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Langerhans Cells in a Bronchiolar-Alveolar Tumour of Lung

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Summary. Large numbers of dendritic cells similar in structure to Langerhans cells of normal epidermis and other epithelia were observed within nodules of a tumour identified as a bronchiolar-alveolar tumour of primary alveolar origin.

Though Langerhans cells have never yet been seen in normal lung, it is argued that the present observations provide circumstantial evidence for their probable presence, perhaps in very small numbers, in this situation. Such a suggestion would also render more explicable the characteristic feature of pulmonary lesions of Histiocytosis-X.

Apparently unrelated pathological conditions sometimes present unexpected common features linked with a more general problem. Bronchiolar-alveolar carcinoma of the lung has no apparent relation with Histiocytosis-X, and one would not necessarily expect examination of the one condition to extend understanding of the other. However, the present paper demonstrates that a previously unreported feature of bronchiolar-alveolar carcinoma can have a direct bearing not only upon the problem of the pathogenesis of pulmonary lesions of Histiocytosis-X, but also upon the closely-linked, but more general problem of the nature of the Langerhans cells of normal epidermis, the ontogeny and function of which, are obscure (Langerhans, 1868; Breathnach, 1965; Breathnach and Wyllie, 1967; Wolff, 1972).

A characteristic, indeed diagnostic feature of pulmonary and extra-pulmonary lesions of Histiocytosis-X is the occurrence of cells containing cytoplasmic organelles (X-granules) of highly individual shape and structure (Basset and Turiaf, 1965; Basset and Nezelof, 1969; Gianotti *et al.*, 1968; Nezelof *et al.*, 1973; Wolff, 1972). So far, the origin and nature of these cells remains to be established, but perhaps the most interesting and puzzling thing about them is their remarkable, though not total, resemblance to Langerhans cells. So close, in fact, is the resemblance, that one is tempted to suggest that the two cells are identical, or, at any rate, that the characteristic cells of Histiocytosis-X (H-X cells) are derived from, or represent pathological variants of normal Langerhans cells. This suggestion would be consistent with the occurrence of cutaneous lesions of Histiocytosis-X (Letterer-Siwe's disease) since Langerhans cells are present in normal skin, both epidermis and dermis (Zelickson, 1965; Breathnach, 1971). It is, however, difficult to explain the pulmonary or osseous lesions on this basis because, despite numerous ultrastructural studies, Langerhans cells have never been reported as present in normal lung or bone. It might, of course, be suggested that the pulmonary lesions are metastatic to primary skin lesions (Nezelof *et al.*,

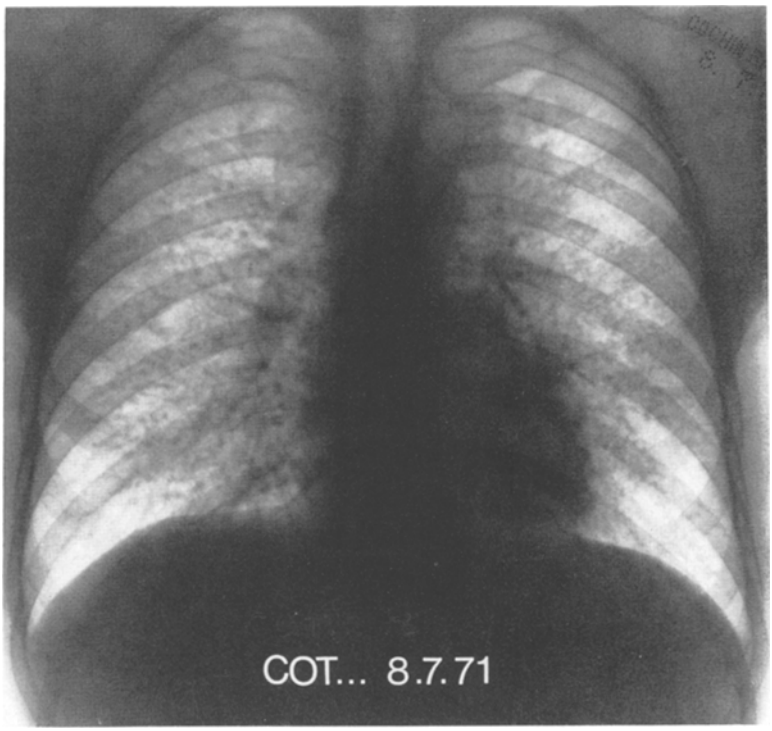

Fig. 1. Chest radiograph taken a few days before biopsy

1973), but there is no real evidence to support this view, which is somewhat speculative. In fact, in many instances, the only lesions to appear are located in the lung, especially in young adults.

