EFFECTS OF HYPOTHYROIDISM ON THE BROWN ADIPOSE TISSUE OF ADULT RATS: COMPARISON WITH THE EFFECTS OF ADAPTATION TO COLD

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SUMMARY

Hypothyroidism was induced in adult rats by oral absorption of methimazole and its effects on brown adipose tissue (BAT) were studied. Hypothyroidism partially mimicked the effects of chronic exposure to cold: BAT weight and its DNA content were increased and the mitochondrial components (proteins, phospholipids) of the tissue were greatly enhanced when expressed per unit of fresh tissue weight. Moreover, hypothyroidism had the same effects as adaptation to cold on the fatty-acid composition of both total and mitochondrial phospholipids. Basal respiratory rate and total cytochrome C oxidase activity of the tissue were also increased. However, the increase in the concentration of the '32 000 mol. wt protein', a polypeptide which regulates the dissipation of heat by BAT, was smaller and non-selective in hypothyroid rats. The amount of this protein was increased per mg tissue, but not per mg mitochondrial proteins, as in rats adapted to cold. Furthermore, in contrast with the large mobilization of the lipid stores in BAT of euthyroid animals, the BAT lipid stores of hypothyroid rats were not mobilized during the first hours of exposure to cold. It may be concluded that (a) hypothyroidism induces several alterations in BAT which are characteristic of an active thermogenic state (this may be because of the response of the organism to the deficiency of thermogenesis induced by hypothyroidism), (b) this potential increase in thermogenic capacity in the BAT of hypothyroid rats has probably a limited physiological role, since thyroid hormones are necessary for the mobilization of the tissue lipids which are the fuel for production of heat and (c) these data provide evidence for a limited role of thyroid hormones in the trophic response of BAT during adaptation to cold.

INTRODUCTION

In mammals, brown adipose tissue (BAT) is the only organ whose major function is to produce heat. Since the thyroid hormones, thyroxine (T₄) and tri-iodothyronine, are known to play an important role in homeothermy, their involvement in the regulation of BAT activity has often been studied in animals either receiving T₄ or made hypothyroid by various means (see review in Hémon, Ricquier & Mory, 1976a; Barnard, Mory & Néchad, 1980).

Both chronic administration of T₄ and chronic exposure to cold induced a growth in BAT. However, the hypertrophy of BAT observed in T₄-treated rats is qualitatively very different from that observed in cold-adapted rats. For example, there is no activation of the oxidative metabolism of the tissue and an accumulation of lipids is observed (Lachance,
1953). Further experiments have confirmed that experimental hyperthyroidism does not reproduce the effects of exposure to cold (for review see Barnard et al. 1980). It has also been found that injection of T₄ during exposure to cold prevents most of the modifications normally occurring in BAT (Ricquier, Mor & Hémon, 1976; Ricquier, Mor, Néchad & Hémon, 1978).

Hypothyroidism also increases the weight of BAT. It has been known since the nineteenth century that human cretinism is accompanied by abnormal deposits of fat (Curling, 1850) and it was later recognized that tumour-like formations in cretinous babies are made of brown fat (Shattock, 1909). In young cretinous rats an enlargement of BAT is also observed and the tissue exhibits several abnormalities (Hémon, 1976; Hémon, Ricquier & Mor, 1976b; Ricquier & Hémon, 1976). One of the most striking features of BAT from cretinous rats is its incapacity to respond normally to noradrenaline, the physiological activator of heat production in this tissue. Since dibutyl cyclic AMP increases the activity of BAT metabolism, this lack of response to noradrenaline has been interpreted as an impairment of cyclic AMP synthesis (Hémon et al. 1976a).

In adult thyroidectomized rats, Sellers, Flattery & Steiner (1974) found that BAT was slightly enlarged and the lipid-free dry mass of the tissue was increased. Since athyroid animals exposed to a low temperature died rapidly, thyroidectomized rats receiving the lowest dose of T₄ which allows survival were adapted to cold by Sellers et al. (1974). The development of BAT in these mildly hypothyroid rats bred at 4 °C was similar to that observed in control animals. The authors have thus suggested that T₄ plays only a permissive role in the response of BAT to cold.

