The Ultrastructure of Brown Adipose Tissue in Perinatal Rats

An early electron microscopic investigation of brown adipose tissue (BAT) from newborn rats[1] has shown that many of its mitochondria contain large matrix inclusions, while these are missing in mature adipocytes from adult rats[2]. In the present study the perinatal development of BAT was investigated to obtain information on the evolution and the eventual fate of the mitochondrial matrix inclusions.

Interscapular brown adipose tissue was fixed in ice cold 3% phosphate buffered glutaraldehyde and post-fixed at 4 °C in 1% osmium tetroxide in Millonig buffer, dehydrated in ethanol and embedded in Epon. Sections were double stained with uranyl acetate and lead citrate.

In 18 day fetuses (4 days before term) brown adipocytes can first be distinguished from undifferentiated connective tissue cells by small deposits of glycogen particles and the presence of lipid droplets. At this early stage already some of the mitochondria, which are rather large, contain inclusions measuring 400–1200 Å. They subsequently increase in number and grow in size. At the time of birth their development reaches its peak and they can be seen, often in multiples, in most mitochondria of many cells. A maximum number of 10 have been observed in a single organelle. Their average diameter now measures 1500 Å (900–2000). Their structure has become more complex; most of them appear to consist of tubular elements wound into tight balls (Figure 1). During prenatal development mitochondria also become more numerous; masses of glycogen accumulate and lipid droplets increase considerably in size. Within 1–2 days after birth mitochondrial inclusions disappear (Figure 2). During this time much smaller and less structured elements, which might be residues of inclusions, are often seen in the matrix. Mitochondria increase further in size and in many oblong forms cristae are reoriented from a mainly longitudinal to a predominantly transverse direction; glycogen disappears within several hours (compare Figures 1 and 2).

BAT is abundant in neonatal mammals[3,4] and probably has an important thermogenetic function during the period of adaptation to extrauterine life[5]. The question therefore arises whether the postnatal ultrastructural changes in BAT might, at least in part, be due to the sudden drop in environmental temperature from an intrauterine temperature of 37 °C to a nest temperature of about 32 °C. If this is the case, then exposure of neonatal rats to a cold environment should accelerate, and exposure to a warm environment should retard the devolvement of the postnatal cellular transformation in BAT. Experiments done to test this supposition gave confirmative results. All the changes, comprising the disappearance of the inclusions, which normally occur in BAT within 1–2 days, are achieved within the limits of 3 h when neonates are exposed to 20–22 °C, whereas BAT remains unaltered, except for a small decrease in glycogen, during exposure to 37 °C for 3 h.

Results of further experiments are in accord with the available physiologic evidence implicating norepinephrine as the mediator of cold-induced changes in BAT[6–8]. By treating new-born rats with norepinephrine (0.5 mg/kg s.c.) and keeping them in a warm environment of 37 °C, changes are induced in BAT within 3 h which closely reproduce the cold effect. The β-blocking agent Trasicor®[9] (50 mg/kg s.c.) abolishes the effect of cold exposure.

Theophylline (120 mg/kg s.c.) also can substitute for cold exposure: within 3 h it leads to disappearance of most matrix inclusions, reorientation of mitochondrial cristae, as well as glycogen and lipid loss from BAT of neonatal rats kept warm, indicating that cyclic 3′, 5′-adenosine monophosphate may mediate the response.

The results from these in vivo experiments are confirmed by evidence obtained from experiments in vitro. Brown adipocytes from fat pads incubated in Krebs Ringer phosphate for 1 h at 37 °C in presence of norepinephrine ($5 \times 10^{-6} M$) are transformed similarly as in vivo. In presence of Trasicor® ($2.5 \times 10^{-5} M$) norepinephrine is ineffective. Theophylline (10 mM) and dibutyryladenosine 3′, 5′-phosphate (5 or 10 mM) also produce changes in vitro which mimic the effect of cold in vivo.

