*Biochimica et Biophysica Acta*, 1015 (1990) 441–449
Elsevier



BBABIO 43144

# Postnatal appearance of uncoupling protein and formation of thermogenic mitochondria in hamster brown adipose tissue

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(Received 26 June 1989)
(Revised manuscript received 29 September 1989)

Key words: Uncoupling protein synthesis; Brown adipose tissue; Thermogenesis; Mitochondrion; Neonatal

**Brown adipose tissue of developing hamster was characterized by western blotting, enzyme activity measurements and immunoelectron microscopy. During the first postnatal week the tissue contained significant amounts of differentiating mitochondria and comparable quantities of active cytochrome oxidase and ATP synthase. The uncoupling protein appeared on the 7/8th day and its specific content increased 80-times between day 8 and day 17. In parallel, the specific content and activity of cytochrome oxidase increased 3-times but ATP synthase decreased 2-times. The total content of uncoupling protein and of cytochrome oxidase in interscapular brown adipose tissue increased 360- and 11-times, respectively. Analysis of isolated mitochondria showed that the observed differences result mainly from changes of the enzymic equipment of the mitochondrial membrane. During the same interval, propylthiouracil-insensitive 'type II' thyroxine 5′-deiodinase activity in brown adipose tissue increased 10-times. It was concluded that the thermogenic function of the hamster brown adipose tissue develops after the first postnatal week due to highly differentiated synthesis of mitochondrial proteins leading to replacement of preexisting, uncoupling protein-lacking nonthermogenic mitochondria by thermogenic ones, similarly as shown in brown adipose tissue of the embryonic mouse and rat (Houštěk, J., et al. (1988) Biochim. Biophys. Acta 935, 19–25).**

## Introduction

Brown adipose tissue thermogenesis represents the major defence mechanism against cold in many newborn mammals [1,2]. Depending on animal species, state of development, thermal and dietetic conditions, the thermogenic potential of brown adipose tissue has to be subtly regulated [2]. The main prerequisite of intensive heat production are functionally competent thermogenic mitochondria. These are characterized by low ATPase activity and high oxidative capacity [3] paralleled by a high content of the critical thermogenic component – the tissue-specific uncoupling protein [4]. Obviously, the changes in enzymic equipment of the mitochondrial membrane could represent one of the major regulatory mechanisms.

It has recently been demonstrated [5] in embryonic mouse and rat that the thermogenic ability and energetics of brown adipose tissue change very substantially within a few hours or days. A characteristic feature of this process is an increase of respiratory enzymes, decrease of ATPase and most importantly, the occurrence and intensive synthesis of the uncoupling protein.

In contrast to the mouse and rat, representing the altricical type of neonate [6], newborn hamsters are immature, with respect to thermoregulation in particular [2]. In fact, immediately after birth they behave rather like poikilotherms [7,8] and one of the reasons appears to be the late development of brown adipose tissue thermogenesis [2,6,9]. In the present report it is shown that the thermogenic function of brown adipose tissue does not begin to develop until after the first postnatal week. Similarly to recruitment of thermogenesis in the mouse and rat [5,6], the critical event is the initiation of synthesis of the uncoupling protein leading to the formation of thermogenic mitochondria.

Abbreviations: $F_1$, $F_1$-ATPase, catalytic part of mitochondrial $H^+$-translocating ATP synthase; $F_0F_1$, $H^+$-translocating ATP synthase; COX, cytochrome oxidase; UP, uncoupling protein.

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A part of this work has already been published as a symposium abstract [10].

## Materials and Methods

Pregnant golden syrian hamsters (*Mesocricetus auratus*) were kept in separate cages at room temperature and under natural lighting (March, April; 12–13/12–11 h, light/dark), with food (DOS 2B diet supplied with corn, sunflower and rye seeds) and water ad libitum. Newborn hamsters and pups of the required age (days after birth) were collected from several litters (one or two animals from each litter) and decapitated and the interscapular brown adipose tissue was dissected out. In some experiments, the brown adipose tissue of adult, cold-adapted hamsters (3 weeks at 4° C) and the liver of adult, warm-adapted (22° C) rats (Wistar I pav) were also used.

Homogenates (10%) were prepared from pooled tissue samples from 9–12 animals in each group using 0.25 M sucrose/10 mM Tris-HCl/1 mM EDTA (pH 7.4). The same medium was used for isolation of mitochondria [11]. For morphological studies, the tissue was excised from animals with body weight corresponding to the mean body weight in a given age group.

The content of the uncoupling protein, cytochrome oxidase and $F_1$-ATPase in aliquots of homogenates (40 $\mu$g protein) or isolated mitochondria (10 $\mu$g protein) was measured by immunoblotting as described previously [5]. Specific antisera were raised in rabbits against isolated hamster uncoupling protein [5], rat heart cytochrome oxidase [12] and bovine heart $F_1$-ATPase [13]. Immunodecorated nitrocellulose sheets were scanned in reflected light (600 nm) and the content of each antigen was evaluated. $F_1$-ATPase was quantified from the content of $\alpha$ and $\beta$ subunits, cytochrome oxidase from the content of subunit II (see Fig. 4). As a standard, isolated mitochondria of cold-adapted adult hamsters, containing 0.7 nmol of uncoupling protein dimer ($2 \times 32$ kDa), 0.65 nmol cytochrome oxidase (200 kDa) and 0.02 nmol $F_1$-ATPase (380 kDa) per mg protein, were used.