In the present work, the role of T₄ in the capacity of BAT to produce heat was investigated with adult hypothyroid rats. Some aspects of BAT composition and metabolism were studied and especially two indices of the level of thermogenic activity in BAT: (a) the phospholipid composition of the tissue since it varies qualitatively and quantitatively according to thermal status (Ricquier, Mor & Hémon, 1975; Ricquier et al. 1976), and (b) the so-called ‘32000 mol. wt protein’, a particular component of BAT mitochondrial membranes. This protein, which plays a major role in the regulation of heat dissipation in BAT (for review see Nicholls, 1979), is increased in mass during adaptation of the rat to cold (Ricquier & Kader, 1976). Guanosine diphosphate (GDP) is a specific ligand of the 32000 mol. wt protein and the GDP binding to isolated mitochondria was determined since this is an index of the thermogenic capacity of the tissue (Heaton, Wagenvoord, Kemp & Nicholls, 1978).

Surprisingly, it was found that hypothyroid rats bred at room temperature exhibited several changes similar to those observed in euthyroid rats adapted to cold. These results have been presented in a preliminary form at a congress (Mory, Ricquier, Pesquiés & Hémon, 1977).

**MATERIALS AND METHODS**

**Animals and treatments**

Male rats of the Sprague–Dawley strain fed with a semi-synthetic diet (Ricquier et al. 1975) were used and were exactly 3 months old at the time of death.

Hypothyroidism was induced by 1-methylimidazole-2-thiol (methimazole; Fluka, CH-9470 Buchs, Switzerland) added to the drinking water at a concentration of 0.1% for 5 weeks. Hypothyroid rats kept at 25 °C were compared with euthyroid animals bred at the same temperature or adapted to cold by exposure to 5 °C for 5 weeks. The effects of acute exposure to cold were studied in hypothyroid and euthyroid rats bred at 25 °C and exposed for 6 h to 5 °C before killing. Cold-adapted hypothyroid rats were obtained by giving methimazole to animals previously adapted to cold. These rats were exposed to cold for 5 weeks but received methimazole only during the last 3 weeks of this period.
Biochemical analysis

Rats were killed by decapitation and their blood was collected in chilled heparinized tubes. Plasma was collected after centrifugation at 4°C, and stored at −80°C before use. Radioimmunoassay of plasma T₄ was carried out in triplicate using kits supplied by Byk-Mallinckrodt, D-6057 Dietzenbach-Steinberg, Western Germany (the method used rabbit antibodies against T₄-bovine serum albumin). After careful dissection the thyroid glands and interscapular BAT were weighed and the gross composition of BAT (water, proteins, total lipids, phospholipids) was determined as previously described (Ricquier et al. 1976). Fatty-acid composition of BAT phospholipids was determined by gas–liquid chromatography (Ricquier et al. 1975). The method of Burton (1956) was used to measure the DNA content of the tissue.

In-vitro respiration of the tissue was determined by measurement of the initial oxygen consumption rate of tissue fragments in Krebs–Ringer phosphate buffer (pH 7.4) at 37°C using a Clark electrode (Gilson, F-95400 Villiers le Bel, France). Cytochrome C oxidase activity (EC 1.9.3.1.) was measured in homogenates of total tissue by a polarographic method (Schnaitman, Erwin & Greenawalt, 1967).

The mitochondrial fraction of the tissue was isolated by differential centrifugation and the yield of mitochondrial extraction measured by the recovery of cytochrome C oxidase activity as described by Ricquier et al. (1975). Protein content, phospholipid content and fatty-acid composition of the mitochondrial fraction were determined as in the total tissue. Analysis of the polypeptide composition of mitochondria was performed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The proportion of protein of 32 000 mol. wt in mitochondrial protein was determined by scanning the gels after staining with Coomassie blue R250 (Ricquier & Kader, 1976). The GDP binding to isolated mitochondria was measured using equilibrium dialysis (Lin & Klingenberg, 1980).