The observation reported here, i.e., the occurrence of cells resembling normal Langerhans cells in a bronchiolar-alveolar tumour of lung, is relevant to the problem outlined above, and it also has intrinsic interest from the point of view of lung tumour pathology.

Clinical Details: Materials and Methods

The patient, a male aged 39, first presented in February 1971 with recurrent episodes of spinal pain, initially confined to the neck, but later spreading to the thoracic and lumbar regions. Symptomatic treatment produced no relief, and loss of weight and general malaise were the chief complaints. Three months after first reporting, respiratory symptoms appeared, with cough, sputum, and rhinitis, but without pyrexia. A chest X-ray at this time showed diffuse micronodular reticulation, predominantly in the upper and middle zones of both lungs (Fig. 1), and an irregular translucent zone in the posterior part of the body of the 6th thoracic vertebra. On physical examination, there was neither dyspnoea, cyanosis, nor finger clubbing, and respiratory and cardio-vascular systems were normal. There was slight hepatomegaly, and some scoliosis of the thoracic spine with limitation of lateral flexion. Sedimentation rate was 12–36 mms per hour and the Mantoux test was positive (10 μ). Repeated sputum examinations for M. tuberculosis proved negative, and respiratory function tests revealed only slight restrictive disease. The patient had never been a cigarette smoker, and

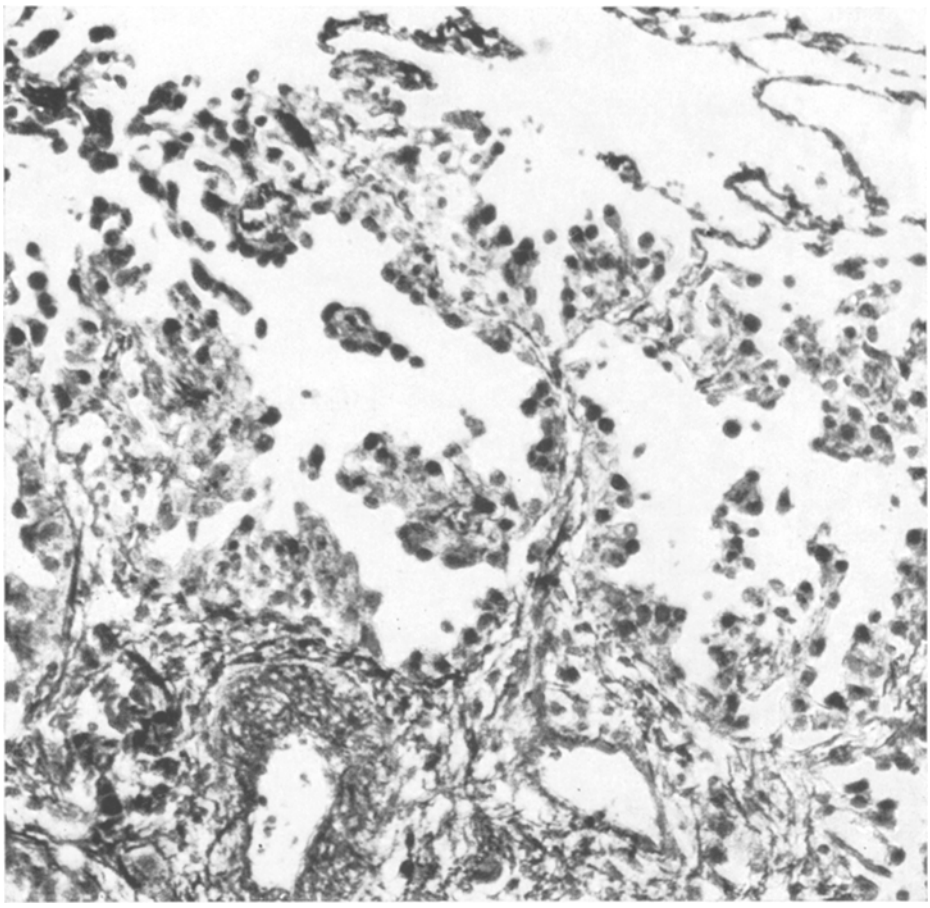

Fig. 2. Light microscopy: part of a tumour nodule with remaining alveolar pattern. Staining: Haematoxylin and eosin. ×125