Light and electron microscopy were performed as described previously [5]. Immunoelectron microscopy was performed using a modification of the described methods [14,15] as follows: The tissue was fixed for 24 h at 4° C in 8% paraformaldehyde, 0.12 M phosphate buffer (pH 7.3), washed for 15 min with PBS (0.15 M NaCl/0.02 M $NaHPO_4$ (pH 7.4)) and saturated with 2.1 M sucrose in PBS ($4 \times 5$ min). Ultrathin cryosections (50–80 nm) were saturated with 10% fetal calf serum in PBS (2 h at 4° C, 30 min at room temperature) and incubated with primary antibody for 30 min (anti-uncoupling protein or anti-$F_1$-ATPase rabbit antiserum diluted 1 : 200 with 5% fetal calf serum in PBS). After washing in PBS ($3 \times 5$ min) the sections were incubated for 25 min with secondary (goat anti-rabbit) antibody-colloidal gold probe (GAR G10 Jansen), diluted with 5% fetal calf serum 1 : 20. After washing with PBS ($6 \times 5$ min) and redistilled water ($3 \times 2$ min), the samples were contrasted by uranyl acetate-oxalate (0.15 M oxalic acid/2% 5′-uranylacetate (pH 7.5) ($NH_4OH$)), washed with redistilled water ($3 \times 3$ min) and impregnated by 1.8% methylcellulose and 0.3% uranyl acetate for 10 min. Samples were examined in a JEM 100B electron microscope.

Cytochrome oxidase activity was measured spectrophotometrically [16]. Hydrolytic activity of mitochondrial oligomycin-sensitive ATPase ($F_0F_1$-ATPase) was measured spectrophotometrically in the presence of ATP-regenerating system [17]. Thyroxine 5′-deiodinase activity was measured in the presence of propylthiouracil in aliquots of homogenates stored in liquid nitrogen using [3′,5′-$^{125}$I]thyroxine (1200 Ci/g; IZINTA, Hungary) purified by paper electrophoresis before the experiment [18]. Protein was measured by the method of Lowry et al. [19] using bovine serum albumin as standard.

## Results

### *Growth of brown adipose tissue during postnatal development*

The wet weight of interscapular brown adipose tissue (Fig. 1) increased 28-times (from 2 to 56 mg) and the total protein content 54-times (from 0.09 to 5.35 mg) between the first and 20th postnatal day. The most pronounced growth was noticed after the 9–10th day, following a plateau at the end of the first week. The relative contribution to the total body weight increased more than 2-times and the changes in brown adipose tissue/body weight ratio were biphasic, with the first maximum on the 6–7th day followed by a continuous increase after the 12th day.

### *Morphological changes of brown adipose tissue*

Differentiation of hamster brown adipose tissue started postnatally. During the first postnatal week cells had an unilocular appearance of white-fat-cells (Fig. 2A), but gradually small islands of typical multilocular brown adipocytes were formed, particularly near the lobe surface (Fig. 2B). During the second week the number of multilocular adipocytes quickly increased and after the 10th day they dominated over the unilocular cells (Figs. 2C and D). Most of the unilocular cells were occupied by a single large fat droplet and the cytoplasm with elongated nucleus formed a thin rim at the outer circumference (Fig. 2A–D asterisk). Further differentiation led to a gradual replacement of the single lipid droplet with numerous small droplets. However, typical multilocular adipocytes were also formed directly from undifferentiated preadipocytes (see Figs.

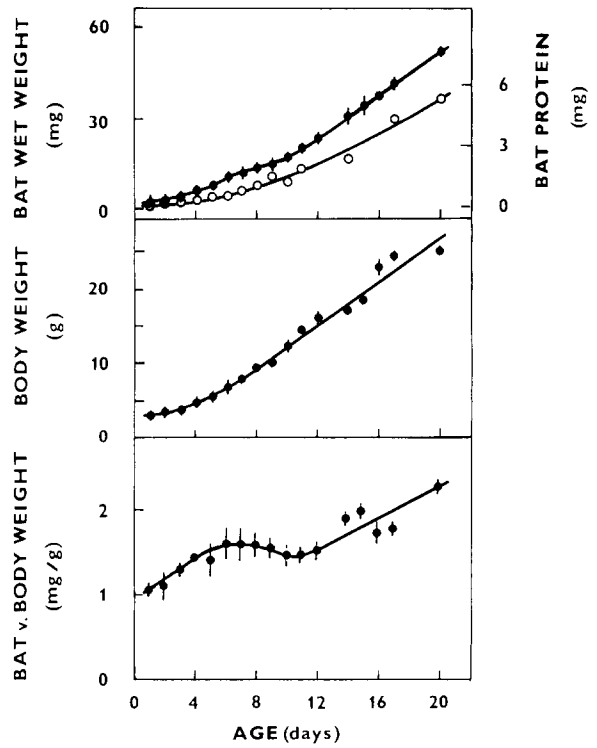


Fig. 1. Growth of interscapular brown adipose tissue (BAT) in hamster. The data (●) are the mean values ± S.E. from 5–12 animals in each age group except for BAT protein (○) which represents the average value calculated from protein content in homogenate of pooled interscapular BAT of 9–12 animals.

3A, 2A–D). This is in agreement with previous studies [20] showing that in hamster brown adipose tissue the mature adipocytes develop either from undifferentiated mesenchymal cells or by transformation of unilocular fat cells.

During the first postnatal week adipocytes contained only a few small mitochondria (Fig. 3A). During the second week both the size and number of mitochondria increased and numerous mitochondrial cristae developed (Fig. 3B, C). After the 14th day (Fig. 3D) mitochondria represented the most characteristic and dominating structures of brown adipocytes, filling most of the cytoplasmic space among the triacylglycerol droplets, to which they were often closely apposed. They become large and ovoid, with an average diameter of approximately 0.7 $\mu$m and numerous highly packed parallel cristae often extending through the whole mitochondrion. However, even at this stage some less differentiated mitochondria could be found.