Results are presented as means (±S.E.M.) and the Mann–Whitney U test was used to determine statistically significant differences.

RESULTS

Growth and thyroid status of the animals

Body weight was decreased similarly by adaptation to cold and by hypothyroidism, the smallest rate of growth being observed in cold-adapted hypothyroid rats. Adaptation to

Table 1. Effects of hypothyroidism and/or adaptation to cold on thyroid weight, plasma level of thyroxine (T₄) and gross composition of interscapular brown adipose tissue (BAT) of rats. Values are means (±S.E.M.)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Euthyroid (25°C)</th>
<th>Hypothyroid (25°C)</th>
<th>Euthyroid (5°C)</th>
<th>Hypothyroid (5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>340±9</td>
<td>249±14**</td>
<td>259±11</td>
<td>239±5**</td>
</tr>
<tr>
<td>Thyroid weight (mg)</td>
<td>15.0±1.1</td>
<td>61.8±3.8**</td>
<td>16.6±1.4</td>
<td>67.9±3.5**</td>
</tr>
<tr>
<td>Plasma T₄ (ng/ml)†</td>
<td>27±5</td>
<td>&lt;1**</td>
<td>36±4</td>
<td>&lt;1**</td>
</tr>
<tr>
<td>BAT weight (mg)</td>
<td>341±33</td>
<td>445±47**</td>
<td>730±5</td>
<td>75±85</td>
</tr>
<tr>
<td>Water (%)</td>
<td>36.9±2.6</td>
<td>39.0±2.2</td>
<td>50.7±2.8</td>
<td>45.9±0.9</td>
</tr>
<tr>
<td>Total lipid (%)</td>
<td>52.4±3.3</td>
<td>46.3±3.5</td>
<td>34.9±1.9</td>
<td>39.9±1.8*</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>6.7±0.3</td>
<td>9.7±0.7**</td>
<td>10.3±0.9</td>
<td>6.7±0.2**</td>
</tr>
<tr>
<td>Phospholipid (%)</td>
<td>0.86±0.16</td>
<td>2.55±0.25**</td>
<td>1.94±0.4</td>
<td>1.71±0.23</td>
</tr>
</tbody>
</table>

Gross composition of BAT is expressed as a percentage of the fresh tissue weight.

*P<0.05. **P<0.01 compared with values for euthyroid animals kept at the same temperature (Mann–Whitney U test).

†Similar results were obtained with the competition protein-binding assay for T₄ of Vigouroux (1972), i.e. 30 ng/ml in euthyroid rats at 25°C (two rats, eight determinations) and 0.2 ng/ml in hypothyroid rats at 25°C (three rats, eight determinations).
cold was found to have no significant effect on thyroid weight and T₄ levels in plasma. Methimazole induced hypothyroidism in animals bred at 25°C or adapted to 5°C: thyroid weight was increased fourfold whereas plasma levels of T₄ were undetectable with our radioimmunoassay (Table 1). In hypothyroid cold-adapted rats, hypothyroidism was associated with a slight decrease in internal temperature: colonic temperature, measured with a thermocouple inserted 8 cm into the colon, was 37.6 ± 0.2°C in euthyroid rats adapted to 5°C and 36.9 ± 0.2°C in hypothyroid rats which had been kept at the same temperature (n = 6, P ≤ 0.05).

Comparison between the effects of hypothyroidism at 25°C and of adaptation to cold

Interscapular BAT from cold-adapted rats increased in weight (Table 1) and this was accompanied by an increase in its DNA content (Table 2) and the modifications of composition usually described (for review see Smith & Horwitz, 1969; Barnard et al. 1980). For example, both the total and relative protein contents of the tissue were increased (Table 1) and the enhancement of total and relative phospholipid content (Table 1) was associated with an alteration in fatty-acid composition (Fig. 1).