gave no history of previous respiratory disease. He had been employed for 25 years as an electrician in buildings belonging to the French Atomic Energy Centre, and had been subjected to radiation-level tests only during the last 7 years of this period. Two years previous to examination, he had been exposed to significant levels of radiation as the result of an accident, but after a few weeks, blood and urine tests returned to normal.

Frozen sections of material obtained by lung biopsy (part of which was set aside for further light microscopic and electron microscopic examination) revelaed tumour tissue of an adeno-carcinomatous papillary type, with numerous intra-vascular growths.

Despite cytotoxic therapy, the patient's condition deteriorated rapidly, and he died 10 months after first presentation with evidence of liver and C.N.S. metastases. At autopsy, multiple nodules were found throughout both lungs, with metastases in liver, spleen, dorsal and lumbar spine, left adrenal and the C.N.S.

Specimens for light microscopy obtained at biopsy and autopsy were fixed in 10% formaldehyde, and paraffin sections were stained with haematoxylin and eosin, Alcian blue, and PAS. For electron microscopy, small blocks of biopsy material were fixed following the Hirsch and Fedorko (1968) technique, in buffered 2.6% glutaraldehyde mixed with 1%

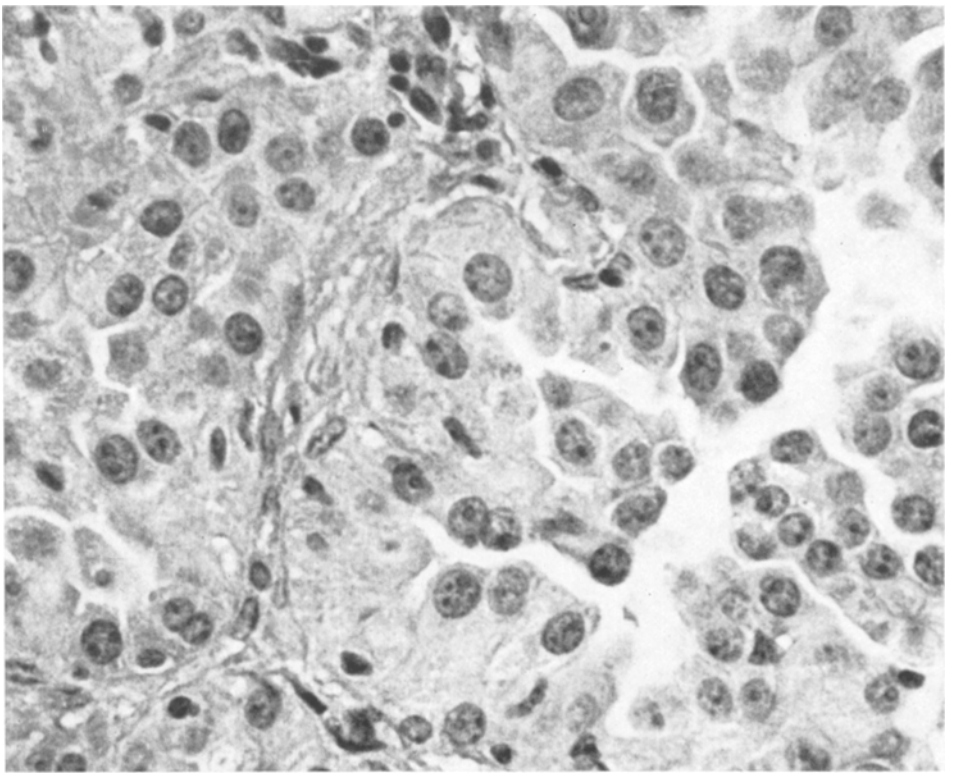

Fig. 3. Light microscopy: Part of a more compact tumour nodule. Staining: Haematoxylin and eosin. ×320

OsO_4, and bulk-stained in sodium acetate buffered 0.25% uranyl acetate at pH 6.3 for thirty minutes. They were then washed in saline, dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon. Semi-thin sections were stained by the Richardson *et al.* (1960) technique, and thin sections stained with lead citrate and uranyl acetate were examined in a Siemens Elmiskop 101 electron microscope.

