At strong alkaline pH ferricytochrome *c* is also a low-spin complex. This spectrum shows 3 *g* values: $g_1 = 2.73$; $g_2 = 2.14$; $g_3 = 1.77$. But the individual lines are much sharpened. A strong acid ferricytochrome *c* solution (pH 0.7) shows 2 absorption centres. One has a *g* value near 6 and the other near 2. The absorption with the *g* value 6.3 originates from a high-spin state. Such an absorption is also typical for other hemoproteins in the high-spin state[3,9]. The absorption at $g = 2.1$ may originate from some low-spin state content. Therefore acid ferricytochrome *c* is not a pure high-spin complex but a thermal equilibrium between the high-spin and the low-spin state. This result is in accordance with the magnetic susceptibility of this compound measured by BOERI, EHRENBERG, PAUL and THEORELL[10]. Ferricytochrome *c* compounds as well as azide and cyanide compounds give electron spin resonance spectra with 3 *g* values nearby 2 which are typical for low-spin complexes. The *g* values for the azide compound from ferricytochrome *c* are $g_1 = 2.77$; $g_2 = 2.27$; $g_3 = 1.85$ and the *g* values for the cyanide compound are $g_1 = 3.04$; $g_2 = 2.30$; $g_3 = 2.01$. It is of interest that the azide ligand reacts better with ferricytochrome *c* at a neutral pH than in alkaline solution. GEORGE and co-workers[11] also found that the equilibrium constant of ferricytochrome *c* azide compound enlarges in the acid range. The fluoride compound of ferricytochrome *c* gives a pure high-spin complex spectrum with a large absorption at $g = 6.1$ and a small absorption with a *g* value of 1.97. As ferricytochrome *c* possesses a large pK value and the fluoride ion is bound only in acid solution[12] the fluoride compound of ferricytochrome *c* was generated by adding KHF_2.

A comparison of the electron spin resonance spectrum of ferricytochrome *c* about pH 7 with other hemoproteins such as hemoglobin[3], peroxydase[4] and catalase[9] shows that ferricytochrome *c* is more in the low-spin state than the other hemoproteins. This can be explained by the ferrihemochromogenic binding of the fifth and sixth coordination groups of the iron which is favoured by the crevice structure of the cytochrome *c* protein in which the hem disc is placed.

Zusammenfassung. Ferricytochrom *c* aus Pferdeherz wurde bei einer Temperatur von 77°K mit der Methode der Elektronenspinresonanz untersucht. Aus den Elektronenspinresonanzspektren ist zu entnehmen, dass Ferricytochrom *c* bei neutralem und alkalischem pH ein Low-spin-Komplex ist, bei stark saurem pH ist es dagegen ein Mischkomplex der High- und Low-spin-Form. Ferricytochrom-*c*-Fluorid ist ein reiner High-spin-Komplex; dagegen sind die Azid- und Zyanidkomplexe Low-spin-Komplexe.

H. REIN, O. RISTAU and F. JUNG

Institute of Pharmacology, German Academy of Sciences, Berlin-Buch (DDR),
26 January 1968.

[9] F. HACKENBERGER, O. RISTAU and H. REIN, Studia biophys. *2*, 287 (1967).
[10] E. BOERI, A. EHRENBERG, K. G. PAUL and H. THEORELL, Biochim. biophys. Acta *12*, 273 (1953).
[11] P. GEORGE, S. C. GLAUSER and A. SCHEJTER, J. biol. Chem. *242*, 1690 (1967).
[12] H. THEORELL, Ergebn. Enzymforsch. *9*, 232 (1943).

Brown Adipose Tissue in Young Mice: Activity and Role in Thermoregulation

The distribution, structure and function of brown adipose tissue has been widely studied in the last few years[1-3]. Its role in hibernation, cold acclimation and especially in the heat production of young animals has been reviewed by HULL[4].

The present study is concerned with the development of interscapular brown adipose tissue in young mice. The succinic dehydrogenase activity in this tissue was measured at different age levels. Local temperature measurements from brain, brown body and subcutis were also performed.

