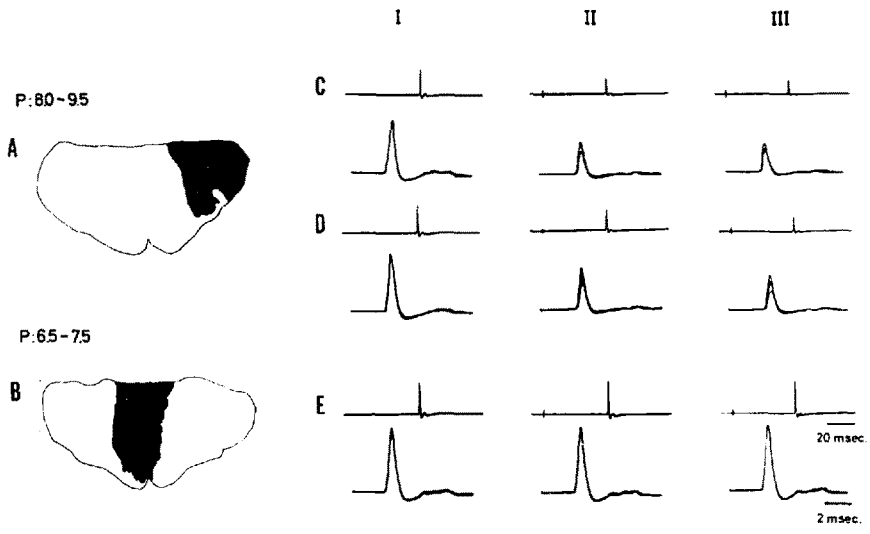

Fig. 2. Effect of brain stem lesion upon the cutaneous inhibition. A and B; filled areas are part of lesion of brain stem at indicated position. Column I; MSR evoked by the tibial nerve stimulation alone. Column II; MSR after the conditioning stimulation of the ipsilateral SR nerve at an interval of about 50 msec. Column III; MSR after that of the contralateral SR nerve at the same interval as Column II. Line C, before; Line D, after the lesion of lateral part of the medulla shown in A. Line E; after lesion of medial part shown in B. Upper traces were obtained at a slow sweep speed and lower ones at a fast sweep speed.

lateral part of the brain stem was cut with fine scissors after the removal of the cerebellum by suction under physostigmine effective state. As seen in Figure 2, the MSR had been depressed to about 50% by the conditioning stimulation of either ipsilateral or contralateral SR nerve at a conditioning-testing interval of about 50 msec before lesion in this preparation (C). After lesion of the lateral part of the medulla (A) the cutaneous inhibition changed little (D), while it was completely abolished by section of the medial part of it (B, E). This clearly shows that the medial part of the medulla is concerned with the cutaneous inhibition, but the localization of physostigmine and/or acetylcholine sensitive mechanism remains unknown.

Zusammenfassung. Nachweis einer verstärkten Spinalreflex-Hemmung bei der decerebrierten Katze durch Physostigmin. Der Reflex wird über N. radialis superficialis hervorgerufen und durch Atropin völlig aufgehoben, während Dihydro-β-erythroidin keine Wirkung hatte.

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Effect in vivo of Norepinephrine on the Membrane Resistance of Brown Fat Cells[1]

The cold-induced increase in thermogenesis of brown fat appears to be mediated by norepinephrine (NE) derived from sympathetic nerve terminals[2]. One of the first events measurable in this metabolic activation is the depolarization of the membrane of the brown fat cell. This change in membrane potential can be elicited by NE applied either in situ[3] or in vitro[4,5]. Similar changes are also seen following electrical stimulation (in vivo) of the sympathetic nerves to the brown fat pad[3]. That this depolarization is at least partially associated with activation of adenyl cyclase was suggested[3] following the finding that: a) the depolarization induced by electrical stimulation is abolished after treatment of the animal with the adrenergic antagonist, propranolol; and b) theophylline, although eliciting a thermogenic response from the brown fat, did not result in a depolarization of the membrane[3]. Recently, observations obtained in vitro have confirmed these results and have been similarly interpreted[5].

As an explanation of the underlying basis for this depolarization, 2 possible mechanisms may be considered; namely, an increase in membrane permeability and/or a direct change in the activity of an electrogenic pump (Figure 1). Hence, the present study was undertaken to determine whether the NE-induced shift in ionic distribution was indeed accompanied by an increase in the permeability of the membrane.

