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Depletion of Fats from the Brown Adipose Tissue Cells of Rats Dead from Cold Exposure

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Summary. Lipid content in the brown and the white interscapular adipose tissue of the rat was assayed after severe cold exposure resulting in death. The brown fat cells were depleted of lipids at the end of the exposure lasting for 3—5 hrs, whereas the white fat cells were still full of fat. The phenomenon could be confirmed microscopically, by thin layer chromatography, and quantitatively. The decline of lipid content was about 50% in the brown adipose tissue and nil in the white adipose tissue. The results indicate the importance of the brown adipose tissue for survival in cold exposure, while the white adipose tissue of the rat is less reactive to cold stress.

Zusammenfassung. Nachdem Ratten intensiver Kälte ausgesetzt wurden, die zum Tode führte, wurde der Gehalt an Lipiden im braunen und im weißen interscapularen Fettgewebe untersucht. Die braunen Fettzellen enthielten am Ende der 3—5stündigen Kälte-Exposition keine Lipide mehr, während die weißen Fettzellen noch völlig mit Fett angefüllt waren. Das Phänomen konnte mikroskopisch, mit Hilfe einer Dünnschicht-Chromatographie und quantitativ bestätigt werden. Die Abnahme des Lipid-Gehalts betrug etwa 50% im braunen und war gleich Null im weißen Fettgewebe. Die Ergebnisse bekunden die Bedeutung des braunen Fettgewebes für das Überleben in starker Kälte, während das weiße Fettgewebe der Ratte auf Kältebeanspruchung nicht reagiert.

Key words: Hypothermia death — cold exposure, lipid content in brown and white fat cells.

Introduction

Genuine hypothermia death is difficult to prove definitely; the diagnosis has to be made by exclusion of any other causes and by inference from the circumstances. The necropsy findings are mainly unspecific organ changes in the liver, the adrenals and the alimentary canal caused by stress (see also Fischer and Spann, 1965; Pugh, 1966). Exposure to cold environment is a threat against the maintenance of homeothermia. To fight the cooling, the organism reduces the loss of heat and increases its production. An experimental study of the latter aspect in rats is reported in this paper.

Contribution of fat from the adipose tissue is essential for the maintenance of the body temperature in cold exposure. Muscles, the liver and the heart produce heat by oxidizing fatty acids liberated from the adipose tissue. The brown adipose tissue — but not the white — is in itself able to oxidize fatty acids and to produce heat (for literature, see Smith and Horwitz, 1969).

Fat depletion in the brown adipose tissue of the rat occurs within 2 hrs of severe cold exposure (Cameron and Smith, 1965). In extreme conditions resulting in death the process is more emphasized.

This was proved by the present experiments, where the rats were kept in cold until dead. Both the brown and the white adipose tissue was obtained from the interscapular region.

Materials and Methods

Experimental Animals. Forty adult (weight 195—250 g) Sprague-Dawley rats of both sexes were used. The rats were deprived of food for 16 hrs before the experiment. Half of the rats were exposed to cold in a box at —20°C until dead, which took 3—5 hrs. The other half served as controls, and they were kept at room temperature (21°C) until killed.

After death, the interscapular growths of brown and white adipose tissue were excised and cut to pieces.

Histology. One piece of the brown fat sample, which also contained a small amount of white fat, was quenched in liquid nitrogen. Sections at 7 micra were cut in a cryostat (—20°C), fixed overnight in 10% buffered formalin, and stained with Oil Red O for the lipids (Pearse, 1969). Counterstaining was made with hematoxylin.

Lipid Studies. After the sample had been weighed, the lipids were extracted into a 2:1 mixture of chloroform and methanol (Folch *et al.*, 1957). After grinding, the ratio of tissue to solvent was adjusted to be 1:20, and the homogenate was filtered. The filtrate was washed once with 1/5 volume of saline. The chloroform phase was separated and diluted to obtain the original ratio.

The Amount of Total Lipids. 0.5 ml of the extract was evoporated in preweighed tubes and the remainder was reweighed. The percentage of lipids of the tissue wet weight was calculated. The mean and the standard deviation of each group were determined. The results were analysed by the *t*-test.

Thin Layer Chromatography. 50 µl of the chloroform extract was pipetted on the plate. Thus the samples were comparable with each other. The plates were coated with 250 µ of silica gel (Kieselgel G, Merck). The solvent was a mixture of light ether, ether, acetic acid, and water (75:25:1:1). The developing time was 40 min. The spots were visualized by spraying the plates with ammonium sulphate sulfuric acid and heating them at 180°C for 1 hr. For identification of the lipid fractions, a mixture of reference compounds were run on each plate.