*Content of cytochrome oxidase, the uncoupling protein and $F_1$-ATPase*

To quantify oxidative, phosphorylating and thermogenic capacity of the tissue we measured the content of cytochrome oxidase, $F_1$-ATPase and the uncoupling protein in homogenates by means of immunoblotting. In agreement with the appearance of mitochondria (see above), cytochrome oxidase and $F_1$-ATPase antigens were already present during the first postnatal week, while the antigen of the uncoupling protein was absent. The method used can detect less than 0.05 pmol of the uncoupling protein in one sample, which is sufficient to measure at least 300-times less of the uncoupling protein than in homogenates of adult hamster brown adipose tissue. As shown in Figs. 4 and 5, the uncoupling protein appeared on the 8th day and then rapidly increased, reaching a maximum on the 17th day. During this interval the specific content of uncoupling protein increased from 0.002 to 0.168 nmol/mg protein, and the total content (expressed per intercapsular brown adipose tissue in one animal) increased from 0.002 to 0.732 nmol. Similarly, the specific content of cytochrome oxidase increased from 0.043 to 0.119 nmol/mg protein and the total content from 0.047 to 0.518 nmol. The antiserum prepared against the rat heart enzyme [12] readily cross-reacted with subunits II, IV and V of the brown adipose tissue enzyme (see Fig. 4). No reaction with the smaller subunits was observed, although the antiserum cross-reacts with subunits VI and VII of the heart enzyme, possibly due to the tissue specificity of the isoenzyme present [21,22]. The development of $F_1$-ATPase (Fig. 4) was completely different. $F_1$-ATPase was already present during the first week, similarly as cytochrome oxidase, but its specific content continuously decreased between the 7th and 20th day from 0.008 to 0.004 nmol/mg protein. The corresponding total content changed from 0.009 to 0.021 nmol.

Small but sufficient amounts of mitochondria for immunoblotting experiments could be isolated from interscapular brown adipose tissue beginning with 7–8th postnatal day, this being at variance with Sundin et al. [9] who were unable to isolate mitochondria before the 12th day. As is shown in Figs. 4 and 5, changes in the specific content of the above three proteins were analogous to those found in homogenates. Between the 8th and 20th day the uncoupling protein and cytochrome oxidase increased almost in parallel from 0.078 to 0.5 and from 0.107 to 0.464 nmol/mg protein, respectively, while $F_1$-ATPase decreased from 0.026 to 0.014 nmol/mg protein. Apparently, the changing enzyme profile of the hamster brown adipose tissue results mostly from the changing equipment of the mitochondrial membrane. As indicated by $F_1$/COX ratio in homogenates and in isolated mitochondria in Fig. 5, a substantial part of the oxidative capacity can be used for ATP synthesis at the end of the first postnatal week. However, this ability later disappears as the $F_1$/COX ratio markedly decreases (13 times in the homogenate and 8 times in mitochondria until the 20th day). On the contrary, the UP/COX ratio in the homogenate rapidly increases (70-times) and a less pronounced increase is also found in isolated mitochondria. Thus, on the 20th

