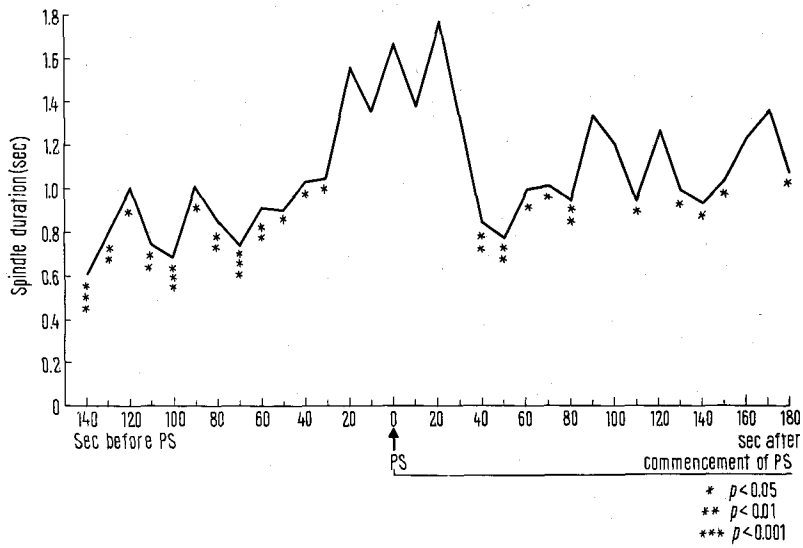

Average evoked spindle duration before and during paradoxical sleep. (Significance levels given for the comparisons between the average evoked spindle durations and this duration at time 0, using the *t*-test.)

response) reaches a maximum[11]. The coincidence of these events may be an expression of the increased tendency towards synchronization and rhythmicity in the areas of the cortex, thalamus, and striatum, three structures whose functional inter-relationship is hardly to be doubted. With increasing duration of the slow wave phase, both the spontaneous and evoked spindles virtually vanish, and the EEG is dominated by slow, high amplitude potentials.

As is shown in the Figure, the second increase in the duration of evoked caudate spindles begins some 30–60 sec before the onset of the typical PS. This is also the point at which the arousal threshold reaches a maximum, and at which the ponto-geniculo-occipital potentials appear[12]. After a further 10–30 sec the hippocampal potentials show an increased frequency with a clearer rhythmicity, and at the same time long, spontaneous, high amplitude spindles appear in all of the caudate and cortical traces. At this point the duration of the evoked spindles reaches a maximum. In this phase there is a greater tendency to rhythmicity and the evoked spindles frequently appear as an amplification of the background activity.

On the other hand, during periods of arousal, as represented in the EEG, spindles cannot be elicited. The explanation for these different responses to stimulation may lie in the function of the ascending reticular formation. During PS this system is strongly inhibited, as is shown by the raised EEG-arousal threshold[12–16], whereas during arousal itself, the ascending reticular system exerts an inhibitory effect on the caudate nucleus, resulting in abolition of the evoked spindles[17].

Zusammenfassung. Bei Ratten wurde über $8^1/_2$ Stunden alle 10 Sek durch elektrischen Reiz eine Kaudatum-Spindel ausgelöst. Die Dauer der einzelnen Spindeln wurde dem EEG-Bild zugeordnet. Bei statistischer Auswertung ergaben sich 2 Maxima für die Spindeldauer, das eine in der Einschlafphase (Übergangsphase + Spindelphase), das zweite beim Übergang vom Slow-wave-Schlaf zum paradoxen Schlaf.

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Norepinephrine-Induced Thermogenesis: Effect of Interscapular Brown Fat[1]

The function of brown adipose tissue (BAT) as a thermogenic effector during cold stress has been well documented[2–4]; however, its role during extended periods of cold exposure has not yet been resolved. In the rat, prolonged cold exposure is followed by adaptive responses among which is the shift from shivering to nonshivering thermogenesis (NST)[5]. Accompanying this transition are trophic changes in BAT resulting in an elevation of the thermogenic capacity of the tissue[2,3]. Notably, much of this heat is applied locally to the thoracocervical spinal cord, the thoracolumbar autonomic structures, and the heart[2,6]. The significance of this distribution is emphasized by the recent demonstration of temperature sensors in the thoracocervical spinal cord[7–9]. That is, the finding that warming of these thermosensitive areas is followed by an inhibition of shivering[7–9] has led to the proposal that during acute cold exposure, heat conveyed from the BAT to the cord may be an important link in controlling the

onset of shivering. Similarly, during prolonged cold exposure, heat from BAT may affect these thermosensitive areas such that shivering is progressively suppressed; i.e., the gradual disappearance of shivering associated with the development of cold acclimation may reflect a thermogenic influence of BAT[2].