The references were: Cholesterol (Merck), Cholesterol palmitate (Merck), Triolein (Calbiochem), Lecithin (Merck), Palmitic acid (Merck).

Results

Histology

Cold-exposed rats showed changes in the brown fat. Lipid droplets had disappeared from the multilocular cells in almost every case. In some cases peripherally located multilocular fat cells contained traces of lipid material. Unilocular fat cells at the periphery of the fat lobules, on the other hand, contained plenty of fat. No sign of depletion was noticed there either (Fig. 1). The white adipose tissue was always intact.

Control rats had always plenty of lipid droplets in the multi- and unilocular fat cells of the brown fat (Fig. 1). The white fat cells were similarly always full of lipid.

Amount of Total Lipids

The depletion of fat from the brown fat was confirmed quantitatively. The mean lipid content in the cold-exposed rats was about one half of that in the controls. No depletion had occurred in the white fat (Table 1). The decline of total lipids in the brown fat was statistically highly significant.

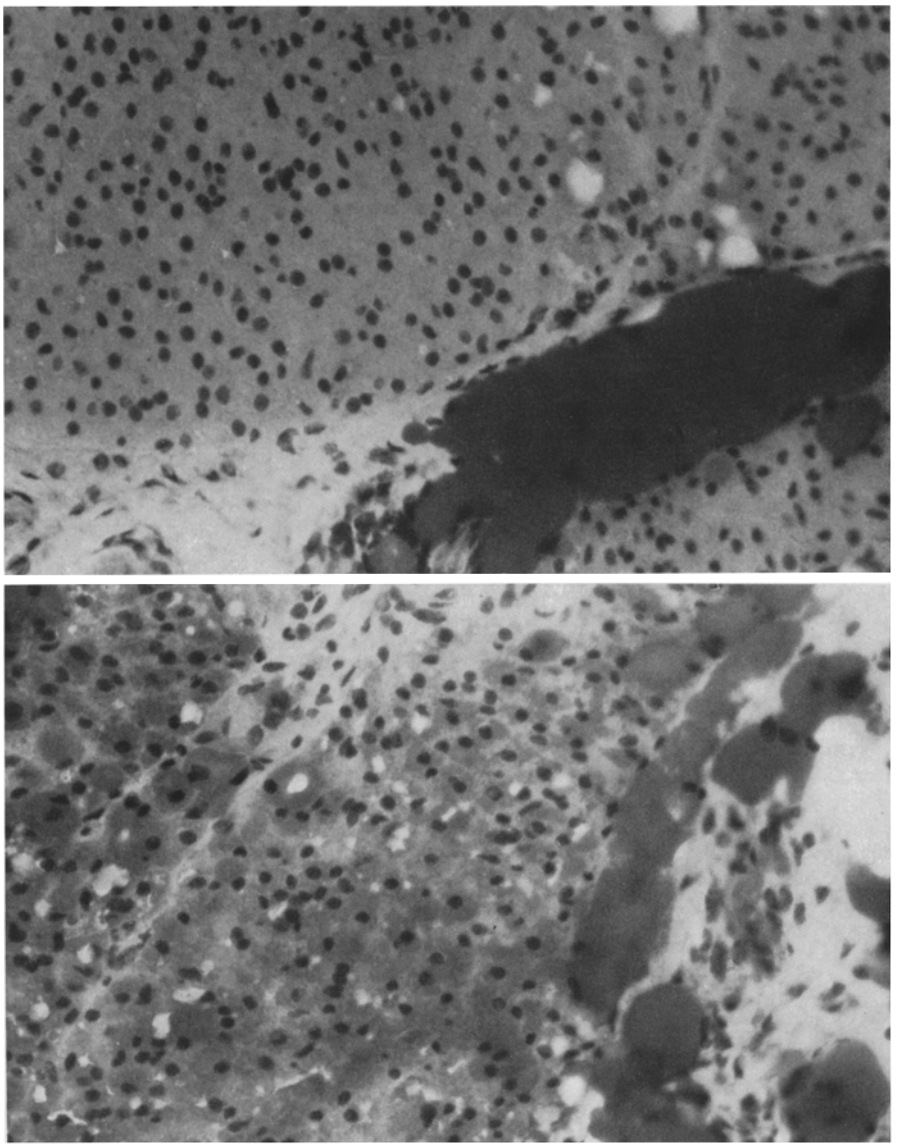

Fig. 1. *Top:* Interscapular adipose tissue of a rat dead from hypothermia. The brown fat cells (upper part) are completely devoid of lipids. The unilocular white fat cells (lower corner right) are full of fat. Oil Red O, 250 ×. *Bottom:* Interscapular adipose tissue of a control rat. Both the brown (left) and the white (right) fat cells are filled with lipid droplets. Oil Red O, 250 ×

