Postnatal recruitment of brown adipose tissue is induced by the cold stress experienced by the pups

An analysis of mRNA levels for thermogenin and lipoprotein lipase

Maria-Jesus OBREGÓN,*† Anders JACOBSSON*, Todd KIRCHGESSNER,‡ Michael C. SCHOTZ,‡ Barbara CANNON* and Jan NEDERGAARD*§

*The Wenner-Gren Institute, Biologihus F3, University of Stockholm, S-106 91 Stockholm, Sweden;

†Departamento Endocrinología Experimental, Instituto de Investigaciones Biomédicas, Facultad Autónoma de Medicina (U.A.M.), Arzobispo Morcillo 4, E-28029 Madrid, Spain;

and ‡Research Veterans Administration, Wadsworth Medical Center, Los Angeles, CA 90073, U.S.A.

In order to investigate the postnatal recruitment process, gene expression in the brown adipose tissue of rat pups was followed during the first 20 h of life. In normal pups, the level of mRNA coding for the uncoupling protein thermogenin increased markedly but gradually within the first 24 h. Lipoprotein lipase and actin mRNA levels were relatively low and remained constant. In pups exposed to thermoneutral temperature (35 °C) for the first 12 h after birth, no increase in thermogenin mRNA or lipoprotein lipase mRNA was observed, whereas in pups exposed to 28 °C a clear increase in both thermogenin and lipoprotein lipase mRNA levels was found. Actin mRNA levels were not affected by the environmental temperature under these circumstances. It was concluded that the postnatal recruitment in brown adipose tissue is a consequence of the cold stress experienced by the newborn pups. Thus, postnatal recruitment is not ontogenically predetermined.

INTRODUCTION

In the newborn mammal, the need for extra heat production is high, and most mammalian newborns have at birth or acquire during the perinatal period a high capacity for nonshivering thermogenesis (Nedergaard *et al.*, 1986). In newborns of species such as rats, a process is initiated (here referred to as 'recruitment') which in the immediate postnatal period leads to an increased capacity for thermogenesis in the brown adipose tissue. The recruitment process involves a series of events (Cannon & Nedergaard, 1986), including cell proliferation and mitochondriogenesis, as well as an increased ability of the tissue to take up substrate for thermogenesis from the circulation [i.e. an increased activity of lipoprotein lipase (Cryer & Jones, 1978; Boulangé *et al.*, 1981)]. The increased capacity for combustion is obtained by an increase in the amount of the rate-limiting factor for thermogenesis: the uncoupling protein thermogenin (Sundin & Cannon, 1980; Cannon *et al.*, 1981).

As discussed in detail recently (Nedergaard *et al.*, 1986; Cannon *et al.*, 1988), the regulation of the recruitment process observed after birth is not yet clarified and, as stated, "a clear demonstration of whether the initial recruitment process is ontogenically determined or a response to the environment is still required" (Nedergaard *et al.*, 1986).

We have earlier shown, on a time-scale of days, that a postnatal increase in thermogenin mRNA occurs very abruptly: from the late fetal stage to within 1 day after birth there is a more than 10-fold increase in the amount of the mRNA for thermogenin, and the level thereafter remains constant for at least several days (Obregón *et al.*,

1987). The aim of the present project was thus to investigate whether this abrupt increase in thermogenin mRNA level observed 24 h after birth (Ricquier *et al.*, 1986; Obregón *et al.*, 1987) is dependent upon the ambient temperature, or is determined by pre-established ontogenic signals.

Based on the results of the present investigation, it would seem clear that postnatal recruitment of brown adipose tissue is caused entirely by the cold stress experienced by the newborns.

Some of these results have been mentioned in review context (Cannon *et al.*, 1988).

EXPERIMENTAL

Animals

Pregnant Sprague–Dawley rats were obtained from a local supplier (Alab, Stockholm) and housed at 22 °C, if not otherwise indicated. The rats had free access to food (rat pellets, Ewos R3) and water and were on a 12 h/12 h light/dark cycle.