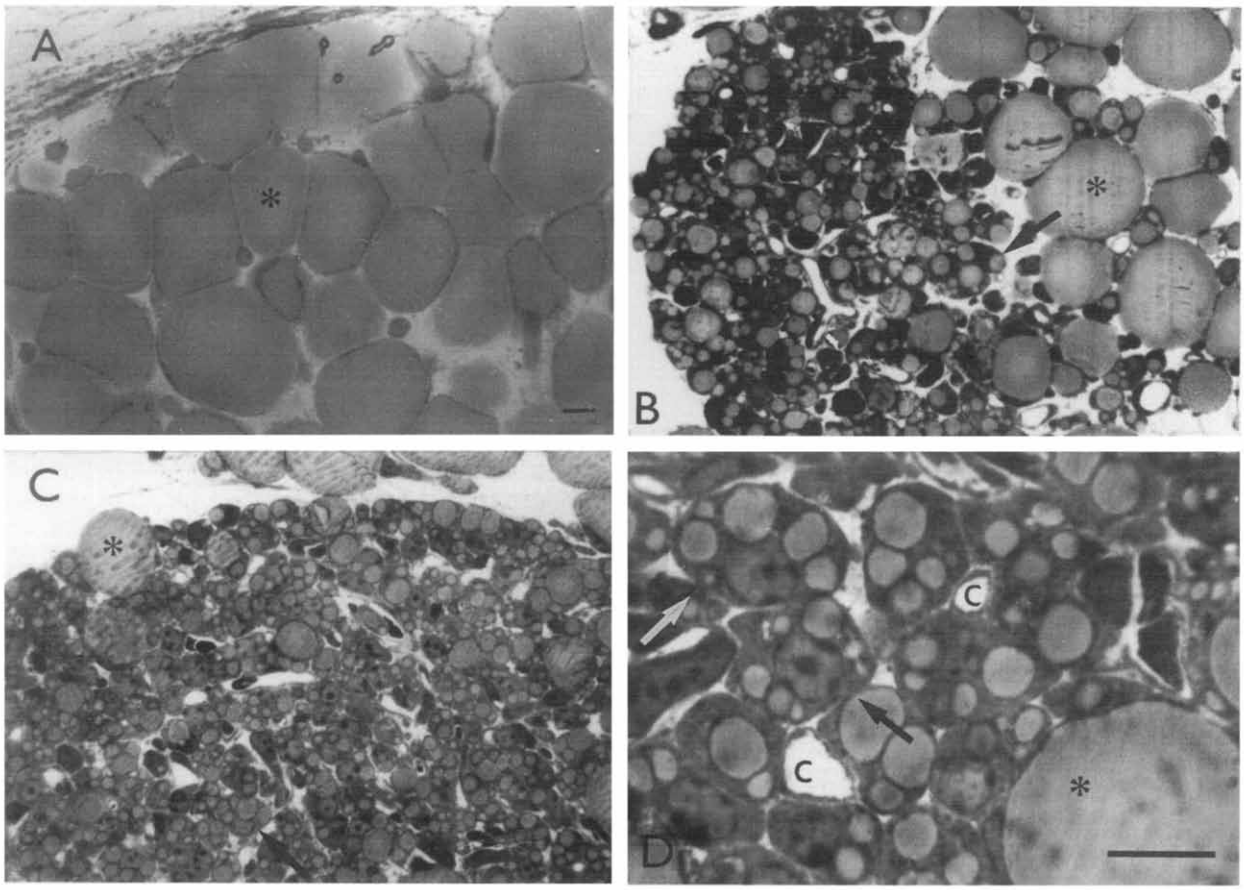


Fig. 2. Postnatal development of hamster interscapular brown adipose tissue (days after birth: A, 2; B, 7; C and D, 10). Typical multilocular adipocytes (arrows) arise from preadipocytes or transform from unilocular fat cells (asterisks). Between adipocytes a dense network of capillaries (c) can be observed. Original magnification of semithin Durcupan sections $4 \cdot 10^2$ times (A–C) or $13 \cdot 10^2$ times (D). Bars indicate 10 $\mu$m.

postnatal day, cytochrome oxidase and the uncoupling protein are present in equimolar amounts, both in the homogenate and in isolated mitochondria.

*Activity of cytochrome oxidase and oligomycin-sensitive ATPase*

As shown in Fig. 6, immunological quantitation of cytochrome oxidase and $F_1$-ATPase was fully confirmed by the enzyme activity measurements. Cytochrome oxidase activity in homogenates increased continuously between the 1st and 40th postnatal day. The sigmoidal character of the increase showed that the most rapid activation occurred during and after the second postnatal week. Activity of oligomycin-sensitive ATPase increased after birth until the maximum was reached on the 7–8th day. On subsequent days the activity quickly decreased, in agreement with changes in the $F_1$-antigen content (Figs. 4, 5). Identical changes were found in isolated mitochondria. It is also evident from Fig. 6 that before the uncoupling protein appears for the first time on the 8th day, the activity of oligomycin-sensitive ATPase fully follows the activity of cytochrome oxidase.

*Activity of 5′-deiodinase of thyroxine*

It was shown before [18] that conversion of $T_4$ to $T_3$ in hamster brown adipose tissue is highly stimulated by short exposure of the animals to cold. Thyroxine 5′-deiodinase activity in homogenates of developing hamster from adipose tissue increased in parallel with the occurrence and increase of the uncoupling protein content. Whereas low activities of 5′-deiodinase were found on the 7/8th day (0.08 pmol/h per mg protein), the activity on the 15th day was about 10-fold (1.05 pmol/h per mg protein).

*Detection of the uncoupling protein and $F_1$-ATPase by immunoelectron microscopy*

When used for immunoelectron microscopy, the antiserum against uncoupling protein readily reacted with mitochondria of adult hamster brown adipose tissue (Fig. 7D) and numerous immunocomplexes, appearing as dense black grains, were found on the inner mitochondrial membrane. The immunostaining was completely absent in other, non-mitochondrial structures, and in mitochondria of other tissues. In contrast to this,