Thin Layer Chromatography

Lipid pattern: Triglycerides (TG) were in general more abundant in the white fat than in the brown. A small spot of FFA and distinct spots of TG and cholesterol were found in both types of fat. Phospholipids were more abundantly present in the brown fat extractions. In addition, faint spots beneath FFA were also visible,

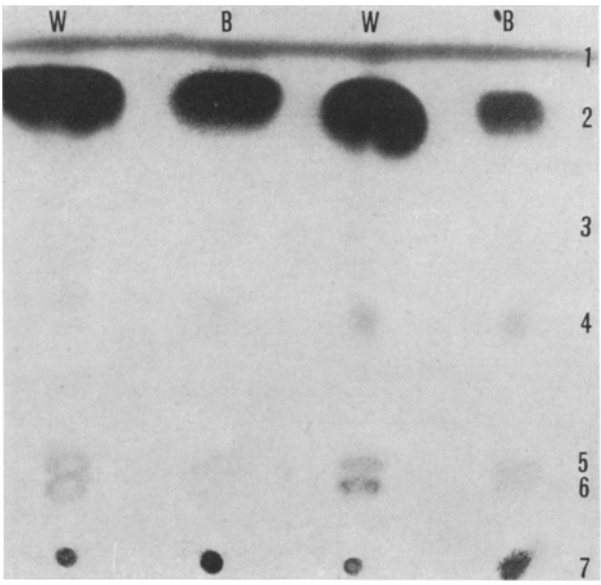

Fig. 2. Thin layer chromatography of the white and the brown adipose tissue. *1* solvent front, *2* triglycerides, *3* traces of free fatty acids, *4* plasmalogens (?), *5* diglycerides, *6* free cholesterol, *7* phospholipids and start. The TG spot of brown fat (*B*) from a hypothermic rat (at the right) is smaller than the corresponding spot of the control rat. No difference can be seen between the TG spots of white adipose tissue (*W*)

Table 1. *Amount of total lipids in the brown and the white adipose tissue. The figures express the percentage of lipids of the tissue wet weight*

	Brown fat mean ± SD		White fat mean ± SD
Cold-exposed 20 rats	7.5 ± 4.6	$p < 0.001$	41.7 ± 12.5
Controls 20 rats	15.7 ± 7.1		40.2 ± 8.6

more distinctly in the brown fat. On the basis of our earlier experience it was assumed to be plasmalogen, but because of the lack of reference material, no final identification could be made. The TG spots of the brown fat were smaller in the cold-exposed rats than in the controls (Fig. 2). This finding confirms the microscopic observation of the disappearance of fat from the multilocular brown fat cells and the presence of lipids in the unilocular cells.

Discussion

The present results confirmed the assumption that multilocular brown adipose tissue cells react strongly to cold exposure by releasing triglycerides. In the experiments resulting in death, the depletion of fat was complete or almost

complete. The unilocular white adipose tissue cells of the same region were not depleted of any fat, which could be shown both microscopically and quantitatively. Thus the difference between the two types of adipose tissue became very clear even in the extreme conditions employed.

The death occurred in an average of 4—5 hrs. Earlier experiments have shown that depletion of fat begins within 2 hrs in the brown fat cells (Cameron and Smith, 1964; Derry *et al.*, 1970). In the present experiments the rats endured quite well for about 3 hrs, after which the decline was rapid. This suggests that the fuel reserves were used up at that moment, and the depletion of fat from brown fat was rapidly followed by death. This question will be studied in future experiments. The survival of man in cold is dependent both on shivering and increased metabolism (e.g. Burton and Edholm, 1969).

The metabolism of fats is different in man from that in rat. Brown fat is only occasionally present in adults (Simon, 1962; Hassi, 1971). Its significance in severe cold stress is not known. Release of fats into the blood during cold exposure also occurs in man which is demonstrated by elevated FFA, triglycerides, and particularly glycerol levels in the plasma (Wilson *et al.*, 1969). It seems that these fats are derived from the white adipose tissue in man.