Near term, the rats were checked at least every second hour, and the start and the end of delivery were recorded. Delivery lasted for approx. 1.5 h, and normally took place between 06:00 and 15:00 h. For time curves, the end of delivery was taken as 'zero' time; this necessarily means that there was a time difference between the first and last pups born. The mean number of pups per litter was 11.5 ± 0.6 (19 litters).

Normal postnatal development. For investigation of normal postnatal development, the litters from nine

§ To whom correspondence should be addressed.

dams were used. These litters remained with the dam, and at 2, 4, 6, 8, 12 and 20 h after birth (starting at time 'zero') two pups were weighed, decapitated and bled, and the interscapular depot of brown adipose tissue was dissected out, pooled and immediately frozen on dry ice. The samples were stored at -80°C for not more than 2 weeks before isolation of RNA (see below).

Effect of ambient temperature on postnatal development.

After the end of the delivery period, and after the whole litter had been cleaned by the dam, the pups were withdrawn from the dam and placed at different temperatures, as indicated below. Ten litters in total were investigated. Half of the litters were placed at 28°C , and half at 35°C in incubators. The pups were placed singly so that huddling could not occur. All pups from one litter were placed either at 35°C or 28°C , and two pups were removed at 2, 4, 6, 8 and 12 h and treated as described above. In order to prevent dehydration, care was taken to maintain high humidity in the 35°C incubator, and generally the pups were given water by mouth after 8 h.

mRNA determinations

Total RNA was prepared from the frozen tissue as previously described (Jacobsson *et al.*, 1985; Obregón *et al.*, 1987), with a guanidine hydrochloride method involving ethanol precipitation. The amount of RNA obtained was determined by spectrophotometry at 260 nm, and the 260/280 ratios were always higher than 1.8. The intactness of the preparation and the absence of DNA were generally checked on agarose minigels. The total amount of RNA obtained from the two pups ranged from 70 to 200 μg .

The amount of thermogenin mRNA, lipoprotein lipase mRNA or actin mRNA was estimated by slot blots with 4 μg or 10 μg of total RNA, as previously described (Jacobsson *et al.*, 1986, 1987; Obregón *et al.*, 1987), using nick-translated cDNA probes for thermogenin (Jacobsson *et al.*, 1985), lipoprotein lipase (Kirchgeßner *et al.*, 1987) or β -actin. After hybridization of the slot blots, and overnight exposure of the film (Amersham Hyper), the intensity of the autoradiograms was evaluated in a laser densitometer.

RNA isolated from the brown adipose tissue of cold-exposed mice was used as a positive reference sample for thermogenin and lipoprotein lipase; rat brain RNA was blotted as a negative reference sample for thermogenin.

RESULTS

For the present study, we first followed the normal postnatal recruitment process by measuring the tissue levels of mRNA for thermogenin and lipoprotein lipase, and, based on these results, we investigated whether an alteration in the environmental temperature could influence the postnatal events.

Normal postnatal development

The mean body weight of the rat pups which remained with the dam is shown in Table 1(a). It is seen that there was no appreciable change in the body weight during the first 20 h of postnatal life; the body weight was approx. 6 g.

Also given in Table 1(a) is the total yield of RNA. It is evident from the Table that there was a tendency to a decreasing total amount of RNA with increasing

Table 1. Changes in body weight and total mRNA level during postnatal development in rat pups

(a) Normal postnatal development; (b), different environmental temperatures. Values are means $\pm$ S.E.M. of the indicated number of pups (in parentheses) or of RNA preparations (each prepared from the pooled interscapular brown adipose tissue of 2 pups). * $P < 0.05$ versus 28°C , Student's *t*-test.