Results

Light Microscopy

Gross light microscopical examination showed a background of normal alveolar tissue in which tumour nodules were scattered. There appeared to be two types of nodule. The first of these was characterized by alveoli lined by tall pale-staining columnar cells arranged in a single layer (Fig. 2). Nuclei were large and often basal, with prominent nucleoli. There was no evidence of mucus secretion, nor were cilia found. Within air spaces, clusters of tumour cells were often seen. It was not possible to decide whether this appearance was due to free desquamated cells or whether it was the result of tangential sectioning of adjacent involved alveoli. Small groups of tumour cells were also seen within otherwise normal alveolar walls in the neighbourhood of frank tumour nodules.

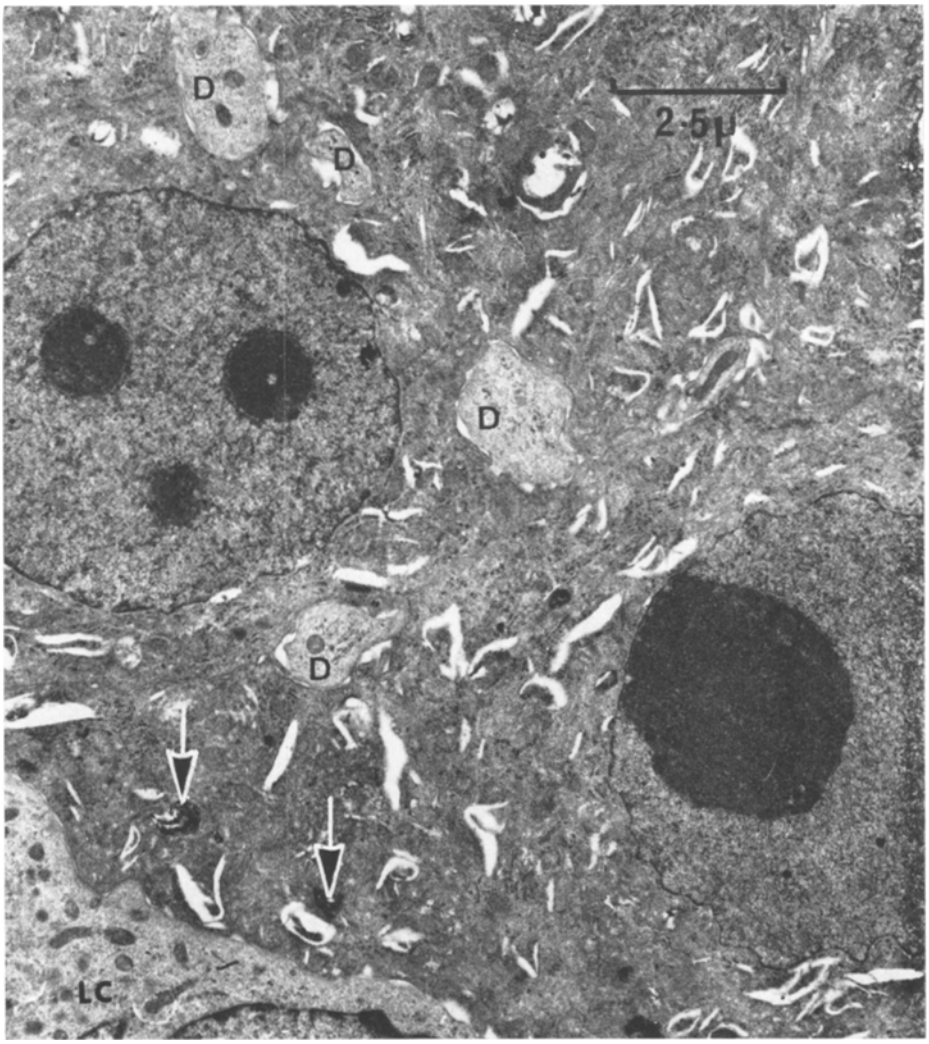

Fig. 4. General appearance of tumour cells with large nuclei, prominent dark nucleoli, cytoplasmic "splits", and a few outlined osmiophilic lamellar bodies (arrows). At bottom left corner there is the body of a "Langerhans cell" (*LC*). Several dendrites (*D*) are interspersed between tumour cells. ×8750

The second type of tumour nodule appeared more compact, alveoli being not only lined, but also filled by closely packed tumour cells of irregular rather than columnar shape, but whose other characteristics, including nuclear and nucleolar staining, were identical to the columnar cells of the first type of nodule (Fig. 3). Thickening and fibrosis of alveolar interstitium were more evident in the second type of nodule and in some places the alveolar pattern itself disappeared. In both forms, blood vascular and lymphatic spread was evident.

