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.

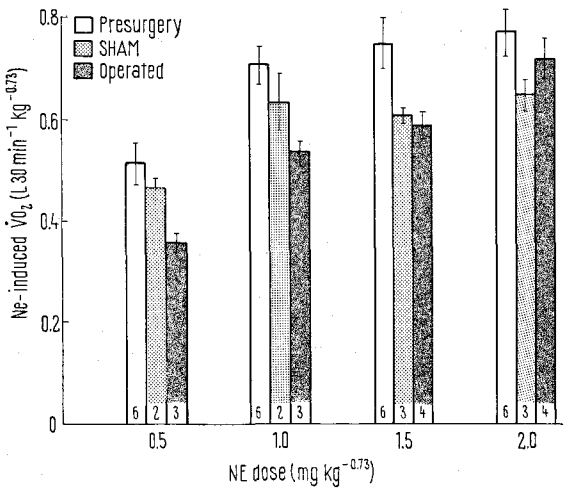

Fig. 2. Effect of increasing doses of NE on the calorigenic response of CA rats. Following surgery, the responses of each rat to the various doses were compared to the preoperative values. From such a comparison the relative (%) changes at each dose were calculated. These were reconverted to units of $\dot{V}O_2$ (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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of lecithin, thereby rendering it less soluble. The specimens were then embedded in Araldite, using dioxan and acetone as dehydrating and thinning agents. With acetone a shortened time-schedule[8] was used. Durcupan (Fluka) water-soluble resin was also sometimes used for embedding; we found that it required 40% more hardener 'C' and a higher embedding temperature (60 °C) than are usual. These methods preserve the LOPB better than do conventional methods.

A prominent feature of micrographs from these specimens has been the presence in the LOPB of series of parallel lines spaced at about 4 nm, referred to here as 4 nmS. Individual measurements of the spacing may vary by ± 15%, but no systematic difference has been found in the spacings in LOPB, in patches at the alveolar wall, or in sediment from lung bubbles. A similar spacing can be found in micrographs by CLEMENTS[9] and DERMER[7]. Using the above methods we have obtained the following results:

1. All LOPB show 4 nmS in some portion of their structure; each lamella consists of several 4 nm layers. (Figure).

2. When the specimen is tilted in a goniometer holder, the places at which 4 nmS is visible shift. This suggests that the LOPB are composed entirely of layers spaced at about 4 nm, which appear on the micrograph only when they are parallel to the electron beam.

3. At the boundary between the alveolar wall and the alveolar space, no continuous lining layer showing 4 nmS has been found, even when a search has been made at varying angles of tilt. There are however localized regions of the surface where several layers spaced at 4 nm can be seen.

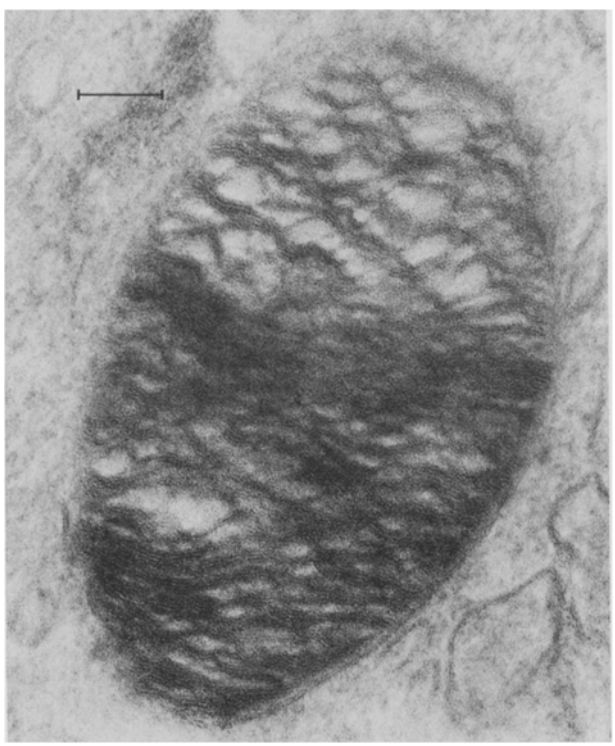
4. In mature foetal mouse lung, LOPB showing 4 nmS are found in the type II cells, and bodies indistinguishable from these are found in the canalicular lumen of the lung. Careful search of the canalicular walls has failed to disclose any sign of 4 nmS or anything else suggesting a surfactant lining. Osmiophilic bodies, of wispy structure, with regular spacings of 4 nm or more, are also sometimes found in the canaliculi.

5. Pellets obtained by centrifuging washings from the lung, which contain dispersed surfactant, show bodies resembling parts of LOPB, with 4 nmS; rather similar bodies, with similar spacing, are found in debris obtained from lung bubbles which have been carefully washed and then collapsed. Other workers[10] have found whorls of regularly spaced parallel lines in the debris from broken lung bubbles. The low surface tension of lung bubbles shows that they are lined with at least one monolayer of surfactant.

6. In a specimen of infected and consolidated lung, LOPB were found in the debris filling the alveoli. There were no traces of a layer with 4 nmS at the walls.

7. In particles of synthetic di-palmitoyl lecithin, fixed with osmium tetroxide and lead ferricyanide, embedded, and sectioned, a variety of spacings from 4.5 nm downwards were found.

We interpret these findings as follows: a) 4 nm spacing is characteristic of surfactant as originally formed in the lung. b) The LOPBs are the source of lung surfactant; it is not formed (as has been suggested[11]) by sloughing of the membranes of the alveolar cells. c) When the LOPB are extruded into an air-filled alveolus, they split up as to cover the interface with pre-formed rafts of surfactant with the hydrophobic groups facing the surface. According to this view, most of the surface has only one or a few monolayers of surfactant; there are thicker local patches representing the remains of LOPB. These patches may adhere to bubbles, and can appear in lung washings. d) With exposure to body or processing fluids, the spacing between mono- or bi-layers of surfactant may increase, so that arrays of lines referred to by some authors as 'myelin figures' are produced.



Osmiophilic body in mouse lung showing 4 nm spacing and coarser lamellae. Glutaraldehyde – osmium – lead ferricyanide fixation, acetone-Araldite embedding. Scale bar 100 nm.

Résumé. On a étudié le poumon au microscope électronique par des méthodes speciales pour ménager les inclusions lamellaries osmiophiliques. On a conclu que la substance tensioactive n'est pas produite par la membrane superficielle des cellules alvéolaires, mais par les inclusions lamellaires des cellules du second type.

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