(a)		
Time after birth (h)	Body weight (g)	RNA (μg)
2	5.86 ± 0.12 (12)	152 ± 19 (6)
4	6.09 ± 0.11 (15)	134 ± 15 (6)
6	6.00 ± 0.16 (12)	127 ± 8 (7)
8	5.89 ± 0.11 (12)	117 ± 13 (5)
12	6.00 ± 0.18 (13)	95 ± 11 (6)
20	5.93 ± 0.12 (13)	101 ± 11 (6)
(b)		
Time after birth (h)	Body weight (g)	RNA (μg)
At 35°C		
2	5.75 ± 0.13 (10)	133 ± 20 (5)
4	5.77 ± 0.19 (10)	120 ± 15 (5)
6	5.61 ± 0.16 (10)	116 ± 11 (5)
8	5.67 ± 0.22 (8)	88 ± 9 (5)
12	$5.45 \pm 0.12^*$ (10)	95 ± 9 (5)
At 28°C :		
2	5.64 ± 0.19 (10)	118 ± 18 (5)
4	5.80 ± 0.10 (10)	100 ± 4 (5)
6	5.73 ± 0.20 (10)	126 ± 10 (5)
8	5.88 ± 0.10 (9)	87 ± 12 (4)
12	6.04 ± 0.15 (9)	71 ± 6 (5)

postnatal time. When analysed statistically, the decrease in RNA yield was found to be significant ($P < 0.05$, $n = 35$) and corresponded to a decrease of 3 μg (2%) per h, with a starting value of 146 μg . Although the isolation was not strictly quantitative, the decrease in RNA yield with time probably reflected a true decrease in total RNA in the tissue and was in agreement with a general tendency to a decreasing RNA yield observed in the transition from the fetal to the newborn state (results not shown).

Thermogenin mRNA. A 5-fold increase in thermogenin mRNA levels was observed between 2 and 20 h postnatally (Fig. 1). Thus, the expression of the thermogenin gene was clearly increased after birth, as observed earlier (Ricquier *et al.*, 1986; Obregón *et al.*, 1987; Cannon *et al.*, 1988).

As seen in Fig. 1, we have here achieved temporal resolution for the postnatal increase in thermogenin expression, and it is clear that the apparent abrupt rise in thermogenin mRNA levels, previously observed to complete 1 day after birth (Obregón *et al.*, 1987), does not occur suddenly after birth, but takes place gradually with a halftime of about 10 h. This is somewhat slower than is seen in cold-exposed adult mice (Jacobsson *et al.*, 1987, 1988).

Fig. 1. Postnatal development of the expression of the thermogenin, lipoprotein lipase (LPL) and actin genes in rat brown adipose tissue

Pups were obtained and RNA prepared as described in the Materials and methods section and the amount of mRNA was determined by the slot blot method. For each mRNA species, the mean level of expression at 2 h was set to 1.0 and the changes were expressed relative to this value. Points are means $\pm$ S.E.M. from four to six preparations. For thermogenin mRNA, *, ** and *** indicate significant differences from the 2 h time-point ($P < 0.05$, < 0.01 and < 0.001 , respectively; Student's *t*-test).

Lipoprotein lipase mRNA. As also seen in Fig. 1, the level of lipoprotein lipase mRNA was not increased postnatally in a sustained way. However, in some experiments (results not shown), we have seen a tendency to an increase in lipoprotein lipase mRNA in later postnatal life (1–2 days *post partum*), and the tissue thus has the potential for expression of the lipoprotein lipase gene (see also below).

Actin mRNA. The level of actin mRNA showed a tendency to increase with postnatal time (Fig. 1). This increase was statistically significant when analysed with regression analysis ($P < 0.05$) and corresponded to an increase of about 2.4 % per h.

This mRNA represents nonmuscular actin, i.e. a household protein which is not expected to increase during differentiation, and thus the increase observed was unexpected. However, as pointed out above, the total yields of RNA decreased during this period, and the relative increase in actin mRNA level and the relative decrease in total RNA is nearly identical (2% per h). Thus, it may be suggested that the increase in actin mRNA is chimaeric, and that the total amount of actin mRNA is unchanged but the amount of ribosomal RNA decreases.

