gical phases of the exocytosis cycle[14] are seen (Figures 1 and 2); besides the usual and filled or granulated DCVs dispersed throughout the cytoplasm (preferentially the subnuclear areas) of the epithelial cells of the NEBs, numerous DCVs concentrate in a much larger number than in the control animals in the vicinity of the basal cell membrane; next DCVs are observed whose membranes become fused with the cell membrane itself (Figure 1, B,C). Consecutively they open at the level of the basal membrane with an extrusion and exocytosis of their contents into the space between the basal cell membrane itself and the basement membranes. At this stage of the secretory cell process, the vesicle may still be observed to contain a small dense core which may become fragmented (Figure 1, A,B), or the vesicle may appear entirely empty (Figure 1, A,D). Finally, we observed areas of the basal epithelial cell cytoplasm which contained vesicles both empty and smaller than the classic DCVs and which are not seen in the normal state. They probably correspond to so-called 'refilling' vesicles[14]. Besides the exocytosis, hypoxia treated animals reveal occasionally a slight and focal mitochondrial lysis and a pronounced development of the Golgicomplex, which is located above the cell nucleus and forms small DCVs and many empty small cisternae.

Discussion. As the apical cell pole of the NEBs immediately contacts the airway lumen and its contents on the one hand, and as a fenestrated blood capillary is closely apposed to their basal or vascular pole on the other hand[10], it appears logical that the NEBs are chemoreceptor organs with a local intrapulmonary secretory activity, one of the substances released within the blood stream of the lungs being serotonin. This identifies the previously unelucidated, intrinsic morphological mechanism explaining the occurrence of a hypoxia induced pulmonary vasoconstriction[1–3] which is in itself not markedly influenced by the nervous system, blood pH and lactic acid, but mediated by humoral substances[6,7], e.g. serotoin[8]. NIDEN et al.[15] have demonstrated that serotonin injected into the pulmonary circulation causes an increase in the oxygen saturation of the pulmonary venous blood. As most of the intrapulmonary bronchial capillary and venous blood is drained off via the pulmonary circulation[16], it may well be that the serotonin secreted by the NEBs during hypoxia causes a vasoconstrictor response with blood shunting from the poor to the better oxygenated and ventilated portions of the lung, providing besides the central and peripheral chemoreceptors a third or locally inbuild intrapulmonary chemoreceptor system which finely adjusts the ventilation to perfusion ($\mathring{V}/\mathring{Q}$) ratios.

Résumé. Les «Corpuscules Neuro-épithéliaux» de l'épithélium respiratoire intrapulmonaire ont ont été étudiés au microscope optique et électronique chez des lapins soumis à des conditions expérimentales d'hypoxie. Dans ce cas ils sécretent à leur pôle vasculaire basal leurs vésicules à noyau dense contenant de la sérotonine. Nous supposons que parmi leurs diverses fonctions neuroréceptrices possibles, ces «Corpuscules Neuro-épithéliaux» forment un système intrapulmonaire chémorécepteur sensible à l'hypoxie, en plus des chémorécepteurs centraux et périphériques (par exemple le corps carotidien) dont l'existence est bien connue. Ils sécretent de la sérotonine et probablement aussi des substances aminées ou peptidiques associées qui influenceraient la vasoconstriction pulmonaire et sont modulés par le système nerveux central.

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Electron Microscopy of the Effects of Histamine and Thermal Injury on the Blood and Lymphatic Endothelium, and the Mesothelium of the Mouse's Diaphragm, Together with the Influence of Coumarin and Rutin[1]

MAJNO et al.[2] showed that 3 mediators of inflammation (including histamine) cause contraction of the endothelial cells of venules. Subsequently[3] a combined light and electron microscopic approach has shown that the contracted cells are those associated with the open junctions which cause the increased permeability of these vessels. In thermal injury, COTRAN[4] showed that the affected cells and open junctions occur in capillaries rather than venules. This has been ascribed to the direct affects of the injury[5,6]. We therefore decided to study the effects of histamine and thermal injury on the contraction of the endothelial cells in both classes of vessels in the diaphragm of the mouse.

Any form of injury which has been tested has been shown to result in the opening of many junctions in the endothelium of the initial lymphatics[7]. There is much evidence indicating that this is largely because of the effects of oedema pulling these vessels open and their cells apart, together with other factors occurring in normal lymphatics[7]. However, because lymphatic endothelium is so similar to that of blood vessels, we decided to examine the effects of the injuries on lymphatic endothelial contraction. Similarly, because of the similarity of the mesothelium to endothelium[8], we decided to examine this too, using that adjacent to the injurious stimuli.