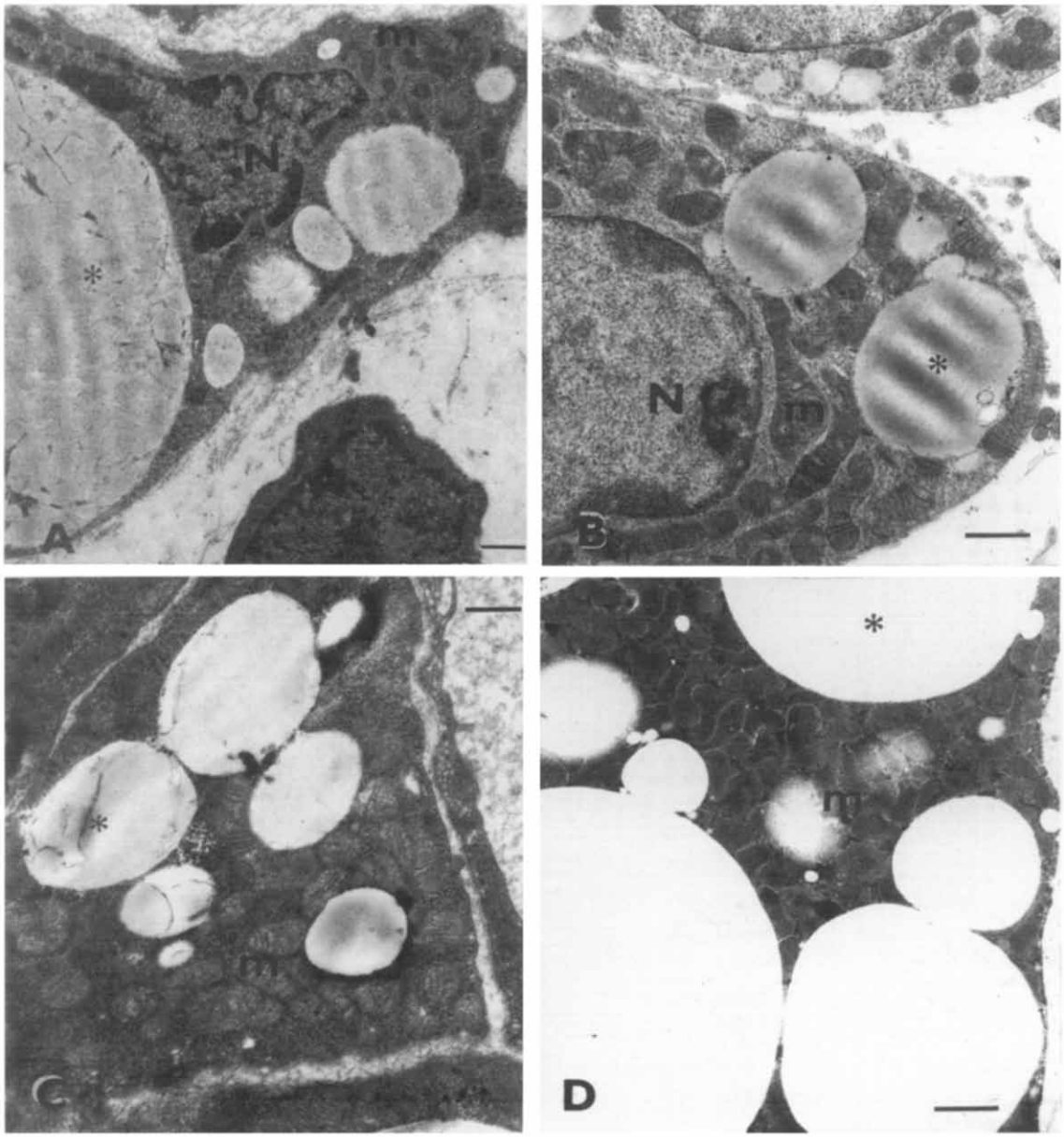


Fig. 3. Ultrastructure of differentiating adipocytes (days after birth: A, 7; B, 8; C, 14; D, 20). Note the increasing number of lipid inclusions (asterisks) and mitochondria (m), which contain numerous regular cristae and are densly packed among lipid droplets after 2 weeks. N, nucleus. Original magnification $7 \cdot 10^3$ times (A) or $9 \cdot 10^3$ times (B–D). Bars indicate 1 $\mu$m.

the anti-$F_1$-ATPase antibody reacted intensively with mitochondria of the adult rat liver, but very weakly with mitochondria of the adult hamster brown adipose tissue (Fig. 7d). Immunostaining of the uncoupling protein in hamster brown adipose tissue continuously changed during development. A few immunocomplexes associated with mitochondria were first found on the 7th day (Fig. 7A) and immunostaining gradually increased on subsequent days (Fig. 7B–D). Significant amounts of the uncoupling protein could be detected in the cytoplasm around the 7th day, indicating accumulation of the protein outside mitochondria. After the 10th day the cytoplasmic reaction disappeared. $F_1$-ATPase immunostaining was positive in mitochondria and also in cytoplasm of brown adipose tissue around day 7 (Fig. 7a). At variance with the uncoupling protein, immunostaining of $F_1$-ATPase rapidly decreased on the following days (Fig. 7b–d).

## Discussion

The main conclusion of this paper is that thermogenic ability of hamster brown adipose tissue develops entirely postnatally due to sudden qualitative changes in

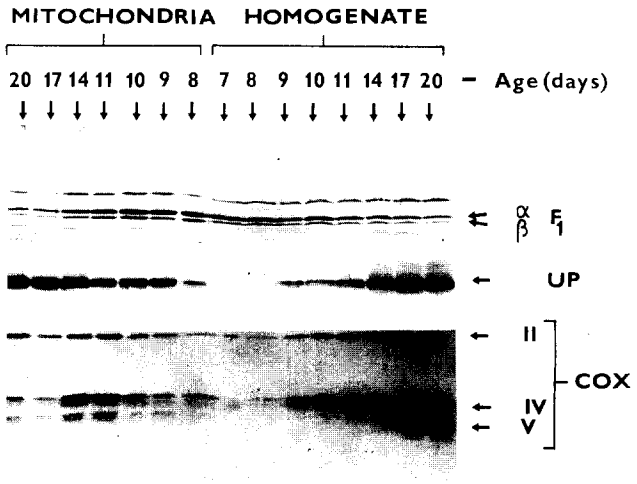


Fig. 4. Immunochemical (western blot) detection of $F_1$-ATPase subunits ($\alpha$, 50 kDa; $\beta$, 48 kDa), uncoupling protein (UP, 32 kDa) and cytochrome oxidase (COX) subunits (II, 27 kDa; IV, 17 kDa; V, 12 kDa) in homogenates (40 $\mu$g protein aliquots) and isolated mitochondria (10 $\mu$g protein aliquots) of hamster brown adipose tissue.

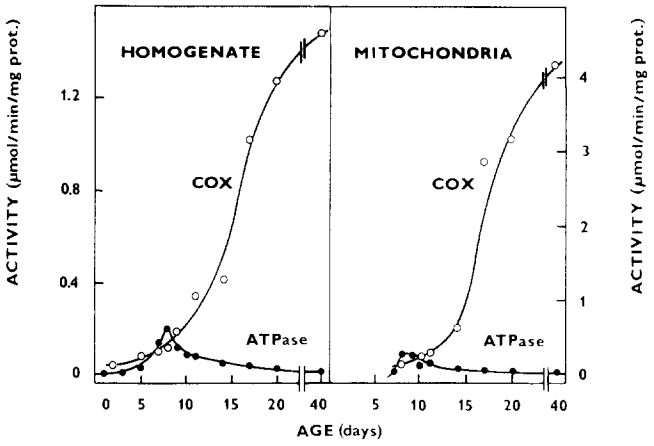


Fig. 6. Activity of cytochrome oxidase (COX, ○) and oligomycin-sensitive ATPase (ATPase, ●) in homogenates and isolated mitochondria of hamster brown adipose tissue. For details see Materials and Methods.

the enzymic apparatus of the mitochondrial membrane. A critical event in this process is the iniciation of uncoupling protein synthesis.