Material and methods. 78 young mice or fetuses, both of NMRI-strain, were used. The number of animals in each age group was 6–12. The interscapular brown fat was removed and weighed. The tissue sections (embedded in paraffin wax) were stained with hematoxylin-eosin. The activity of succinic dehydrogenase complex in tissue homogenates was measured by the method of KUN and ABOOD[5] as described earlier[6]. The incubation was carried out in Thunberg tubes because of the small amount of tissue (1% homogenate). The temperature of brain, brown fat (neck) and caudal part of back was measured using the 'Ellab' (Copenhagen) thermogalvanometer. The thermocouples were placed s.c., and in the brain in the hypothalamus reached through the posterior fontanella. The environmental temperature was at the beginning of the experiment 35°C and the animals were then removed to 26°C.

Results. Figure 1 shows the weight of interscapular brown fat and its succinic dehydrogenase activity at different age levels. The relative weight of brown fat (mg/100 g) has a maximum at birth; it then rapidly decreases and reaches a minimum in 5-day-old mice. The succinic dehydrogenase activity in the brown fat is at maximum and minimum almost simultaneously with the weight. In addition, at the age of 2 weeks it has a second maximum.

It appears from histological examination that the lipid content of the cells and the size of lipid vacuoles show the same trend as was seen in the weight of brown fat and enzyme activity. The lipid vacuoles are large and numerous at birth. At the age of 4 days they decrease in size and

[1] L. NAPOLITANO and D. FAWCETT, J. Biophys. Biochem. Cytol. *4*, 685 (1958).
[2] B. JOHANSSON, Metabolism *8*, 221 (1959).
[3] D. HULL and M. M. SEGALL, Nature *29*, 469 (1966).
[4] D. HULL, Br. med. Bull. *22*, 92 (1966).
[5] E. KUN and L. G. ABOOD, Science *109*, 144 (1949).
[6] K. LAGERSPETZ, H. TARKKONEN and R. TIRRI, Experientia *18*, 552 (1962).

number. However, this development is rapidly reversed, and at the age of 10–13 days the histological picture is very similar to that found at the time of birth.

Temperature measurements from the brown fat and the back were performed with 25 mice aged from 1–10 days. At 35 °C the temperature of the brown body was on average 0.2 °C higher than that of back. At 26 °C both temperatures decreased continuously, but the decrease was smaller in the brown body. After 12 min of exposure to 26 °C the temperature difference between brown fat and back was on average 0.6 °C. The temperature measurements from brain and brown body were performed with 18 mice aged from 2–12 days. Figure 2 shows that in

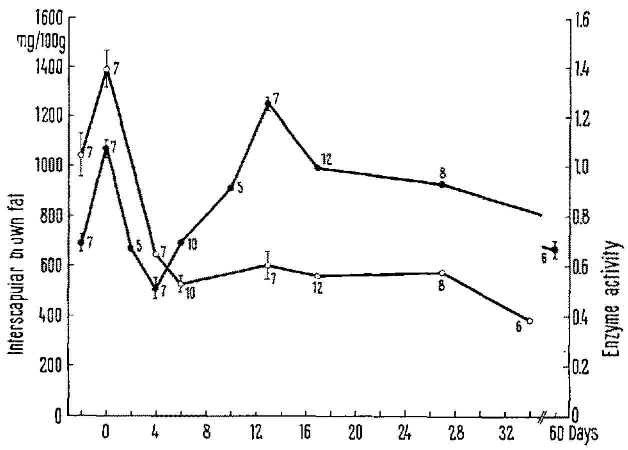

Fig. 1. The relative weight of interscapular brown fat of 19-day-old fetuses and young mice at different age levels (open circles). The activity of succinic dehydrogenase complex at same age levels (black circles). The enzyme activity is expressed as μg of reduced triphenyltetrazolium chloride (TTC)/mg of tissue fresh weight in 10 min.

