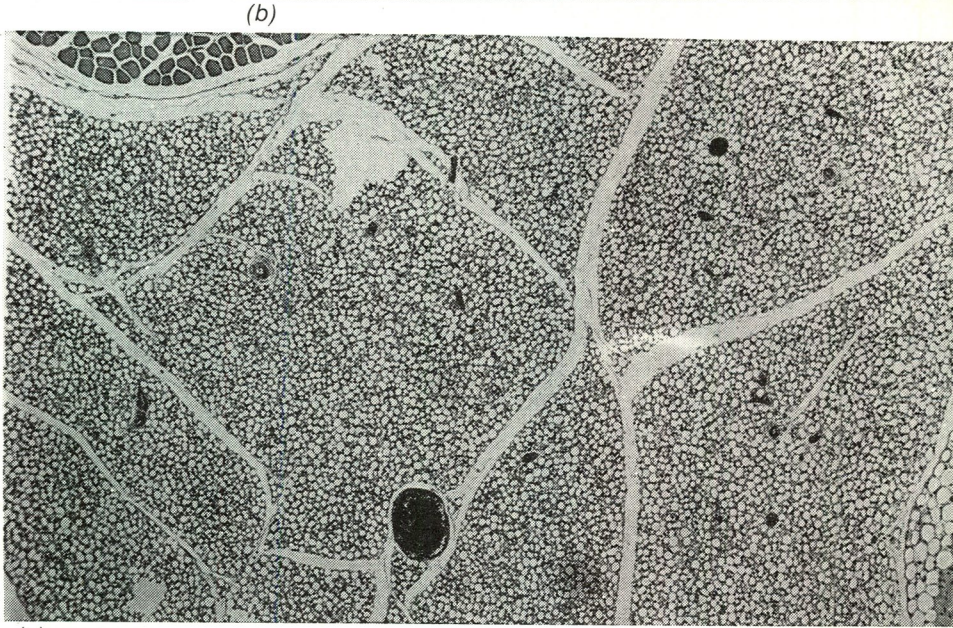
gm and 3.1 gm in the cold (8°C), control (21°C) and hot (33°C) groups respectively.

From the interscapular masses, lobules of BAT extended laterally to the axillae. The lobules at first ran along the inferior border of the scapulae on the serratus anterior, covered by the trapezius muscle and then the lobules extended anteriorly to the scapulae, between the subscapularis and the serratus anterior muscles.

In the cold (8°C) group, lobules of BAT were also seen distributed subcutaneously along the lateral borders of the anterior abdominal wall.

2. Axillary BAT : This occured in the form of lobulated masses which covered the axillary blood vessels and filled the axillary fossae. Delicate lobules were also seen between these vessels. The axillary lymph nodes were embedded in a mixture of WAT and BAT. In the axillae these masses were covered by the pectoral muscles.

The axilary BAT was also seen connected to the cervical BAT by delicate lobular prolongations which intersected the axillary muscles and ran under the clavicles and along the axillary vessels to the posterior and anterior triangles of the neck.



(c)