Table 2. Effect of adaptation to cold, or of hypothyroidism at 25°C, on the DNA content of brown adipose tissue (BAT) of rats. Values are means (± s.e.m.); there were six determinations per group

<table>
<thead>
<tr>
<th></th>
<th>BAT weight (mg)</th>
<th>Proteins (mg/organ)</th>
<th>DNA (μg/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid (25°C)</td>
<td>252 ± 22</td>
<td>22.8 ± 2.0</td>
<td>550 ± 61</td>
</tr>
<tr>
<td>Euthyroid (5°C)</td>
<td>573 ± 55**</td>
<td>77.9 ± 3.5</td>
<td>1066 ± 121**</td>
</tr>
<tr>
<td>Euthyroid (25°C)</td>
<td>306 ± 12</td>
<td>25.7 ± 1.9</td>
<td>505 ± 44</td>
</tr>
<tr>
<td>Hypothyroid (25°C)</td>
<td>417.5 ± 41**</td>
<td>50.9 ± 3.1**</td>
<td>780 ± 53**</td>
</tr>
</tbody>
</table>

**P ≤ 0.01 compared with values for euthyroid rats at 25°C.

Fig. 1. Effects of hypothyroidism and/or adaptation to cold on the fatty-acid composition of phospholipids in brown adipose tissue. The concentration of each fatty acid is expressed as a percentage of the total fatty-acid concentration of the phospholipid. They are classified from left to right by their numbers of carbons and double bonds (C16:0, C16:1, C18:0, C18:1, C18:2, C20:4). The number in each diagram indicates the degree of unsaturation of the fatty acids (percentage of carbon–carbon double bonds in the total number of carbon–carbon bonds). The S.E.M. was always less than 6% of the mean value. The same pattern of variation was observed in mitochondrial phospholipids.
Brown fat in hypothyroid rats

Interscapular BAT from hypothyroid rats at 25°C was modified in the same way as in cold-adapted animals. As observed by Sellers et al. (1974) the tissue was slightly enlarged and total and relative protein contents were increased (Table 1). This development was accompanied by an enhancement of total DNA content (Table 2). Hypothyroidism also induced a large increase in the relative (threefold) and total (fivefold) phospholipid contents of the tissue (Table 1). The fatty-acid composition of the phospholipids showed the same modifications as that in cold-adapted rats (Fig. 1); the proportions of C16:0, C16:1 and C18:1 were decreased, while the proportions of C18:0, C18:2 and C20:4 were increased. This alteration led to an increase in the degree of unsaturation of phospholipids (+31.8%) similar to that in cold-adapted animals (+41.8%).

Effects of hypothyroidism on BAT mitochondria

Hypothyroidism induced a development of mitochondria as indicated by the increase in the mitochondrial contents of proteins and phospholipids (Table 3). The fatty-acid composition of mitochondrial phospholipids was modified as in cold-adapted rats. The degree of unsaturation of these fatty acids was increased (+14.75%) as were total phospholipids of the same tissue samples (+18.9%). The cytochrome C oxidase activity increased in parallel with BAT weight. The in-vitro basal oxygen consumption of the tissue was enhanced, per organ and per unit of fresh weight.

Table 3. Effects of hypothyroidism on some brown adipose tissue (BAT) mitochondrial parameters in the rat. Values are means (±S.E.M.); there were six determinations per group, each determination being obtained with pools of two samples

<table>
<thead>
<tr>
<th>Euthyroid (25°C)</th>
<th>Hypothyroid (25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT weight (mg)</td>
<td>298±20</td>
</tr>
<tr>
<td>Mitochondrial proteins (mg/organ)</td>
<td>7.4±0.8</td>
</tr>
<tr>
<td>BAT weight (mg)</td>
<td>234±12</td>
</tr>
<tr>
<td>O2 consumption (nmol O2/min per organ)</td>
<td>204±25</td>
</tr>
<tr>
<td>Cytochrome C oxidase activity (μmol O2/min per organ)</td>
<td>9.7±0.6</td>
</tr>
<tr>
<td>Mitochondrial phospholipid (mg/organ)</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Degree of unsaturation of mitochondrial phospholipid†</td>
<td>6.1±0.2</td>
</tr>
</tbody>
</table>

*P≤0·05, **P≤0·01 compared with values for euthyroid rats.
† Percentage of carbon–carbon double bonds in the total number of carbon–carbon bonds present in the fatty acids of mitochondrial phospholipids of the tissue. The degree of unsaturation of the total phospholipid in the tissue which was used to prepare this mitochondrial fraction was 5·9±0·1 in euthyroid rats and 7·0±0·1 in hypothyroid animals.