To evaluate this possibility, the effect of NE on the membrane resistance of the brown fat cells was examined in Long-Evans, cold-acclimated (4 ± 1°C, 3–4 weeks) rats. These rats were anesthetized (sodium pentobarbital,

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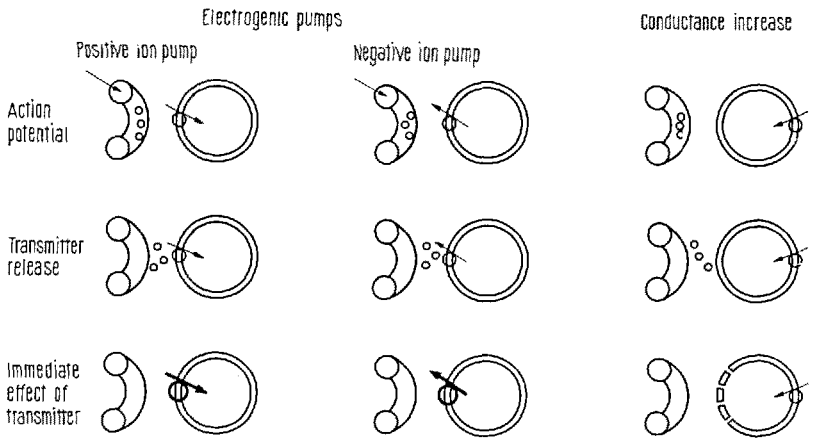

Fig. 1. Diagram of 2 possible mechanisms whereby norepinephrine may effect the depolarization of the brown fat cell membrane. In the sequence of events shown on the right, the release of the transmitter from nerve endings is followed by an increase in the permeability (increase in conductance) of the cell membrane. On the other hand, as depicted on the left, the transmitter may directly alter the activity of an electrogenic membrane pump. Depending on the nature of the pump, this activity may either be increased (as illustrated above) or decreased.

60 mg/kg, i.p.), and the left jugular vein cannulated for administration of NE [l-arterenol bitartrate (Sigma)] and also sustaining doses of pentobarbital. The interscapular brown fat pad was isolated and stabilized, leaving intact the nerve and vascular supplies to the tissue[3]. Intracellular potentials were recorded with glass microelectrodes as previously detailed[3].

Intracellular resistance was measured with a bridge circuit similar to that used by LIBET[6]. This circuit involved application of a square wave voltage across 2 opposite nodes of the bridge while the potential across the remaining 2 nodes was amplified and displayed on an oscilloscope. (In 1 arm of the bridge, a 10^9 ohm resistor was placed in series with the glass microelectrode). When the bridge was balanced, the signal from the differential amplifier appeared as a line broken only by the sharp deflections reflecting the capacitance of the electrode, cell, and cables (e.g., trace 1, Figure 2). On the other hand, when the bridge was unbalanced (as would occur during changes in membrane resistance), the signal appeared on the oscilloscope as a square wave (e.g., trace 3, Figure 2). Thus, utilizing these techniques, the effect of NE on the resistance as well as the potential of the membrane of the brown fat cells was examined.

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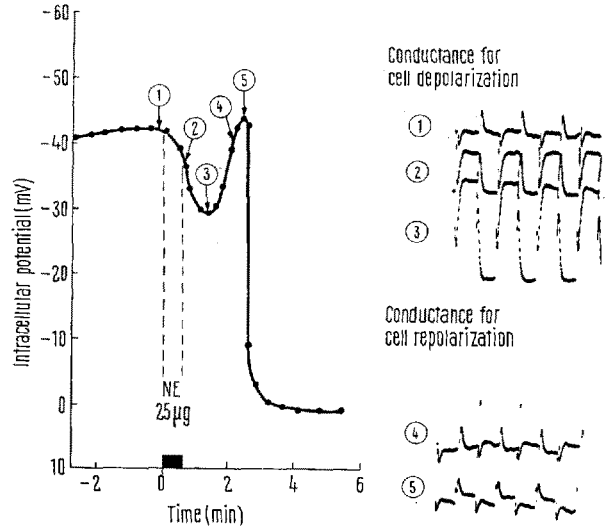

Fig. 2. Effect of NE *in vivo* on the membrane conductance and potential of a brown fat cell. Following the i.v. injection of NE into the rat (time = 0), the membrane of the impaled cell is seen to transiently depolarize [negative potentials are plotted above the time axis]. Changes in the permeability of the cell membrane occur concurrently, as seen on the right where the measure of the membrane conductance is shown for various times during the depolarization-repolarization cycle.