As demonstrated by morphological (Figs. 2, 3), and immunoblotting data (Figs. 4, 5), as well as by enzyme activity measurements (Fig. 6), brown adipose tissue of the newborn hamster undergoes intensive differentiation. During the first postnatal week it contains significant amounts of mitochondria which increase in number and complexity. Although the mitochondria contain considerable and proportional amounts of cytochrome oxidase and $F_0F_1$-ATPase, they completely lack the critical thermogenic component – the uncoupling protein. Similarly to mitochondria of other tissues, they thus have a potential to carry out regular oxidative phosphorylation and have to be considered as nonthermogenic, phosphorylating mitochondria. Beginning with the 8th day, the uncoupling protein appears in the mitochondrial membrane and its content rapidly increases. This is clearly earlier than has been shown in the study of Sundin et al. [9] which was based on isolation of mitochondria and quantification of the uncoupling protein by [$^3$H]GDP binding. The amount of the uncoupling protein then follows the increasing oxidative capacity (see Figs. 5, 6), while the ATPase content and activity decreases. Consequently, thermogenic mitochondria are formed and the overall thermogenic capacity of brown adipose tissue, as expressed by the total content of the uncoupling protein in the interscapular brown adipose tissue, increases 360-times between the 8th and 17th day and the corresponding content of cytochrome oxidase increases 11-times.

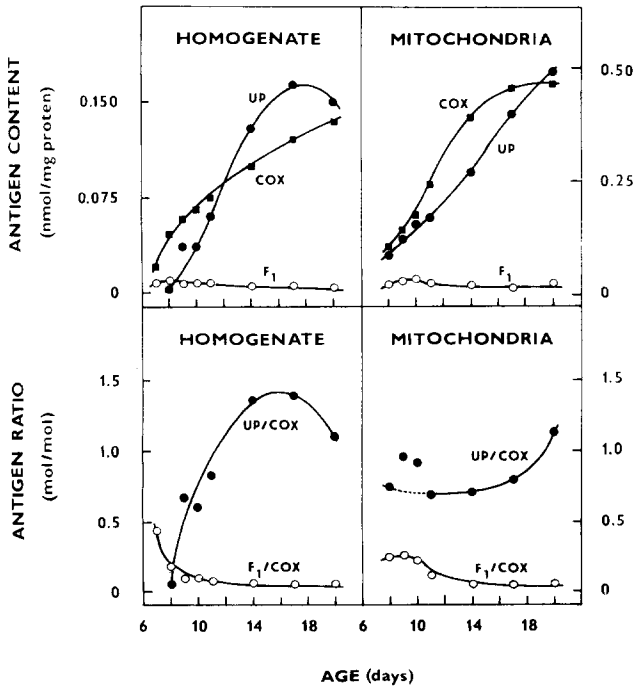


Fig. 5. Quantitative evaluation of cytochrome oxidase (COX, ■), $F_1$-ATPase ($F_1$, ○), uncoupling protein (UP, ●) and their respective molar ratios ($F_1$/COX, ○; UP/COX, ●) in homogenates and isolated mitochondria of hamster brown adipose tissue. Densitometry of immunoblots was performed as described in Materials and Methods.

As numerous mitochondria are already present when the uncoupling protein synthesis begins, transformation of the preexisting nonthermogenic to the thermogenic mitochondria is very likely. However, the production of new thermogenic mitochondria has also to be considered, since the cellular composition of brown adipose tissue is changing substantially (see Results and Ref. 20). The biogenesis of thermogenic mitochondria may be associated with different types of mitochondria existing in parallel and replacing one another during prenatal differentiation od brown adipose tissue in the