In contrast, the amount of 32,000 mol. wt protein, which was selectively increased in cold-adapted rats, was not similarly affected by hypothyroidism. The same percentage of this protein in the mitochondrial proteins was found in euthyroid and hypothyroid rats kept at 25°C (Fig. 2). The total amount of 32,000 mol. wt protein was, however, increased twofold (329 μg/organ in control animals v. 811 μg/organ in hypothyroid rats) since the mitochondrial protein content of the tissue was greatly increased. A similar result was observed with GDP binding on mitochondrial proteins (Fig. 2), which was not affected by hypothyroidism when expressed per mg protein but was increased twofold when expressed per organ (0·95 nm-GDP/organ in euthyroid rats v. 1·75 nm-GDP/organ in hypothyroid rats).
Effect of hypothyroidism on BAT in rats exposed to cold

The hypertrophy of BAT was maintained when cold-adapted rats were given methimazole. The increase in the percentage of phospholipids in the tissue and the fatty-acid composition of these lipids were similar to those observed in euthyroid rats adapted to cold (Table 1 and Fig. 1). A slight deficit in the protein percentage was observed, however, and the tissue contained a larger amount of total lipids.

Effect of short-term exposure to cold in hypothyroid rats

Exposure to 5°C for 6 h induced a large mobilization of total lipids in BAT of euthyroid animals (Table 4). The tissue lost two-thirds of its total lipids while the total water content of the tissue was simultaneously increased. In hypothyroid rats previously bred at 25°C, such cold stress was without any significant effect on the lipid content of BAT.

Table 4. Effects of acute exposure to 5°C for 6 h on the interscapular brown adipose tissue (BAT) of euthyroid and hypothyroid rats kept at 25°C. Values are means (±S.E.M.); there were six determinations per group

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid</th>
<th>Euthyroid + acute cold</th>
<th>Hypothyroid</th>
<th>Hypothyroid + acute cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT weight (mg)</td>
<td>312 ± 11</td>
<td>306 ± 30</td>
<td>370 ± 41</td>
<td>334 ± 50</td>
</tr>
<tr>
<td>Water (%)</td>
<td>33.1 ± 2.7</td>
<td>68.3 ± 2.3**</td>
<td>38.9 ± 2.9</td>
<td>44.7 ± 1.8</td>
</tr>
<tr>
<td>Total lipid (%)</td>
<td>57.9 ± 3.0</td>
<td>22.3 ± 1.4**</td>
<td>45.5 ± 4.7</td>
<td>42.8 ± 2.9</td>
</tr>
</tbody>
</table>

The percentages of protein and phospholipid, which were not affected by acute cold, are not presented.

**P ≤ 0.01 compared with values for respective control rats (Mann–Whitney U test).
Brown fat in hypothyroid rats

DISCUSSION

Similarities between the effects of hypothyroidism and of adaptation to cold

Hypothyroidism mimics, at least qualitatively, most of the effects of adaptation to cold on BAT. These are: increase in the relative content of proteins in the tissue; increase in the DNA content which is an index of hyperplasia; and increase in mitochondrial contents of proteins and phospholipids and an increase in cytochrome C oxidase activity, all of which indicate a development of the mitochondria. Furthermore, hypothyroidism is the only experimental situation which reproduces the effect of adaptation to cold on the fatty-acid composition of BAT phospholipids.