As a consequence, apparent increases in mRNA levels of other genes which do not clearly exceed that seen for actin (such as was the case for lipoprotein lipase mRNA) may not represent true increases in expression. It should, however, be pointed out that the postnatal increase seen for thermogenin mRNA considerably exceeds that of actin and thus represents a true increase in thermogenin gene expression.

Role of environmental temperature

In order to investigate the physiological background for the postnatal increase in thermogenin mRNA levels,

Fig. 2. Effect of environmental temperature on gene expression in brown adipose tissue of newborn rat pups

After delivery, litters were separated from the dam and the pups were placed singly at either 35 °C or at 28 °C. RNA was prepared and mRNA levels were estimated as described in the Materials and methods section. For each mRNA species, the mean level of expression at 2 h at 35 °C was set to 1.0 and the other values expressed relative to this. Results are means $\pm$ S.E.M. of four to five preparations for each point (where not shown, the S.E.M. is smaller than the size of the symbol), *, ** and *** indicate significant differences between mRNA levels of pups at 35 °C and 28 °C at the indicated time points ($P < 0.05$, < 0.01 and < 0.001 , respectively; Student's *t*-test).

we investigated whether it is the environmental temperature which dictates this postnatal recruitment process. Therefore we exposed the newborn pups, in the absence of the dams, to two different temperatures, one considered to be thermoneutral, and one representing a cold stress.

The temperatures chosen were taken from the studies of Thompson & Moore (1968), who showed that 35 °C was thermoneutral for 1-day-old pups, whereas oxygen consumption was approximately doubled when the pups were exposed to 28 °C; lower temperatures did not further increase oxygen consumption. Thus, for our investigations, we chose 35 °C and 28 °C.

Physiological aspects. The pups at thermoneutrality (35 °C) showed hypermotility and appeared vasodilated. In the pups which remained at this temperature for 12 h, there was a tendency to a slight decrease in body weight (Table 1b), probably due to water loss. The brown adipose tissue in these pups was less compact than in normal pups and resembled the tissue as found in fetal rats.

The pups at 28 °C were less motile and appeared vasoconstricted. There was no decrease in body weight. The brown adipose tissue in these pups appeared to be in an activated state; it was visible through the skin (due to the high blood flow), and after the longer periods at 28 °C, the size of the tissue was clearly reduced.

In both groups of pups, the postnatal decrease in yield of total RNA was again observed, and there was no apparent difference in this parameter between the two groups (Table 1b).

Thermogenin mRNA. As seen in Fig. 2a, there were clear differences between the expression of thermogenin mRNA in the two groups. In the thermoneutral pups, the level of thermogenin mRNA was unchanged for the entire experimental period, i.e. the postnatal recruitment was entirely absent. In the pups at 28 °C, there was a clear postnatal increase in the level of thermogenin mRNA, with a time course and an extent similar to that observed in normal pups, living at normal ambient temperature (22 °C) with the dam. This thus demonstrates that the postnatal increase in thermogenin mRNA – and probably the entire recruitment process – is a response to the environmental temperature experienced by the pup. Although the pups at 28 °C probably tended to become hypothermic towards the end of the experiment, it is evident that this did not in itself prevent the response that is expected of animals trying to defend themselves against the cold, i.e. the increase in thermogenin mRNA.

Lipoprotein lipase mRNA. As seen in Fig. 2(b), the expression of lipoprotein lipase mRNA was very similar to that of thermogenin mRNA at the different environmental temperatures. Thus, in the thermoneutral animals, there was no postnatal increase in lipoprotein lipase mRNA, whereas a continually increasing amount of lipoprotein lipase mRNA was found in the pups at 28 °C. This increase in lipoprotein lipase mRNA is similar to that observed during cold stress of adult rats (Mitchell *et al.*, 1989), although the rate of increase seems somewhat lower in the pups.