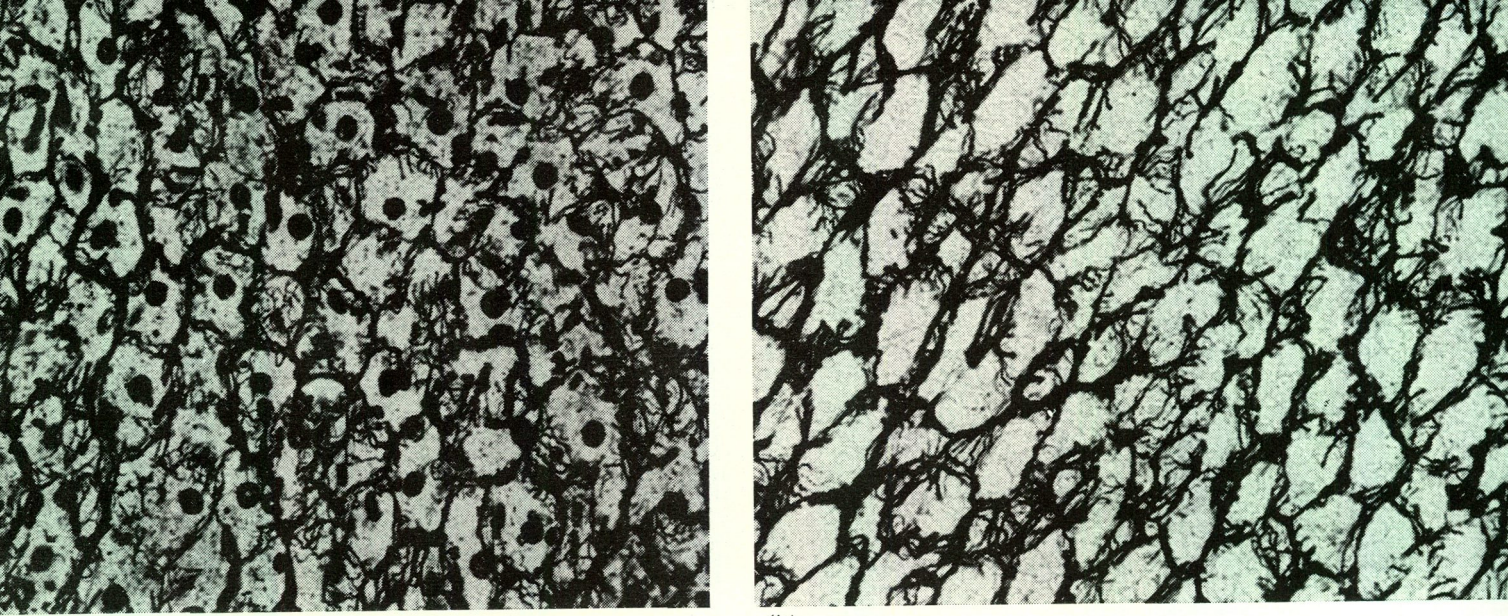
Fig. 1 Photomicrographs (Haematoxylin and Eosin) of the interscapular masses in the (a) cold (b) control and (c) hot groups of mice maintained at 8°C, 21°C, and 33°C respectively

3. Cervical BAT : This was seen as lobulated strips which ran across the ventral side of the neck over the major blood vessels sending delicate prolongations between these vessels and the cervical lymph nodes. It extended over the sternothyroid and sternohyoid muscles and was covered by the parotid gland.

4. Thoracic BAT : In the thorax, BAT was seen as discrete islands around the thymus, heart and the hilum of the lungs. It also formed slender lobules along and on either sides of the thoracic aorta, filling the costovertebral angle and extending into the abdomen.

5. Abdominal BAT : In the abdomen, BAT also occurred in the form of discrete islands along the abdominal aorta and its major branches. It was also found at the hilac of the spleen and kidneys and around the upper third of the ureters.

The amount of axillary, cervical, thoracic and abdominal BAT was difficult to assess not only because



(a) (b)

Fig. 2. Photomicrographs (Reticulin stain) of the interscapular masses in the (a) cold and (b) hot groups of mice maintaind at 8°C and 33°C respectively

the lobules were often too small to be dissected but also because they were mixed with WAT.

B. Microscopic Anatomy : The main distinctive features of interscapular BAT found in mice under the three experimental conditions is shown in the table.

In the cold and control groups, the interscapular, axillary and cervical masses had the typical appearance of BAT. It was covered by a capsule of delicate areolar connective tissue which contained blood vessels, nerves and a variable number of adipocytes. From the capsule, septa of loose vascular connective tissue penetrated the whole brown mass dividing it into irregular but well defined lobes and lobules *(Fig. 1)*

In the BAT of the cold group and to a lesser extent in the control group the blood vessels and capillary network which surrounded the individual adipocytes were found to be packed with red blood cells.

The parenchyma of the brown lobules was composed of closely packed brown adipocytes. These are polygonal cells which occured in groups and clumps interlaced by abundant coarse randomly oriented reticulum fibres *(Fig. 2)*. The average size of these cells was 18 um in the interscapular BAT of the cold group and 23 um in the control group.