It appears from the present study that norepinephrine liberated in the course of cold exposure of new-born rats may act on adenyl cyclase of brown adipocytes. Increased cellular levels of cyclic adenosine monophosphate may lead to activation of phosphorylase and lipase, thus supplying the cells with the necessary substrates for

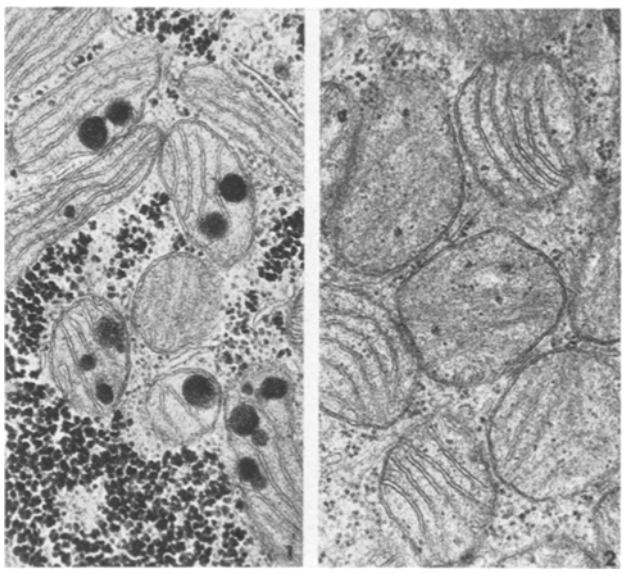


Fig. 1. Brown adipocyte from a new-born rat. Several mitochondria contain large matrix inclusions. In most of them a tubular substructure is visible. In filamentous mitochondrial profiles the cristae are oriented lengthwise. 2 kinds of particles occur in the cytoplasmic matrix, the smaller less dense ones are ribosomes, the larger black appearing ones are glycogen granules. × 49,000.

Fig. 2. Brown adipocyte from a 17 h old rat. The mitochondrial matrix inclusions have disappeared and in their place much smaller granular elements are visible. The mitochondria are somewhat larger and more nearly round. The cytoplasm contains only ribosomes, and no glycogen remains. × 49,000.

[1] L. Napolitano and D. Fawcett, J. biochem. biophys. Cytol. *4*, 685 (1958).
[2] E. R. Suter, J. Ultrastruct. Res., in press.
[3] K. Brück, Naturwissenschaften *54*, 156 (1967).
[4] D. Hull and M. M. Segall, J. Physiol. *181*, 449 (1965).
[5] R. E. Smith, Science *146*, 1686 (1964).
[6] R. E. Moore and M. C. Underwood, J. Physiol. *168*, 290 (1963).
[7] T. Heim and D. Hull, J. Physiol. *187*, 271 (1966).
[8] E. Zeisberger and K. Brück, Pflüger's Arch. ges. Physiol. *296*, 263 (1967).
[9] H. Brunner, P. R. Hedwall and M. Meier, Arzneimittel-Forsch. *18*, 164 (1968).

thermogenesis. Surprisingly, cyclic adenosine monophosphate also seems to mediate postnatal changes in mitochondrial structure. How these could be related to mitochondrial function is at present unknown. Possibly they might be the expression of increased metabolic or thermogenetic capacity[10].

Zusammenfassung. Die prä- und postnatale Entwicklung des braunen Fettes von Ratten wurde untersucht unter besonderer Berücksichtigung der Matrixkörper der Mitochondrien. Diese Einschlusskörper erreichen im Zeitpunkt der Geburt hinsichtlich Grösse und Zahl ein Maximum und verschwinden dann innerhalb von 1–2 Tagen. In vivo und in vitro Experimente mit verschiedenen Umgebungstemperaturen, mit Noradrenalin, Theophyllin, einem β-Rezeptorenblocker (Trasicor®) und Dibutyryl-Adenosinmonophosphat weisen alle darauf hin, dass die physiologischen postnatalen Veränderungen im braunen Fett wenigstens zum Teil mit dem plötzlichen Temperaturabfall zusammenhängen und hormonal ausgelöst werden. Die Matrixkörper sind unter allen Versuchsbedingungen an den Strukturveränderungen der Mitochondrien beteiligt.

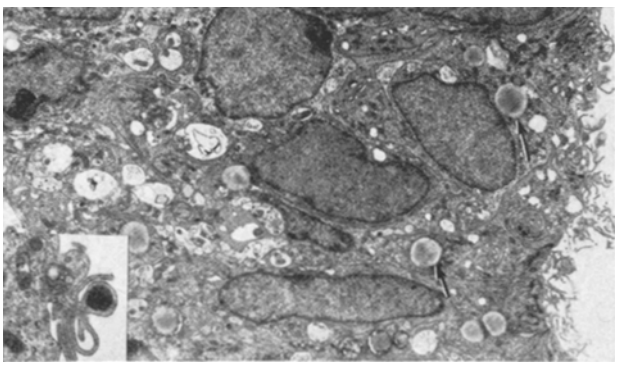
ELSI R. SUTER

Research Laboratories of the Pharmaceutical Department, CIBA Limited, Basel (Switzerland), 23 December 1968.