Actin mRNA. As seen in Fig. 2(c), there was practically no difference between the actin mRNA levels in the thermoneutral and the cold-stressed pups.

DISCUSSION

Thermogenin mRNA

In the present investigation, we have found that the previously observed postnatal increase in thermogenin mRNA levels occurred gradually during the first 24 h of postnatal life. Interestingly, it was found that this postnatal increase was entirely a response to the environmental temperature; thus, pups experiencing a temperature close to that found *in utero* did not show any postnatal increase in the level of thermogenin mRNA,

and only in pups which were functionally cold stressed did the postnatal increase occur.

There are several interesting implications of this. The results indicate that in the newborn, the control of brown adipose tissue recruitment is similar to that occurring in the adult, i.e. it is a response to the cold. As the response is similar in cold-stressed pups placed singly at 28 °C and in normal pups remaining with their dams at 22 °C, it must be concluded that the pups under normal laboratory conditions are sufficiently cold stressed for the increase in thermogenin mRNA to occur. Indeed, probably the entire postnatal recruitment of brown adipose tissue (Nedergaard *et al.*, 1986) is due to the cold stress experienced by the pups. In preliminary experiments, we observed that in pups remaining with the dam and living at 28 °C, a postnatal increase in thermogenin mRNA was still seen within 1 day, indicating that even when the pups are nursed by the dam at an elevated environmental temperature, the environment is still experienced as cold by the pups. Also implicit in these findings is that the recruitment is not an ontogenically determined process, bound to occur at birth, but is a facultative response to the new conditions occurring after birth.

Further, in the adult, there is good evidence that the response to cold (increase in thermogenin mRNA) is mediated via the sympathetic nervous system via β -receptors (but probably also requires α -receptor stimulation) (Bouillaud *et al.*, 1984; Ricquier *et al.*, 1984, 1986; Jacobsson *et al.*, 1986, 1987; Cunningham & Nicholls, 1987). Although not directly demonstrated here, it is most likely that the postnatal increase in thermogenin mRNA in rat pups is also mediated via noradrenaline release from the sympathetic nervous system, and the cells at birth are thus apparently fully competent to respond to such stimulation. This is principally in agreement with the observation of Schneider-Picard & Girardier (1982) that sympathetic stimulation can evoke changes in flavoprotein redox state in pups younger than 12 h. As we see a tendency in cold-stressed pups to an increased level of thermogenin mRNA only 2 h after birth, the pups must be able to analyse the thermal environment directly at birth and respond accordingly. Thus, even at the moment of birth, the pup is not poikilothermic but initiates responses aimed at elevating metabolic heat production and stabilizing body temperature.

Lipoprotein lipase mRNA

In the pups exposed (without the dams) to different environmental temperatures, the lipoprotein lipase mRNA response was similar to that of thermogenin. Thus, in a cold environment, lipoprotein lipase gene expression was elevated, whereas no elevation was found in the thermoneutral environment (Fig. 2). From studies of lipoprotein lipase mRNA levels and activity in adult rats, it has been concluded that brown adipose tissue lipoprotein lipase is under positive control of the sympathetic nervous system mediated via β -adrenergic receptors (Carneheim *et al.*, 1984, 1988; Mitchell *et al.*, 1989). Thus, it is likely that the postnatal increase in lipoprotein lipase mRNA observed here is due to sympathetic stimulation of the tissue. Lipoprotein lipase therefore exhibits a response in accordance with it being a marker for recruitment of the tissue.