The nuclei of the brown adipocyte were usually central, large and spherical and never peripheral or flattened as in the white adipocyte. Occasionally however, some eccentric nuclei were seen. The chromatin material formed a granular network in the meshes of which 1 — 3 prominent nucleoli were seen. In all the sections of BAT examined, the nuclei were similar and mitotic figures were absent. The average size of the nuclei was 6 um in the interscapular BAT of the cold group and 4 um in the control group.

Unlike the large single lipid globule of the white adipocyte with its thin peripheral rim of cytoplasm, the cytoplasm in the brown adipocyte was abundant but varied between the cold and control groups. In both, the cytoplasm was laden with drop-like lipid inclusions. These droplets generally had a uniform rounded shape but their size and number varied both between cells in the same animal and group of animals as well as between the cold and control groups. In the cold group, the cytoplasm of the brown fat cells was coarsely granular, abundant, deeply stained and contained variable numbers of lipid droplets of various sizes. In contrast, the cytoplasm of the cells in the control group was less granular, scanty, lightly stained and contained more lipid droplets than the cold group.

Several other forms of adipocyte were also seen. Occasionally brown adipocytes with one or two relatively larger lipid inclusions and several smaller droplets of various size were seen. In some other cases, the cells were entirely filled with 2 — 3 large inclusions. White adipocytes were also seen between the closely packed brown adipocytes. These forms were more frequently observed in : (i) the capsule and connective tissue septa of BAT (ii) close to large blood vessels (iii) the BAT of the control group (iv) the thoracic and abdominal BAT of the cold and control groups.

The thoracic and abdominal BAT of the cold and control groups had the same structure, but the vascularity was less than that of the

interscapular, axillary, or cervical BAT. On the other hand white adipocytes as well as those forms with 2 — 3 large lipid droplets were more frequent.

In the hot group, the miscroscopic anatom of the 5 above masses appears as that of WAT, but with the lobular pattern preserved. *(Fig. 1)* and the vascularity greatly reduced. In sections, the interscapular masses were formed of closely packed large polyhedral cells mostly occupied by a large single lipid globule. The cytoplasm was very scanty and the nuclei were flattened and displaced to the thin rim. As in the BAT cells, these cells were also surrounded by abundant coarse reticulin fibres of random orientation *(Fig. 2)*. The average size of these cells in the interscapular masses was 35 um.

The biopsy specimens which were obtained from groups of mice maintained at 8°C and 33°C showed the typical BAT and WAT respectively. When transferred to 33°C and 8°C for 4 weeks, the histological apperance had changed to typical WAT in the first group and BAT in the second group.

DISCUSSION

In most mammals, BAT not only has a similar anatomical distribution but also similar histological appeaances (4). In this study, the distribution of WAT and BAT in the adult mouse under three environmental conditions was studied. The anatomical organization of both types were similar in the three groups. The distribution of BAT was divided into 5 groups; interscapular, axillary, cervical, thoracic and abdominal BAT. However, the bulk of the BAT in the mouse was found in the two symmetrical masses forming the interscapular part. This part has been recognized at the most important site of BAT and chnages in the fresh weight of its masses to represent the changes in the fresh weight of total BAT in the body (5). The weight of BAT was also reported to be influenced by exposure to cold environment (6). Thus after 6 weeks of cold acclimatisation the fresh weight of these masses was found to increase 4 times (5). In the present study, the fresh weight of interscapular BAT in th cold (8°C) reared mice was 272 % and 303 % of that in the control (21°C) and hot (33°C) groups. These changes may also partly reflect the degree of vascularity. In the rat the vascularity of BAT was estimated to be 4 — 6 times greater than that of WAT (7).

The distinction between WAT and BAT has been investigated by many authors (4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17). Thus whereas the poorly vascularised WAT is made of cells containing single large globule of fat, the highly vascular BAT is made of smaller cells which contain numerous small droplets. Because of the presence of these droplets, the BAT is often termed multilocular adipose tissue to distinguish it from the unilocular adipose tissue or the ordinary WAT. In addition, the uniform appearence of brown adipocyte nuclei as well as the absence of mitotic figures identifies BAT as a fully differentiated tissue.