It has recently been shown that coumarin and related compounds have the property of considerably reducing high-protein oedemas, including those caused by thermal injuries[9–14]; it is considered that this probably occurs because coumarin induces considerable proteolysis of the extravasated plasma proteins in the tissues[11,12]. While it

Time (min)	Cells[a]	Nuclei[a]	Blood vessels[b] No Venalot	Blood vessels[b] Venalot	Lymphatics No Venalot	Lymphatics Venalot	Mesothelium No Venalot	Mesothelium Venalot	Connective tissue No Venalot	Connective tissue Venalot
0	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	Dark, shrunken and pseudopodia	Contraction +++	Open junctions ++	+++	Dilated and open junctions +	+	Open junctions ++	++	Proteins and oedema +	+
10	Dark, shrunken and pseudopodia	++	++	+++	++	+	++	++	++	+
30	Some pale and swollen	+	+ some stasis and collapse of venous capillaries	++	+++	++	+	+	+++	++
90	Many vacuoles, small vesicles and blebs	0	0	+	+++	++	0	0	++++	++

These results represent the means of 20 nuclei per cell type for each of 3 animals at each time; on a scale of 0 to ++++; the ranges were ~ +. [a] These include the blood and lymphatic endothelium, and the mesothelium. [b] These refer mainly to venules in the case of histamine injury, and mainly to capillaries in the case of burning.

has been shown that coumarin slightly increases blood vascular leakage[11,12] and functions even if the lymphatic system is occluded[11–13], we considered that it would be useful to include a study of coumarin in this series of experiments. This was not only to study any possible effects it might have on cellular contraction, but also to study its effects on oedema in another region.

One group of 36 adult white mice (~ 20 g) were i.p. injected with 17 ml/kg of Venalot® (Schaper and Brümmer, Salzgitter-Ringelheim, Western Germany – each 1 ml contains 1.5 mg of coumarin and 25 mg of trihydroxyethyl-rutin); another 36 received an equal amount of normal saline. 1 h later, 15 in each group had 0.2 mg of histamine PO_4 injected into their right pleural cavity, while another 15 had their diaphragms burned by injecting onto it 10 ml of normal saline (54 °C), via an incision in their right flanks. After 40 sec this was washed out with normal saline at 39 °C. The uninjured animals were used as controls; the injured ones were killed at 1, 3, 10, 30 and 90 min. In all cases portions of diaphragms were excised, fixed in glutaraldehyde, post-fixed in osmium tetroxide, embedded in araldite, stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I. The amounts of cellular contraction were estimated using the established criteria[2,3], with especial attention paid to nuclear pinches; 20 nuclei of each cell type were randomly studied for each animal, and the results noted on a O to ++++ range.

The results are summarized in the Table and illustrated in Figures 1 and 2. The changes seen with histamine and thermal injury were very similar and, for simplicity, are considered together in the Table. It should however be noted that while histamine mostly affected the venular blood endothelium (with only minor cytoplasmic changes) thermal injury more severely affected mostly the capil-

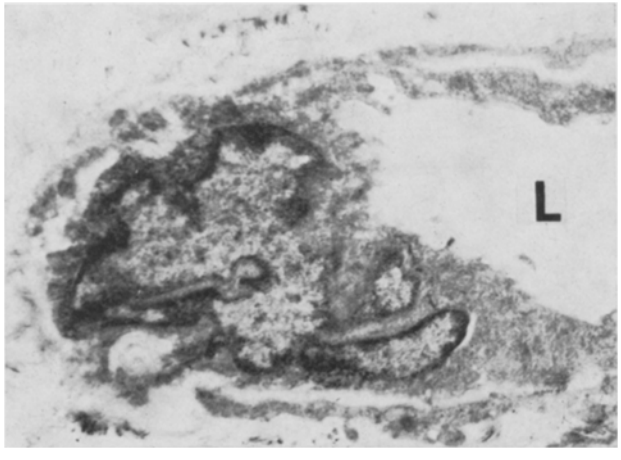

Fig. 1. A lymphatic (L), 10 min after histamine, treated with Venalot. There is a nucleus showing that the cell has contracted by its numerous infoldings. There is relatively little protein in the tissues or the lumen. ×5000.

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laries – presumably because, as in the skin, these lie closer to the surface of the tissue and hence suffer more injury[5,6]. The mesothelium, and the lymphatic and blood endothelium all showed very similar changes, which were naturally most pronounced adjacent to the injurious stimuli. Initially the cells appeared shrunken with dark, electron-opaque, cytoplasm and pseudopodia; after 30 min more and more cells became swollen, pale and oedematous. Some blebs were seen, together with many large vacuoles. Unlike what happens with more severe burns[15], the small vesicles seemed to increase in numbers.