This simple situation was not found, however, in the pups which remained with the dams (Fig. 1). Here, the

thermogenin mRNA increase indicated that the pups had experienced a marked cold stress. Nonetheless, the lipoprotein lipase mRNA was not increased postnatally, as it was when the pups were exposed to 28 °C without the dam (Fig. 2b). This absence of an increase in gene expression was surprising considering the results of Cryer & Jones (1978). Under similar conditions, with pups remaining with the dams, these authors noted a 3-fold increase of the lipoprotein lipase activity up to 16 h after birth. One possible explanation for the discrepancy between the data presented here on gene expression and those on lipase activity could be that in the postnatal state, the translation efficiency of the existing lipoprotein lipase mRNA was increased. An intriguing alternative possibility could be that the lipoprotein lipase activity measured in the tissue could derive from sources outside the tissue itself and be transferred with the blood to the capillary walls in the brown fat. One potential source of this activity could be the milk, which, for unknown reasons, has a high lipoprotein lipase activity (Cryer, 1987; Olivecrona & Bengtsson-Olivecrona, 1987). We observed that suckling generally started 2–3 h after birth and this therefore could affect the development pattern of the lipoprotein lipase mRNA within the time scale studied here. As the intestinal epithelia during early postnatal life are highly permeable to macromolecules (Henning, 1987; Gardner, 1988), the lipase from the milk could pass to the blood of the pups and further to the capillaries of brown adipose tissue. The presence of the lipoprotein lipase activity could then lead to a feedback suppression of the lipoprotein lipase gene expression, while at the same time an increase in lipoprotein lipase activity in the tissue could be measurable (Cryer & Jones, 1978). Although this explanation is hypothetical, it would account both for the discrepancy between the data on lipoprotein lipase mRNA and activity and for the difference between gene expression in the pups remaining with the dam and in the pups transferred to 28 °C, and it is at least likely that the absence of the postnatal increase in lipoprotein lipase mRNA in the pups remaining with the dam is a consequence of the suckling process.

Developmental status

If the conclusions from the observations here on rat pups are extended to the newborn of other species, the following considerations can be made.

Rat pups belong to the group of altricial newborns (Nedergaard *et al.*, 1986), in which the newborn is 'nest-dependent' and not very developed at birth. Probably in all altricial newborns [including the human newborn (Nedergaard *et al.*, 1986; Cannon *et al.*, 1988)], the entire postnatal recruitment of brown adipose tissue is due to a response to the cold stress experienced by the newborn in the transfer from uterine life.

In truly immature newborns, such as hamsters, the recruitment of brown adipose tissue can be suggested to occur following the same pathway as that demonstrated here for the altricial newborns. However, in these newborns, the pups are initially truly poikilothermic and brown adipose tissue is not recruited until long after birth. When the recruitment occurs, we would suggest that it is a response to the environmental temperature, just as for the altricial species, but that this response cannot be elicited until a certain stage of maturity has been reached.

In precocial newborns, such as guinea pigs and lambs, however, the regulation of brown adipose tissue recruitment must be principally different from that described here. In these newborns, the evidence is that the recruitment takes place in the fetal state, while the young animal is still in the uterus (Nedergaard *et al.*, 1986; Casteilla *et al.*, 1987), and it is not possible to envisage that the recruitment under these conditions could be due to a cold stress. Rather, the present experiments have further delineated the differences between postnatal recruitment, which, regardless of whether it occurs in the first hours after birth or any later stage, is due to the cold stress experienced, and the intrauterine recruitment, where regulation must occur by other means.

This study was supported by grants from the Swedish Natural Science Research Council, Veterans Administration, American Heart Association, Greater Los Angeles Affiliate and the National Institute of Health. M.-J.O. was the recipient of a collaborative grant from the Consejo Superior Investigaciones Científicas (Spain) and the Swedish Natural Science Research Council. The authors would like to thank Agneta Bergström for some initial experiments.