Recently, it has been proposed that BAT serves an endocrine function during cold acclimation[10]. This suggestion derived from the observation that removal of the interscapular brown fat pad (IBAT) from cold-acclimated (CA) rats was followed by a decreased calorigenic response to administered catecholamines[10,11]. Since this decrease could not be accounted for by the magnitude of the oxygen consumption of the excised tissue or by the 'normal' rate of deacclimation to cold, it was proposed that BAT secretes a 'cold-acclimation factor' into the general circulation[10]. However, in view of the postulated interaction between BAT and thermosensors in the spinal cord, it may be that the response seen after IBAT excision reflects removal of an important thermoregulatory input to the central nervous system.

Our attempts to distinguish between the 'endocrine' and the 'neural' hypotheses were based on the fact that the venous efflux from the fat pad proceeds: a) by way of the bilateral thoracodorsal veins which empty into the precavals; as well as b) via the unpaired 'Sulzer's' vein to the deep fourth thoracic vein, and thence through the inner vertebral sinuses into the azygous[6]. Thus, ligation of Sulzer's vein should prevent much of the heat produced by the IBAT from being transfered directly to the thermosensitive areas of the cord. On the other hand, any factor secreted by the pad should still be accessible to other tissues via drainage through the thoracodorsals. Therefore, if the decreased calorigenic response to norepinephrine (NE) seen after excision of IBAT results from removal of the influence of a secreted factor, one might expect little decrease in the response of rats without Sulzer's vein. Alternatively, if the neural hypothesis is correct, the magnitude of the NE-induced thermogenesis in rats having had Sulzer's vein ligated should be similar to that of rats without the fat pad.

To test the two possibilities, the NE-induced stimulation of the rate of oxygen consumption ($\dot{V}O_2$) was compared in CA (4°±1°C, 60% relative humidity, 6–8 weeks), male, Long-Evans rats (300–350 g) having been subjected to one of the following surgical treatments: a) removal of the IBAT (group I); b) ligation and severance of Sulzer's vein (group II); and c) sham operated (group III). During the experiment, the $\dot{V}O_2$ of the rat was continuously monitored in a closed system apparatus (Volume Meter, Med-Science Electronics). All measurements were obtained with unanesthetized, unrestrained rats placed in chambers of sufficient size to allow movement. The chamber was maintained at 23°±1.5°C by controlling the temperature of a surrounding water bath. Although the rats were free to move, a relatively stable $\dot{V}O_2$ was reached after 1–2 h in the chamber. At that time, the rat was removed from the apparatus, injected s.c. with NE, and then replaced in the chamber. Since the $\dot{V}O_2$ increased immediately as a result of the handling, a period of 20–30 min was allowed for return to baseline. Moreover, as the response to NE was not usually manifested before 20 min, it was possible to separate out the handling artifact from the effect of the drug.

Using such procedures, the calorigenic response to NE (L-arterenol bitartrate, 1.75 mg/kg$^{0.73}$) was measured in all rats prior to any surgical manipulation. (The NE dose was one which we had previously found to stimulate maximally the $\dot{V}O_2$ of these rats.) Following the measurement, the rat was returned to the cold for at least 1 week, after which it was surgically altered according to one of the 3 treatments indicated above. Surgery was performed under sodium pentobarbital anesthesia, and the metabolic response to NE was subsequently followed on days 2, 5 and 7–8 (postsurgical), the rats being maintained at 23°±1°C.

The preoperative responses (plotted as values for day 0) were similar in all 3 groups of rats (Figure 1). Following surgery however, the magnitude of the NE effect in groups I and II was considerably lower than that in the shams even though the response to the catecholamine increased progressively when the rats were returned to the cold (day 10–11, postoperative). Moreover, throughout this entire period (about 30 days), the decrease of the calorigenic effect of NE in those rats without Sulzer's vein was as great as that in rats having had their fat pad removed (Figure 1).

That this similarity did not reflect necrosis of the fat pad in group II animals was ascertained by histological examination of the pad. Such examination indicated the presence of: a) mildly acidophilic cytoplasm and normal

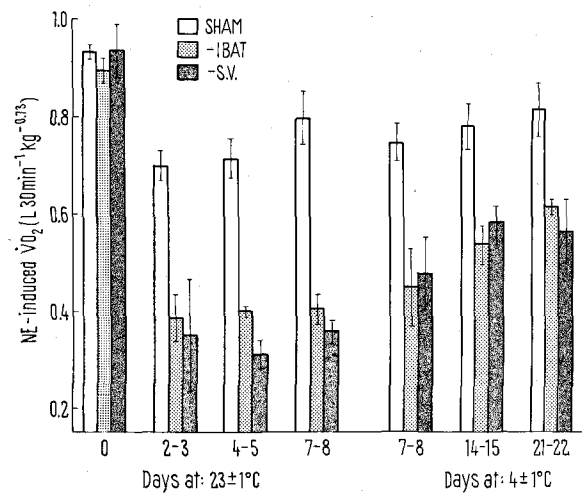

Fig. 1. NE-induced calorigenesis (L O_2 30 min^{-1} kg$^{-.73}$) in CA rats before (day 0) and after surgical treatment (see text). Values represent mean ± standard error; each group was comprised of 4 rats.