Since the interscapular masses are the most important site of BAT in the mouse (5) it is possible according to the results of this study to divide the anantomical organization of these masses into three forms. The typical BAT is seen in the interscapular masses of mice reared at 8°C (cold group) whereas the WAT occured in those maintained at 33°C (hot group). The third was found in mice kept at 21°C (control group) and may represent a transitory stage between the cold and hot group. Therefore one may postulate that the environmental temperature may determine the form of BAT in the mouse. The main distinctive features of the interscapular masses of the control group are : (i) Its fresh weight is heavier that the WAT of the hot group but lighter than the typical BAT of the cold group. (ii) The vascularity is moderate between the highly vascular BAT and the poorly vascular hot group. (iii) The adipocytes in the control group were larger than the brown adipocytes but smaller than those of the hot group. Generally, the size of the cells depends on the animal species (hibernators-nonhibernators), the developmental stage (newborn-adult) and the degree of lipid depletion or loading (4). Fat depletion can be brought about by prolonged starvation (18), exposure to cold (19) or hibernation (9, 20) in which both factors are combined. The brown adipocyte contains few small lipid droplets and abundant cytoplasm, thus it may represent a state of fat depletion. On the other hand, the single lipid globule and the scanty cytoplasm of the adipocytes in the hot group may indicate fat loading. The adipocytes of the control group however, contained many large lipid dropletes but the degree of fat depletion was greater than that of the hot group (4). It contained more white adipocytes as well as many forms of fat cells in different stages of maturation than BAT.

Whether BAT can be converted into WAT is often disputed (2). But Dyer's (21) in vitro study of isolated adipocytes confirmed such conversion. In this analysis BAT found in the cold group and WAT found in the hot group were converted to and from each other by transferring the animals between the hot and cold rooms. Histologi-

cally, this transformation of BAT into WAT seems to be a replacement of WAT rather than a change in the structure i.e. increased lipid loading because (i) the final histological picture of the converted tissue resembled the WAT, (ii) the multilocular pattern of BAT was preserved in the hot group (iii) the arrangement of the reticulin fibres was also preserved. Normally each WAT fat cell is enclosed in fine delicate reticulin fibres whereas in the BAT, the reticulin fibres are coarse and of random orientation (iv) in the connective tissue septa and in areas where the temperature is high such as adjacent to the blood vessels and in the thorax and abdomen, the predominent BAT cells are those with 1 — 3 large lipid droplets and few small lipid inclusions. This may be a transitory stage before the droplets coalesce and from one large single fat globule.

The thermogenetic importance of BAT in cold reared animals is well known (1). However, the hot environment would favour heat loss rather than heat generation. This may again account for the conversion of BAT into WAT which has no thermogenetic properties (22).

	Cold (8°C)	**Control (21°C)**	**Hot (33°C)**
Weight	12.25 gm (SD 0.9)	4.5 gm (SD 0.85)	3.1 gm (SD 0.9)
Type	Typical BAT	Mixed	WAT
Colour	Brown	Light brown	Yellowish
Vascularity	Highly vascular. Vessels are frequent, prominent and packed with rbcs.	Moderately vascular. Vessels are occasional, mildly congested.	Poorly vascular. Vessels few and mostly empty.
Lobular pattern	Evident	Maintained	Preserved
Adipocytes			
1. Shape	Polygonal	Polygonal	Polyhedral
2. Size	18 μm (SD 1.2)	23 μm (SD 1.3)	35 μm (SD 1.2)
3. Cytoplasm	Abundant, deeply stained, coarsely granular.	Scanty, lightly stained, less granular.	Very scanty, reduced to peripheral rim.
4. Droplets	Few of various sizes	Many of various sizes	Single globule
5. Nuclei	Central, large, spherical never peripheral or flattened.	Central, large spherical, occasionally eccentric.	Small, flattened, peripheral.
6. Size of nuclei	6 μm (SD 1.15)	4 μm (SD 1.21)	2 μm (SD 1.35)
7. Reticulin fibres	Abundant, coarse and randomly oriented.		

Table : The main distinctive features of interscapular masses in the cold (8°C), control (21°C) and hot (33°C) groups.

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