REFERENCES

- Bouillaud, F., Ricquier, D., Mory, G. & Thibault, J. (1984) *J. Biol. Chem.* **259**, 11583–11586
- Boulangé, A., Planche, E. & de Gasquet, P. (1981) *Metabolism* **30**, 1045–1052
- Cannon, B., Nedergaard, J. & Sundin, U. (1981) in *Survival in Cold* (Musacchia, X. J. & Jansky, L., eds.), pp. 99–120, Elsevier–North Holland, Amsterdam
- Cannon, B. & Nedergaard, J. (1986) in *Proceedings of the XIIIth International Congress of Nutrition* (Taylor, T. G. & Jenkins, N. K., eds.), pp. 315–319, John Libbey, London
- Cannon, B., Connolly, E., Obregón, M. J. & Nedergaard, J. (1988) in *The Endocrine Control of the Fetus* (Kunzel, W. & Jensen, A., eds.), pp. 306–320, Springer-Verlag, Berlin
- Carneheim, C., Nedergaard, J. & Cannon, B. (1984) *Am. J. Physiol.* **246**, E327–E333
- Carneheim, C., Nedergaard, J. & Cannon, B. (1988) *Am. J. Physiol.* **254**, E155–E161
- Casteilla, L., Forest, C., Robelin, J., Ricquier, D., Lombet, A. & Ailhaud, G. (1987) *Am. J. Physiol.* **252**, E627–E636
- Cryer, A. (1987) in *Lipoprotein Lipase* (Borensztajn, J., ed.), pp. 277–327, Evener Publishers, Chicago
- Cryer, A. & Jones, H. M. (1978) *Biochem. J.* **174**, 447–452
- Cunningham, S. A. & Nicholls, D. G. (1987) *Biochem. J.* **245**, 485–491
- Gardner, M. L. G. (1988) *Annu. Rev. Nutr.* **8**, 329–350
- Henning, S. J. (1987) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed.), pp. 285–300, Raven Press, New York
- Jacobsson, A., Stadler, U., Glotzer, M. A. & Kozak, L. P. (1985) *J. Biol. Chem.* **260**, 16250–16254
- Jacobsson, A., Nedergaard, J. & Cannon, B. (1986) *Biosci. Rep.* **6**, 621–631
- Jacobsson, A., Cannon, B. & Nedergaard, J. (1987) *FEBS Lett.* **224**, 353–356
- Kirchgesner, T. G., Svenson, K. L., Lusi, A. J. & Schotz, M. C. (1987) *J. Biol. Chem.* **262**, 8463–8466
- Mitchell, J. R., Carneheim, C., Jacobsson, A., Kirchgesner, T., Schotz, M. C., Alexson, S., Cannon, B. & Nedergaard, J. (1989) in *Obesity in Europe 88* (Björntorp, P. & Rössner, S., eds.), pp. 209–213, John Libbey, London

- Nedergaard, J., Connolly, E. & Cannon, B. (1986) in *Brown Adipose Tissue* (Trayhurn, P. & Nicholls, D. G., eds.), pp. 152–213, Edward Arnold, London
- Obregón, M. J., Pitamber, R., Jacobsson, A., Nedergaard, J. & Cannon, B. (1987) *Biochem. Biophys. Res. Commun.* **148**, 9–14
- Olivecrona, T. & Bengtsson-Olivecrona, G. (1987) in *Lipoprotein Lipase* (Borensztajn, J., ed.), pp. 15–58, Elsevier Publishers, Chicago
- Ricquier, D., Mory, G., Bouillaud, F., Thibault, J. & Weissenbach, J. (1984) *FEBS Lett.* **178**, 240–244
- Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J. & Penicaud, L. (1986) *J. Biol. Chem.* **261**, 13905–13910
- Schneider-Picard, G. & Girardier, L. (1982) *J. Physiol. (Paris)* **78**, 151–157
- Sundin, U. & Cannon, B. (1980) *Comp. Biochem. Physiol.* **65B**, 463–471
- Thompson, G. E. & Moore, R. E. (1968) *Can. J. Physiol. Pharmacol.* **46**, 865–871

Received 26 September 1988/2 December 1988; accepted 6 December 1988