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chromatin dispersion in the multilocular fat cells; b) patent blood vessel lumens; and c) vessel walls apparently free of any form of necrosis. Moreover, no signs of edema, hemorrhage or inflammatory reaction were seen.

Thus, the observation that the NE-induced calorigenesis in rats having had Sulzer's vein severed was similar to that in rats without their fat pad indicates that the decreased effect of NE cannot be explained by the loss of a modifying factor secreted by the IBAT into the general circulation. Alternatively, the results support the suggestion that IBAT may be involved in regulating the thermogenic response of CA rats to NE via local warming of the thermosensitive areas of the spinal cord.

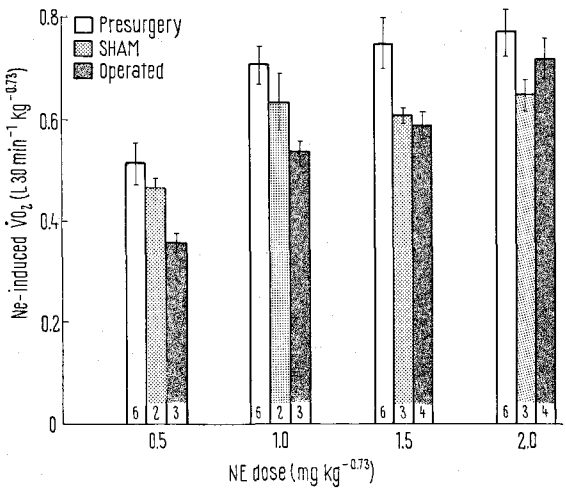

Fig. 2. Effect of increasing doses of NE on the calorigenic response of CA rats. Following surgery, the responses of each rat to the various doses were compared to the preoperative values. From such a comparison the relative (%) changes at each dose were calculated. These were reconverted to units of $\dot{V}O_2$ (L O_2 30 min^{-1} kg$^{-0.73}$) by taking as 100%, the average presurgical response for the given dose. The value within each bar represents the number of animals for which measurements were obtained.

With respect to the nature of this interaction, the results presented do not distinguish between a decreased sensitivity to NE (i.e., a lower response to a submaximal NE dose) and/or a decreased capacity of the surgically-treated rat to respond to NE (*i.e.*, a lower response to a maximum NE dose). However, that the sensitivity to NE may be decreased following removal of the fat pad or Sulzer's vein is suggested by preliminary studies wherein such rats were challenged with doses of NE ranging from 0.5 to 2.0 mg/kg$^{0.73}$ (injected through an implanted intraperitoneal cannula). As summarized in Figure 2, the NE-induced response of these surgically-treated rats differed from that of the shams only at the lower doses of NE. However, in view of the limited number of rats examined and the large variability seen, additional data are needed before concluding that removal of the IBAT affects the sensitivity of the CA rat to NE rather than the potential of the animals to respond. Nevertheless, the observation that responses of rats without IBAT were similar to those without Sulzer's vein appears inconsistent with the previously proposed[10] endocrine function of the tissue.

Résumé. La réduction de l'effet calorigénique de la noradrénaline chez des rats adaptés au froid, dont la veine de Sulzer a été ligaturée et sectionnée, est semblable à celle obtenue chez des rats sans graisse brune interscapulaire. Etant donné l'absence de signe de nécrose de la graisse brune chez les rats à veine ligaturée, cette observation ne parle pas en faveur d'une fonction endocrine de ce tissu.

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Electron Microscopy of the Lung Surfactant

There is strong evidence[1] that the alveoli of the lung are lined with a substance (surfactant) rich in di-palmitoyl lecithin[2], whose function is to lower the surface tension. Circumstantial evidence[3] points to the lamellated osmiophilic bodies (LOPB), contained in the type II cells, as the source of surfactant. Most electron micrographs of the lung[4], however, show no trace of a layer of surfactant at the alveolar surface. Others show a structureless material[5,7], or one showing geometrical patterns with spacings up to 20 nm. It is likely that in most of these cases the surfactant has been shifted from its position during fixation, or has been altered or removed by the organic liquids used in embedding. We have re-investigated the problem in mouse lung, using methods designed to circumvent these difficulties.

To prevent, as far as possible, the movement of superficially located surfactant, we have used either vacuum-collapsed lung, or lung whose alveolar air has been evacuated and replaced with boiled linseed oil (which hardens during embedding, and in which di-palmitoyl lecithin is insoluble). The specimens were fixed in glutaraldehyde and osmium tetroxide; they were then placed in a mixture[7] of lead nitrate and potassium ferricyanide solutions, M/240 with respect to $Pb_3(FeCy_6)_2$, with 5% excess ferricyanide ion to prevent blotchy precipitation. The lead ferricyanide forms a 'tricomplex'[7] with the polar groups

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