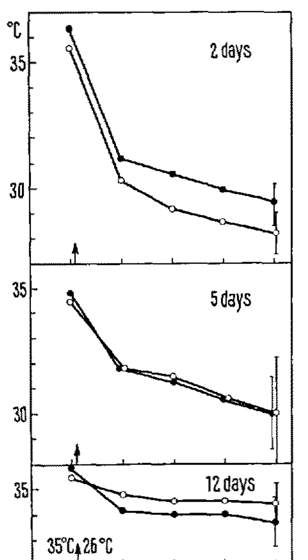

Fig. 2. Brain (black circles) and brown fat (open circles) temperatures in 2-, 5- and 12-day-old mice. At the arrow the animals were removed from 35–26 °C. Each symbol represents 6 animals. At the age of 2 days the temperature difference of brain and brown fat after 40 min at 26 °C is significant, $p < 0.05$, but not at the age of 12 days, $p < 0.2$.

2-day-old mice the brain temperature is 1.2 °C higher than that of brown fat at 26 °C. In 5-day-old mice there is no difference in brain and brown fat temperatures, but at the age of 12 days the brown body temperature is about 0.8 °C higher than that of brain at 26 °C.

Discussion. The changes in the weight, structure and succinic dehydrogenase activity of brown fat might well be explained in the light of the findings of NAPOLITANO and FAWCETT[1] and HULL[4]. Using electron microscopy, they have found that the lipid vacuoles in the brown fat of new-born mice and rats are large and numerous as well as tightly surrounded by large mitochondria and thus well suited for rapid oxidation of fats (see also SMITH[7]). When HULL exposed the rats to a cool environment for 48 h, the lipid vacuoles became very small and scattered about the cytoplasm, which was filled by numerous mitochondria. The correlation found in the present study between the succinic dehydrogenase activity and the lipid content of the brown fat during the postnatal development, obviously depends on the use of the stored lipids as the main substrate for oxidative metabolism.

The temperature measurements from brain, brown interscapular fat and subcutis of the back show that in young mice the brown adipose tissue does not respond to cool environment as strongly as in new-born rabbits[8] and guinea-pigs[9]. Also the relative weight of the interscapular brown fat is smaller in new-born mice (1.4 g/100 g) than in new-born rabbits (2.4 g/100 g)[8].

LAGERSPETZ[10] has reported that in young mice, which are almost poikilothermic, the motor activity is an important mechanism of heat production during the first postnatal days. It is obviously of greater importance in this respect than the brown adipose tissue. However, the heat production of the brown fat apparently contributes to the maintenance of the brain temperature at a higher level in young mice, which during the first 2 weeks of postnatal life, have relatively low body temperature[11]. At the age of 12 days the heat produced in the brown fat is perhaps more efficiently distributed and used in other parts of the body because of better insulation at that age.

Zusammenfassung. Gewicht und die Bernsteinsäuredehydrogenase-Aktivität des braunen interskapularen Fettgewebes wurden bei Foeten und jungen Mäusen gemessen. Beide Variabeln zeigen ein Maximum bei der Geburt und ein Minimum im Alter von 5 Tagen. Die Temperaturmessungen des Gehirns und des braunen Fettgewebes zeigen, dass bei neugeborenen Mäusen bei 26 °C die Temperatur des Gehirns höher ist als diejenige des braunen Fettgewebes, während im Alter von 12 Tagen das braune Fettgewebe wärmer ist.

H. TARKKONEN and HELKA JULKU

Zoophysiological Laboratory, Department of Zoology of the University, Turku 2 (Finland), 12 March 1968.

[7] R. E. SMITH, JANE ROBERTS and K. J. HITTELMAN, Science *154*, 653 (1966).
[8] M. R. J. DAWKINS and D. HULL, J. Physiol. *169*, 101P (1963).
[9] K. BRÜCK, Naturwissenschaften *54*, 156 (1967).
[10] K. LAGERSPETZ, Annls Med. exp. Biol. Fenn. *44*, 71 (1966).
[11] K. LAGERSPETZ, Experientia *18*, 282 (1962).