[10] The author wishes to thank Dr. W. STÄUBLI for his valuable criticism and to acknowledge the excellent technical assistance of Miss URSULA MANN and Miss BARBARA GAERISCH.

Über ein spezielles Ependym im 3. Ventrikel der Ratte

Elektronenmikroskopische Untersuchungen am Ependym verschiedener Tiere haben gezeigt, dass es sich um ein Gewebe mit vielen Besonderheiten handelt[1–3]. Entsprechende Untersuchungen bei der Ratte sind bisher nur in geringem Umfang durchgeführt worden[4,5]. Vorliegende Mitteilung soll diese Lücke schliessen helfen und berichtet über spezielle Ependymzellen an der Seitenwand des 3. Ventrikels über dem Nucleus arcuatus. Das Ependym ist hier mehrreihig, stark verzahnt und besteht aus Tanizyten, die durch etwa 1 μ grosse mit homogenem Inhalt gefüllte Blasen ausgezeichnet sind (Figur). Diese Blasen haben keine nähere Beziehung zu den anderen Zellorganellen der Ependymzellen. Ausserdem fallen in den an der freien Zelloberfläche gelegenen häufig bizarr gestalteten Cytoplasmavorstülpungen dunkle Einschlüsse auf (Figur, Inset). Ob es sich bei den Blasen und Einschlüssen um Zellstrukturen im Dienst sekretorischer oder resorptiver Vorgänge handelt, kann bisher nicht entschieden werden. Bemerkenswert ist jedenfalls, dass das endoplasmatische Retikulum dieser Zellen relativ spärlich entwickelt ist, dafür aber zahlreiche freie Ribosomen vorkommen. Die Mitochondrien sind stabförmig und cristareich. Abgesehen von diesen zytologischen Besonderheiten, ist die innige Verbindung dieser Ependymzellen mit zahlreichen marklosen Nervenfasern kennzeichnend. Diese liegen teils zwischen den Zellen, teils stülpen sie sich in das Zytoplasma der Ependymzellen ein. Einige Nervenfortsätze erreichen fast die Ventrikeloberfläche. Beim Vergleich der verschiedenen Nervenendigungen können solche mit typischen synaptischen Bläschen von anderen mit Bläschen dichten Inhaltes unterschieden werden. Offen bleiben muss, ob es sich in letzterem Fall um Elementargranula (neurosekretorische Granula) oder katecholaminhaltige Vesikel handelt. Schliesslich fällt auf, dass sich zwischen die Oberflächenzellen Tanizytenfortsätze schieben, die von tiefer gelegenen Zellen stammen und das Ventrikellumen erreichen. Zusammengenommen gleicht die geschilderte Ependymregion einem neuroependymalen Kontaktfeld, wie es in dieser Ausprägung bisher von keinem anderen Ependymbezirk der Ratte bekannt ist[6].



Ependym über dem Nucleus arcuatus einer erwachsenen Ratte. Beachte die verschiedenen Blasen mit homogenem Inhalt (Pfeil). Gesamtvergrösserung × 5700. Inset: stark vergrösserte Zytoplasmafortsätze der Zelloberfläche mit dichtem Einschluss.

Summary. The ependyma cells situated above the nucleus arcuatus are characterized in the rat by various peculiar vesicles and close interdigitations with nerve fibres. Possibly, here is a field of neuroependymal contact.

A. MITRO

Anatomisches Institut der Universität, 87 Würzburg (Deutschland), 18. November 1968.

[1] V. M. TENNYSON and G. D. PAPPAS, Z. Zellforsch. mikrosk. Anat. *56*, 595 (1962).
[2] K. H. BLINZINGER, Acta neuropath., Berl. *1*, 527 (1962).
[3] H. LEONHARD, Z. Zellforsch. mikrosk. Anat. *74*, 1 (1966).
[4] M. W. BRITHMAN and S. L. PALAY, J. Cell Biol. *19*, 415 (1963).
[5] A. HIRANO and H. M. ZIMMERMANN, Anat. Rec. *158*, 293 (1967).
[6] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.