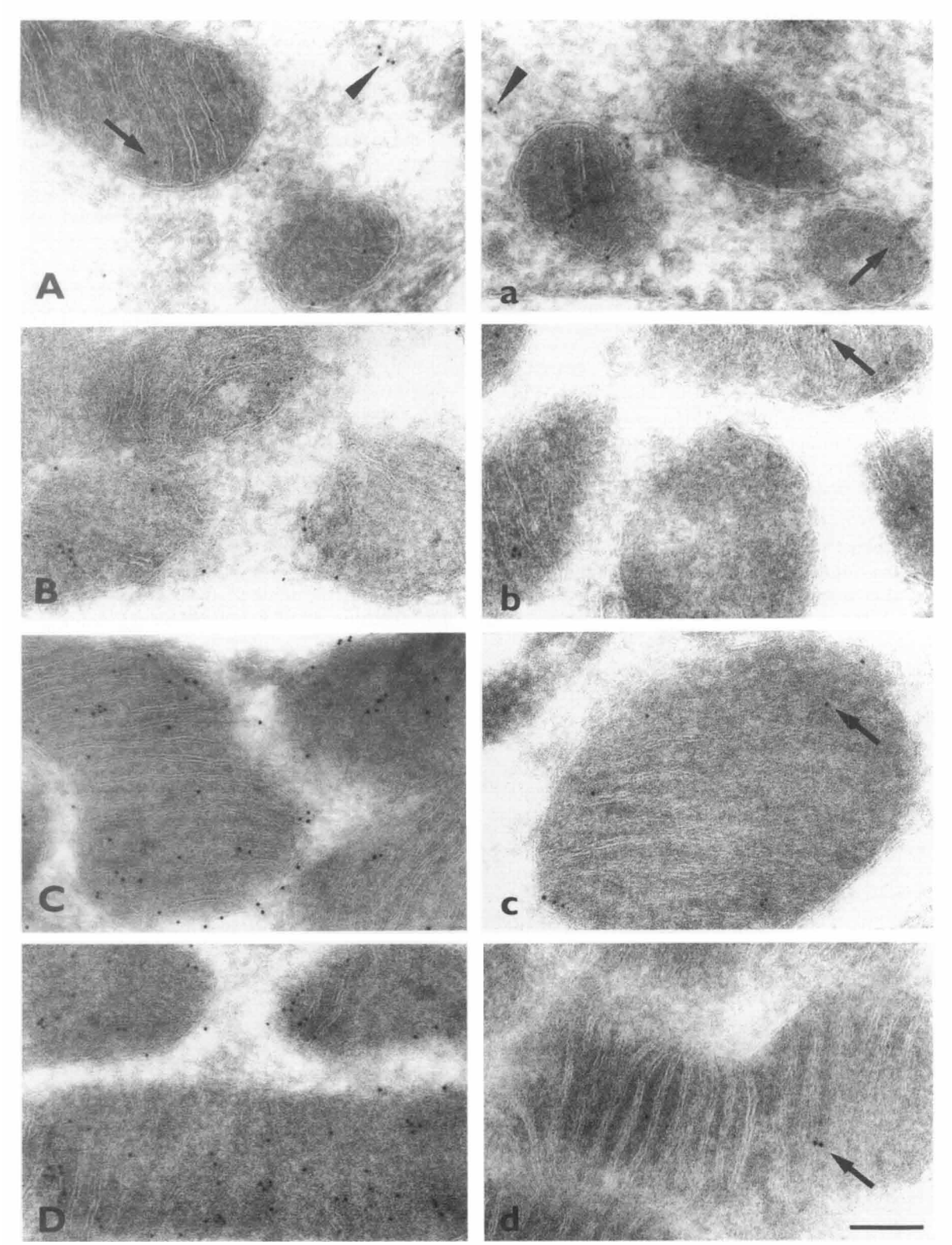


Fig. 7. Immunoelectron microscopy of the uncoupling protein (A–D) and $F_1$-ATPase (a–d) in developing hamster brown adipose tissue (days after birth: A, a, 7; B, b, 10; C, c, 17; D, d, adult). Immunocomplexes bound to the mitochondrial membranes are indicated by arrows, soluble immunocomplexes by arrow-heads. For immunostaining, 10 nm gold particles were used. Bar indicates 0.2 $\mu$m. For details see Materials and Methods.

mouse and rat [5,23]. An analogous heterogeneity of mitochondria could also exist in postnatally developing hamster brown adipose tissue. This is indicated by immature and well-differentiated mitochondria occurring in parallel (see Fig. 3), as well as by immunoblotting experiments. Quite clearly, the mitochondria isolated from brown adipose tissue of 8- to 11-day-old animals showed a much higher content of the uncoupling protein and UP/COX ratio than expected from corresponding data obtained in homogenates (Fig. 5, 6). As the efficiency of the isolation procedure was apparently low in samples from younger animals it is likely that the differential centrifugation method, originally developed for mitochondria from highly active brown adipose tissue [11], preferentially isolates one type of mitochondria that are well differentiated and thermogenic.

The observed changes in mitochondrial energetics clearly result from different rates of synthesis of individual mitochondrial proteins. This process closely resembles the development of thermogenesis and thermogenic mitochondria in altricical type of newborns [6], such as the rat, mouse, sheep and cow, although it occurs there prenatally, at the latest period of embryogenesis [5,24–27]. As to the exact beginning of uncoupling protein synthesis in the hamster, immunoblotting first detected measurable amounts of the protein on the 8th postnatal day and immunoelectron microscopy revealed first molecules on the 7th day. This is quite understandable as detection of individual protein molecules by immunostaining is much more sensitive than immunoblotting.

Immunoelectron microscopy also demonstrated the uncoupling protein and $F_1$-ATPase in the cytoplasm. The presence of soluble, unassembled protein antigens might indicate the accumulation of newly synthesized molecules in the cytoplasm before their incorporation. Quantitative aspects, however, have to be considered with caution as some of the presumably soluble proteins might belong to invisible small membrane fragments, and reacting epitopes of unassembled proteins may be more numerous and more accessible for the antibody than those of the membrane-assembled ones.

The immunological approach yields information about the quantity of the antigen-protein measured rather than about its function. Nevertheless, in case of cytochrome oxidase and $F_1$-ATPase immunoblotting data were in perfect agreement with enzyme activity measurements. In addition, the measurements of the oligomycin-sensitive ATPase activity demonstrate that the observed changes in the $F_1$-ATPase content are paralleled by changes in the $F_0$ portion (membrane part) of the enzyme. Therefore, the process of formation of thermogenic mitochondria in hamster brown adipose tissue clearly indicates that the uncoupling protein 'replaces' $F_0F_1$-ATPase in the mitochondrial membrane not only functionally but also physically.