Release of fats from the adipose tissue is triggered by adrenergic nerves. Noradrenaline from the nerve endings activates lipase, which splits triglycerides. Liberation of fats by cold can be prevented by beta-receptor blocking drugs, e.g. isoproterenol (Derry *et al.*, 1971). Intact beta-receptors are essential for the survival of rats in cold (Estler and Ammon, 1969). Similar mechanisms probably also exist in man. Injection of catecholamines elevates serum FFA (Havel and Goldfien, 1959). Furthermore, the excretion of catecholamines into urine seems to increase during cold exposure (Wilson *et al.*, 1969). Lipolysis in human white adipose cells is enhanced by adrenaline and noradrenaline (Östman *et al.*, 1969).

Liberation of fatty acids into plasma is, however, not unique for cold stress. It also occurs in agonia (Gostomzyk and Frei, 1969) and after trauma (Warner, 1969), i.e.whenever extra fuel is needed for the production of energy by oxidation. Increased excretion of catecholamines is often present at the same time (Laves and Berg, 1965).

As regards the diagnosis of genuine hypothermia death, a great deal of investigation on specific questions is needed. At the present state of knowledge it is apparent that many tests must be applied; assays of serum lipids and catecholamines, investigation of the adrenal glands, the adipose tissue, and the liver to mention the most important ones. Any actual situtation is further complicated by the intake of alcohol and physical exhaustion, two stress conditions that can trigger the same physiologic mechanisms as cold exposure.

References

Burton, A. C., Edholm, O. G.: Man in a cold enviroment. New York: Hafner Co. 1955 (reprinted 1969).

Cameron, I., Smith, R. E.: Cytological responses of brown fat in cold exposed rats. J. Cell Biol. **23**, 89—100 (1964).

Derry, D. M., Ranson, J., Morrow, E.: Effect of isopoterenol on brown and white fat of the adult rat. Can. J. Physiol. Pharmacol. **49**, 8—13 (1971).

Estler, C.-J., Ammon, H. P. T.: The importance of the adrenergic beta-receptors for thermogenesis and survival of acutely cold-exposed mice. Can. J. Physiol. Pharmacol. **47**, 427—434 (1969).

Fischer, H., Spann, W.: Pathologie des Trauma. München: Bergmann 1967.

Folch, J., Lees, M., Stanley, G. H. S.: A simple method for the isolation and purification of total lipides from animal tissue. J. biol. Chem. **226**, 497—509 (1957).

Gostomzyk, J. G., Frei, G. F.: Unveresterte freie Fettsäuren im Serum und agonales Kreislaufversagen. Z. klin. Chem. **7**, 505—508 (1969).

Hassi, J.: Histochemical investigation on brown adipose tissue (BAT) in man at different ages. Scand. J. clin. Lab. Invest. 27., Suppl. **116**, 4 (1971).

Havel, R. J., Goldfien, A.: The role of the sympathetic nervous system in the metabolism of free fatty acids. J. Lipid. Res. **1**, 102—108 (1959).

Laves, W., Berg, S.: Physiologisch-chemische Untersuchungen bei gewaltsamen Todesarten. Agonie. Lübeck: Schmidt-Römhild 1965.

Maickel, R. P., Matussek, N., Stern, D. N., Brodie, B. B.: The sympathetic nervous system as a homeostatic mechanism. I. Absolute need for sympathetic nervous function in body temperature maintenance of cold-exposed rats. J. Pharmacol. exp. Ther. **157**, 103—110 (1967).

Pearse, A. G. E.: Histochemistry. Theoretical and applied, 3. Ed. Vol. 1. London: Churchill 1968.

Pugh, L. G. C. E.: Accidental hypothermia in walkers, climbers, and campers: Report to the medical commission on accident prevention. Brit. med. J. **1966 I**, 123—129.

Simon, G.: Genise et structure du tissu adipeux chez l'homme. Acta anat. (Basel) **48**, 232—241 (1962).

Smith, R. E., Horwitz, B. A.: Brown fat and thermogenesis. Physiol. Rev. **49**, 330—425 (1969).

Warner, W. A.: Release of free fatty acids following trauma. J. Trauma **9**, 692—699 (1969).

Wilson, O., Laurell, S., Tibbling, G.: Effect of acute cold exposure on blood lipids in man. Fed. Proc. **28**, 1209—1215 (1969).

Östman, J., Efendić, S., Arner, P.: Catecholamines and metabolism of human adipose tissue. Acta med. scand. **186**, 241—246 (1969).

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