Ricquier & Hémon (1976), comparing young cretinous rats with age-matched euthyroid animals, found that hypothyroidism had no effects on the composition of BAT phospholipids. Suckling rats bred at room temperature, however, are animals far from their thermal neutrality and thus present an active BAT which has similar characteristics to that of cold-adapted adult animals (Barnard et al. 1980). If the fatty-acid composition of phospholipids in the BAT of cold-adapted rats (Ricquier et al. 1976) is compared with the composition observed in euthyroid suckling rats (Ricquier & Hémon, 1976), we can conclude that these compositions are similar. Thus there is no contradiction with the present data: hypothyroidism in young cretinous rats had no significant effects on the fatty-acid composition of BAT phospholipids since control animals of the same age also presented the composition observed during adaptation to cold.

Hypothyroidism also induced an increase in the amount of 32 000 mol. wt protein in BAT of 107% per organ and 45% per unit of fresh tissue weight. This increase was smaller than in cold-adapted euthyroid rats, however, and furthermore there was no selective increase in this protein: the proportion of 32 000 mol. wt protein in mitochondrial proteins was not affected by hypothyroidism. This result was confirmed by the measurement of GDP binding to isolated mitochondria, which was enhanced per organ but not per mg of mitochondrial proteins. Nevertheless, according to the findings of Heaton et al. (1978), this increase in GDP binding could be the index of an enhancement of the potential capacity of BAT from hypothyroid rats to produce heat.

Differences between the effects of hypothyroidism and of adaptation to cold

In a normal rat, the metabolism of BAT can be stimulated by the sympathetic nervous system. Noradrenaline released by nerve endings activates lipolysis and the fatty acids so produced are the fuel for thermogenesis (Smith & Horwitz, 1969). In hypothyroid rats, the lipid stores in the BAT were not mobilized during cold stress. This suggests that the BAT of adult hypothyroid rats, like the BAT of the young cretinous rats previously studied (Hémon, 1976), was unresponsive to catecholamines. Thus the hypertrophic BAT of hypothyroid rats may have a limited physiological role. However, the BAT of hypothyroid rats was not found to be totally inactive and its basal respiratory rate was even increased when compared with the rate observed in the BAT of euthyroid animals (26% per g tissue). This can be explained by the presence of lipoprotein lipase activity in BAT, which is low in euthyroid rats bred at room temperature but greatly increased by hypothyroidism (Hémon et al. 1976b). Thus, the uptake of fatty acids by brown adipocytes could be increased by hypothyroidism, providing substrates for the metabolism of the organ. We note that Ikemoto, Hiroshige & Itoh (1967), who studied hypothyroid mice, have found similar results: the weight and the oxygen consumption of BAT from their animals were increased but the tissue was unable to respond to noradrenaline.

Determination of BAT trophic response in hypothyroid rats

Table 5 summarizes the results of the present work and compares them with data obtained previously by ourselves and other authors on hypothyroid rats bred at room or cold...
temperature. A coherent pattern of variation can be observed for most of the parameters quoted in Table 5. The pattern suggests that the effects of thyroid status on BAT are consequences of variations in the thermal status of the animals. Both hypothyroid rats bred at 25°C and euthyroid rats exposed to cold are animals faced with the problem of maintaining their body temperature, and these two situations induce similar changes in BAT. In contrast, injections of T₄ increase production of heat in the organism. Subsequently, the need for greater heat production in T₄-treated rats exposed to cold is probably negligible and it was observed that experimental hyperthyroidism impairs the effect of exposure to cold on BAT.