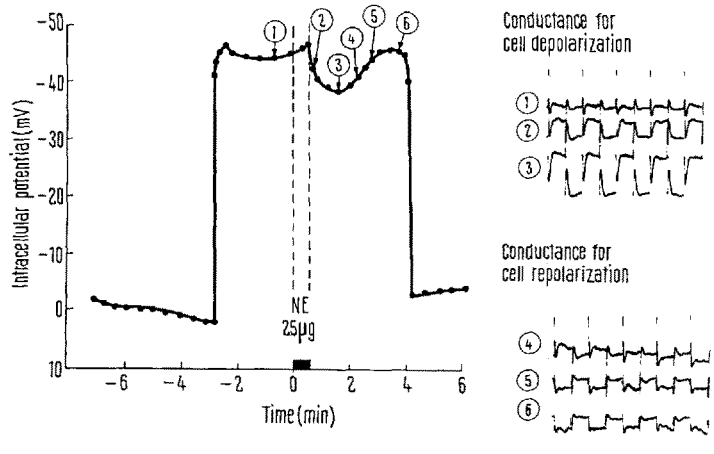

Fig. 3. Effect of NE, injected at time = 0, on the membrane conductance and potential of a brown fat cell. Differences between the response of this adipocyte and that depicted in Figure 2 reflect the variance in recordings.

Injection of NE (20–65 μg/kg i.v.) was followed by the expected depolarization of the impaled cell (Figures 2 and 3). Concurrent with this depolarization came a decrease in the resistance of the cell membrane, the resistance reaching a minimum at the time of maximum depolarization (trace 3, Figures 2 and 3). As the cell repolarized, the membrane resistance returned toward its pre-stimulus level (traces 4–6, Figures 2 and 3). Such changes in membrane conductance were noted in all brown fat cells examined. These observations thus indicate, that following NE injection an increase in the permeability of the membranes of the cells occurs concurrently with the depolarization.

The demonstration of this increased permeability lends support to the view that activation of the brown fat cell is accompanied by an increased energy requirement[2,3,7,8]. That is, if the membrane remained relatively impermeable, the depolarization-repolarization sequence might have involved the transfer of only a few ions and required little additional energy. However, as a result of the increased permeability, the ionic fluxes across the membrane must be substantially greater (i.e., the membrane behaves as if it were a 'sieve') and thus, considerably more energy may be required to restore the ionic distribution to the pre-stimulus conditions. Such an increased work load would be consistent with our previous suggestion, i.e., that maintenance of the elevated rates of oxygen consumption as observed when brown fat is activated in vivo may be sustained by an increased energy turnover rather than dissociation of cellular respiration from energy conservation[2,3,7–9].

Résumé. La dépolarisation de la membrane des cellules adipeuses brunes, produite par l'administration in vivo de noradrénaline, est aussi accompagnée d'une augmentation de la perméabilité mesurée par une augmentation de la résistance membranaire.

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Syndroma of Forced Weaning in Nursing Female Rats

Functional and morphological changes, which are obviously associated with milk production, occur in the organism of rats during lactation: Hyperphagia is accompanied by hyperplasia of the mucosa of the small intestine[1], changes occur in the liver[2], bone[3], adrenals[4], in serum lipoproteins[5], etc. It would, therefore, not be surprising if the forced weaning of the offspring led to more severe disturbances in the equilibrium of this increased metabolism.

As early as in 1936, Pugsley[6] demonstrated hypercalcuria after removal of the young. In our rats, the sudden interruption of suckling led to hyperphosphataemia and the development of postreproductive arteriopathy[7]. Nephrocalcinosis, appearing acutely within 24 h after forced weaning, has been detected as a further anatomical finding in our rats[8].

Recently, we investigated the serum levels of sodium, potassium, magnesium, glucose, cholesterol and β-lipoproteins after forced weaning.

Material and methods. 60 female Wistar rats aged 3–4 months were divided in 2 equal groups (according to the diet used). Each of these rats suckled 12 young of her first litter and forced weaning was carried out on the 21st day of lactation. On this day one half of the rat mothers was killed. The second half of each of the two groups was killed on the 22nd day after delivery, i.e. 24 h after forced weaning.

In the blood serum the above-mentioned components were estimated by following methods: glucose by means of the colorimetric method with *o*-toluidine[9], sodium and potassium by flame photometry, magnesium with the colorimetric method using thiazol yellow[10], cholesterol with the modified colorimetric method according to Pearson[11] and β-lipoproteins by means of the turbidimetric method[12].

Diets: 1. The so-called Larsen diet contained 1500 mg/100 g of calcium and 600 mg/100 g of phosphorus. 2. The diet DOS-II contained 1000 mg/100 g of calcium and 850 mg/100 g of phosphorus. The detailed composition of both these diets was given in our previous paper[13].

Control animals: In both groups there were 20 virgin female rats of the same age, fed 6–8 weeks before killing by the appropriate diet.

Results and discussion. No significant differences in food consumption were ascertained between analogical groups of diet 1 compared with diet 2.

The results are given in the Table. No significant differences were found after forced weaning in the levels of glucose, sodium and magnesium. Potassium showed a mild but significant decrease the day after forced weaning

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