The nuclei of all 3 classes of cells showed much evidence of cellular contraction (the venules in the case of histamine, and the capillaries with thermal injury). At maximum, some 30% of nuclei were affected. The contraction occurred early in all cases and passed off by 90 min. This is similar to what has been observed by intra-vital microscopy with venules[3]. The contraction of the mesothelium and of the lymphatic endothelium is important since these tissues are not subjected to the pressure of the blood. This is additional evidence that this is an active contraction and not just passive recoil[2]. The association of this contraction with the opening of the intercellular junctions, particularly in the mesothelium, indicates that it is the active contraction which opens the junctions. (Although it must be remembered that there are normally some open mesothelial junctions[16].) Of course, the poorly supported lymphatic endothelial junctions are easily opened by this contraction as well as by all the other factors normally affecting them, including the oedema[7].

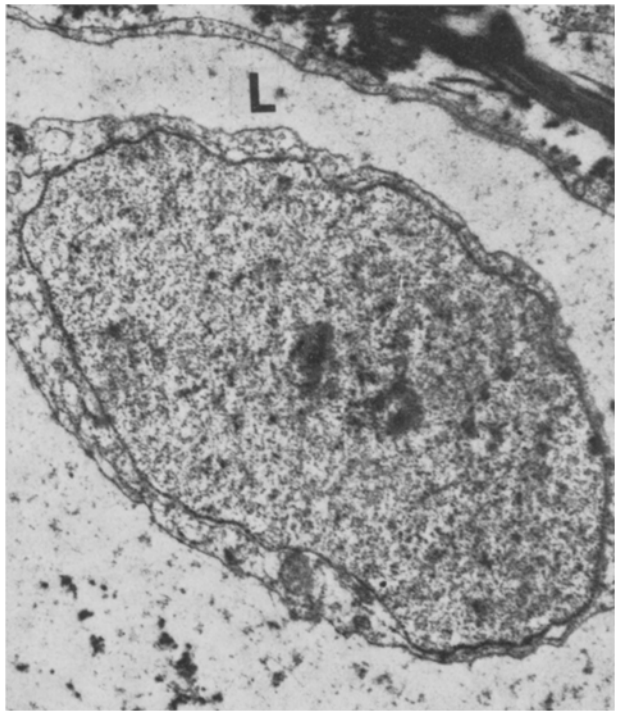

Fig. 2. A lymphatic, 30 min after burning, without Venalot treatment. The nucleus has become ovoid, with little indication of cellular contraction after this period. There is a considerable amount of protein in the tissues and in the lumen. ×8000.

The Table shows that Venalot causes some increase in the number of open blood vascular junctions as has been recorded elsewhere[11,12]. It did not appear to have any effect on the cellular contraction itself, although the slight increase in the numbers of open blood vascular junctions suggests that this may indeed be the case, but that our numbers of observations were insufficient to detect it. Any increased leakage of protein occasioned by this is more than compensated for by its action in removing protein (and hence oedema) from the tissues. This allows the lymphatics to be less dilated and to have fewer open junctions since, although some cells contract, many of them do not, and any lessening of the oedema will lessen the tension in the anchoring filaments, the dilation of the lymphatics and the separation of their endothelial cells[7].

Zusammenfassung. Hitze und Histamin vermögen eine Kontraktion der Endothelzellen feiner Venen zu erzeugen. Hitze und Histaminschädigung des Mäusezwerchfells (Endothelfensterung) führt zu Kontraktionen von Endothelzellen und Mesothelialzellen. Venalot, ein Produkt aus Cumarin und Trihydroxyethyl-Rutin, vermag das Oedem zu beseitigen.

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Combined Surgical and Radiation Injury VIII: The Effect of the Gnotobiotic State on Wound Closure

Despite extensive clinical and experimental investigation, the mechanism of wound healing has not been completely elucidated. Previous studies have demonstrated a delay in wound contraction following whole body X-irradiation in the midlethal range. Retardation in wound closure was most marked when the rodent was irradiated 4 days prior to wounding[1]. The irradiation induced mortality was significantly increased by wounding. Partial bone marrow shielding[2] bone marrow transplantation[3] or the administration of radioprotective compounds[4] decreased the mortality and partially corrected the radiation induced retardation in wound contraction. A major factor underlying the augmented mortality and delayed wound healing pattern in the whole body irradiated animal may be the decreased resistance to infection characteristic of the hematologic syndrome. Although the wound healing abnormality was not corrected by the administration of antimicrobials[5], suggesting that bacterial

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