Development and adaptive changes of the thermogenic function, particularly the synthesis of the uncoupling protein, is under hormonal control, where both $\alpha$ and $\beta$ adrenergic receptors and thyroid hormons are involved [28,29]. The increase of specific mRNA for the uncoupling protein and its activated synthesis, as well as the synthesis of several other proteins, require active 5′-deiodination of thyroxin directly in brown adipose tissue (type II enzyme) [30,31]; $T_3$ administration cannot be substituted for this. The need for thyroid hormones has also been demonstrated during perinatal development of rat brown adipose tissue [27] and in tissue culture experiments [32]. The induction of uncoupling protein synthesis in fetal rat and bovine species corresponds to the high activity of 5′-deiodinase in brown adipose tissue [33,34], which is one order of magnitude higher with respect to adult animals. As has also been shown in the present report, a similar 10-fold increase of 5′-deiodinase activity correlates with the onset of synthesis of the uncoupling protein in the developing hamster. All these data support the view that active 5′-deiodinase of thyroxine is absolutely essential in the brown adipose tissue for long-term regulation of brown adipose tissue thermogenesis by means of biosynthetic processes which modulate the structure and function of mitochondria in particular.

## Acknowledgements

Antibodies against cytochrome oxidase were generously provided by Dr. Š. Kužela. Excellent technical assistance of V. Fialová is gratefully acknowledged.

## References

1 Nicholls, D.G. (1976) FEBS Lett. 61, 103–110.
2 Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1–64.
3 Houštěk, J., Kopecký, J. and Drahota, Z. (1978) Comp. Biochem. Physiol. 608, 209–214.
4 Lin, C.S., Klingenberg, M. (1982) Biochemistry 21, 2950–2956.
5 Houštěk, J., Kopecký, J., Rychter, Z., Soukup, T. (1988) Biochim. Biophys. Acta 935, 16–25.
6 Nedergaard, J., Connolly, E., Cannon, B. (1986) in: Brown Adipose Tissue (Trayhurn, P., Nicholls, D.G., eds.), pp. 152–213, Edward Arnold, London.
7 Hissa, R. (1968) Ann. Zool. Fenn. 5, 345–383.
8 Rink, R.D. (1969) J. Exp. Zool. 170, 117–123.
9 Sundin, U., Herron, D. and Cannon, B. (1981) Biol. Neonate 39, 141–149.
10 Houštěk, J., Bednár, J., Janíková, D., Soukup, T. and Kopecký, J. (1988) Abstracts 14th International Congress of Biochemistry, Prague, July 10–15, Abstr. TH:487.
11 Hittelman, K.J., Lindberg, O. and Cannon, B. (1969) Eur. J. Biochem. 11, 183–192.
12 Kolarov, J., Wielbursky, A., Mendel-Hartwig, I. and Nelson, B.D. (1981) Biochim. Biophys. Acta 652, 334–336.

13 Kužela, Š., Luciaková, K. and Lakota, J. (1980) FEBS Lett. 114, 197–201.
14 Tokuyasu, K.T. (1980) Histochem. J. 12, 381–403.
15 Tokuyasu, K.T. (1986) J. Microsc. 143, 139–149.
16 Wharton, D.C. and Tzagoloff, A. (1967) Methods Enzymol. 10, 245–250.
17 Kopecký, J., Guerrieri, F. and Papa, S. (1983) Eur. J. Biochem. 131, 17–24.
18 Kopecký, J., Sigurdson, L., Park, I.R.A., Himms-Hagen, J. (1986) Am. J. Physiol. 251, E1–E7.
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–276.
20 Néchad, M. (1986) in Brown Adipose Tissue (Trayhurn, P. and Nicholls, D.G., eds.), pp. 1–30, Edward Arnold, London.
21 Kuhn-Neutwig, L. and Kadenbach, B. (1985) Eur. J. Biochem. 149, 147–158.
22 Kadenbach, B., Kuhn-Neutwig, L. and Büge, U. (1987) Curr. Top. Bioenerg. 15, 113–161.
23 Cipolini, M., Cinti, S., Brunestti, L., Bosello, O., Osculati, F. and Björnstorp, P. (1985) Exp. Cell Res. 159, 261–266.
24 Freeman, K.B. and Patel, H.V. (1984) Canad. J. Biochem. Cell Biol. 62, 479–485.
25 Casteilla, L., Champigny, O., Bouillaud, F., Robelin, J. and Ricquier, D. (1989) Biochem. J. 257, 665–671.
26 Casteilla, L., Forest, C., Ricquier, D., Robelin, J., Lombert, A. and Ailhaud, G. (1987) Am. J. Physiol. 252, E627–E636.
27 Obregon, M.-J., Pitamber, R., Jacobsson, A., Nedergaard, J. and Cannon, B. (1987) Biochim. Biophys. Res. Commun. 148, 9–14.
28 Jacobsson, A., Nedergaard, J. and Cannon, B. (1986) Biosci. Rep. 6, 621–631.
29 Himms-Hagen, J. (1983) in: Mammalian Thermogenesis (Girardier, L. and Stock, M.J., eds.), pp. 141–177, Chapman & Hall, London.
30 Bianco, A. and Silva, J.E. (1987) Am. J. Physiol. 254, 4225–E263.
31 Bianco, A. and Silva, J.E. (1987) J. Clin. Invest. 79, 295–300.
32 Rehnmark, S., Kopecký, J., Jacobsson, A., Néchad, M., Herron, D., Nelson, B.D., Obregon, M.-J., Nedergaard, J. and Cannon, B. (1989) Exp. Cell Res. 182, 75–83.
33 Iglesias, R., Fernandéz, J.A., Mampel, T., Obregon, M.J. and Villarroya, F. (1987) Biochim. Biophys. Acta 923, 233–240.
34 Giralt, M., Casteilla, L., Vinas, O., Mampel, T., Iglesias, L., Robelin, J. and Villarroya, F. (1989) Biochem. J. 259, 555–559.