Table 5. Comparison between the effects of adaptation to cold and of thyroid status on various parameters in the brown adipose tissue of the rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adaptation to cold v. room temperature</th>
<th>Hypothyroidism v. euthyroidism</th>
<th>Hyperthyroidism v. euthyroidism</th>
<th>Adaptation to cold in hypothyroid rats v. adaptation to cold in euthyroid rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DNA content</td>
<td>+ (1)</td>
<td>+ (2)</td>
<td>+ (3)</td>
<td>- (3)</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>+ (4)</td>
<td>+ (2)</td>
<td>- (5, 6)</td>
<td>- (6)</td>
</tr>
<tr>
<td>O₂ consumption per organ</td>
<td>+ (4)</td>
<td>+ (2)</td>
<td>0 (7)</td>
<td>no data</td>
</tr>
<tr>
<td>Cytochrome C oxidase total activity</td>
<td>+ (1)</td>
<td>+ (2)</td>
<td>0 (5, 8)</td>
<td>- (8)</td>
</tr>
<tr>
<td>Lipoprotein–lipoase activity/mg tissue</td>
<td>+ (9)</td>
<td>+ (9)</td>
<td>0 (9)</td>
<td>no data</td>
</tr>
<tr>
<td>'32000 mol. wt protein' concentration and/or guanosine–diphosphate binding on this protein</td>
<td>+ (10, 11)</td>
<td>0 (2)</td>
<td>0 (12)</td>
<td>- (12)</td>
</tr>
<tr>
<td>Phospholipid concentration</td>
<td>+ (6)</td>
<td>+ (2)</td>
<td>0 (6)</td>
<td>- (6)</td>
</tr>
<tr>
<td>Fatty-acid unsaturation in phospholipids</td>
<td>+ (6)</td>
<td>+ (2)</td>
<td>- (6)</td>
<td>- (6)</td>
</tr>
</tbody>
</table>

+ indicates an increase of the parameter, — a decrease and 0 that it is absolutely or almost unchanged.

Numbers in parentheses indicate references: (1) Thomson, Habec, Nance & Beetham (1969); (2) results from this work; (3) Mory, Ricquier & Hemon (1975) and/or Mory, Ricquier & Hemon (1980); (4) Smith & Roberts (1964); (5) Heick, Vachori, Kallai, Begin-Heick & Leblanc (1973); (6) Ricquier et al. (1976); (7) Lachance (1953); (8) Ricquier et al. (1978); (9) Hemon et al. (1976); (10) Ricquier & Kader (1976); (7) Desautels, Zabor-Behrens & Himms-Hagen (1978); (12) Sundin (1981).

This hypothesis raises the question of the nature of the mediation between the thermal status and BAT. Several authors have shown that hypothyroidism induces an activation of sympathetic activity. This phenomenon, which is thought to be an attempt to maintain normal body temperature, has been observed at the level of the whole organism (Johnson, Flattery & Schönbaum, 1967; Coulombe, Dussault & Walker, 1977) or of particular organs such as the heart (Tedesco, Flattery & Sellers, 1977) and BAT (Zenker, Goudonnet & Truchot, 1976). Furthermore, the trophic response of BAT to cold, which is comparable to the trophic response of BAT to hypothyroidism, seems to be mainly controlled by the sympathetic nervous system (Barnard et al. 1980). In the case of hypothyroid rats, however, the results concerning the level of sympathetic activity in BAT are contradictory. Zenker et al. (1976) found an increase in activity of tyrosine hydroxylase, the rate-limiting enzyme in noradrenaline synthesis. Kennedy, Hammond & Hamolsky (1977) described a decrease in this activity associated with a decrease in both the noradrenaline content of the tissue and the noradrenaline turnover. Moreover, we have mentioned above the lack of sensitivity of BAT to noradrenaline when animals are made hypothyroid.

The exact nature of the trophic response of BAT in hypothyroid rats remains unclear. The hypothesis for a role of the sympathetic innervation requires an additional postulate:
sympathetic nerves exert a trophic role over BAT through a mechanism different from the activation of the classic β-receptor–adenyl cyclase complex which is impaired by hypothyroidism. Thus this problem is part of the one concerning the determination of neurotrophism, a problem which has not yet been solved (Smith & Kreutzberg, 1976).

**Conclusion**

According to the finding of Lachance (1953) we must allow the paradox that T₄, which is a well-known thermogenic hormone, cannot induce heat production in the only tissue specialized for thermogenesis. Moreover, Sellers *et al.* (1974) demonstrated that the role of T₄ in BAT activity was only permissive. The present work shows that this role of T₄ is important but seems to be limited, the presence of T₄ being necessary only for a normal metabolic response of BAT to catecholamine. Brown adipose tissue from hypothyroid rats exhibited similar changes to those in cold-adapted rats, a phenomenon that we have interpreted as a defence against cold. This means that most of the changes which occurred in BAT from rats exposed to cold do not require T₄.

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