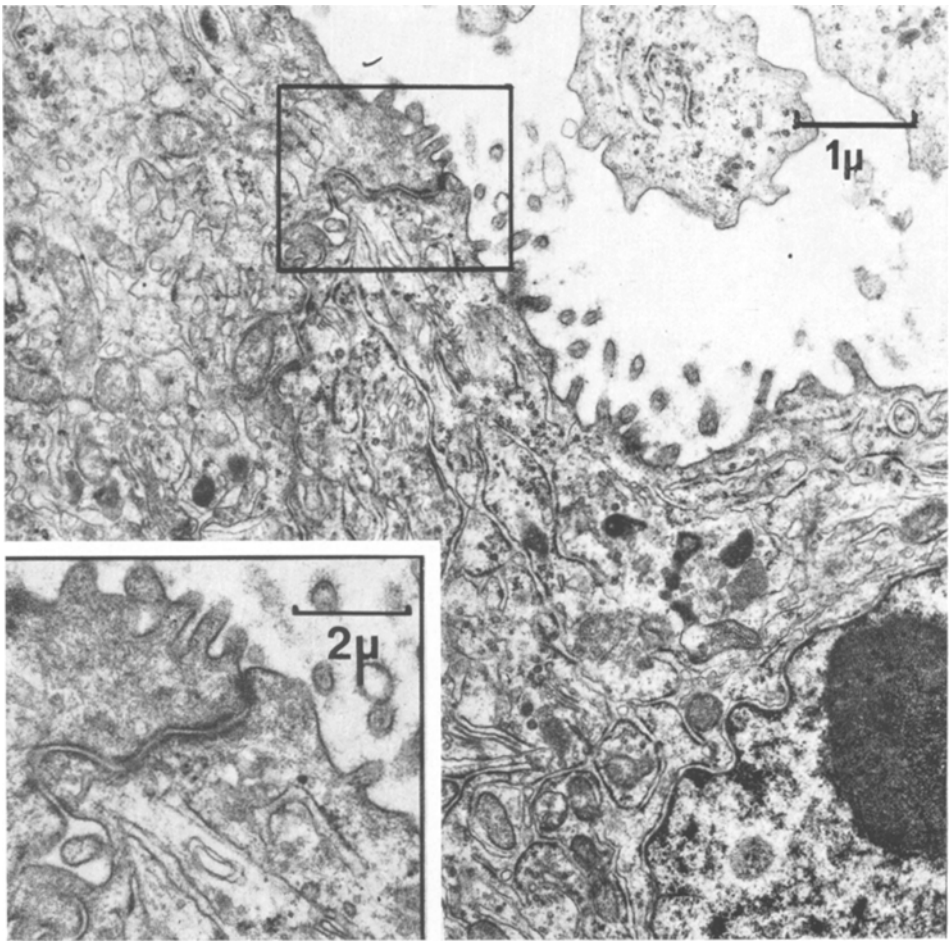

Fig. 5. Detail of two adjacent poorly differentiated tumour cells showing their surface microvilli and (insert) the junctional complex between them. ×15000. Insert: ×30000

A tentative diagnosis of bronchiolar-alveolar tumour was made on the basis of the above observations.

Electron Microscopy

Tumour cells were easily recognizable by their large rounded nuclei, containing one or more extremely electron-dense nucleoli (Figs. 4, 5). Numerous short slender microvilli projected from the cell surface (Figs. 5, 6) and some cells exhibited many "splits" in the cytoplasm (Figs. 4, 10). The splits are probably artifactual, but nevertheless possibly of significance, as in granular pneumocytes (Basset *et al.*, 1971). There was a marked development of the rough and smooth endoplasmic reticulum, of free ribosomes (often forming rosettes) (Figs. 5, 6) and

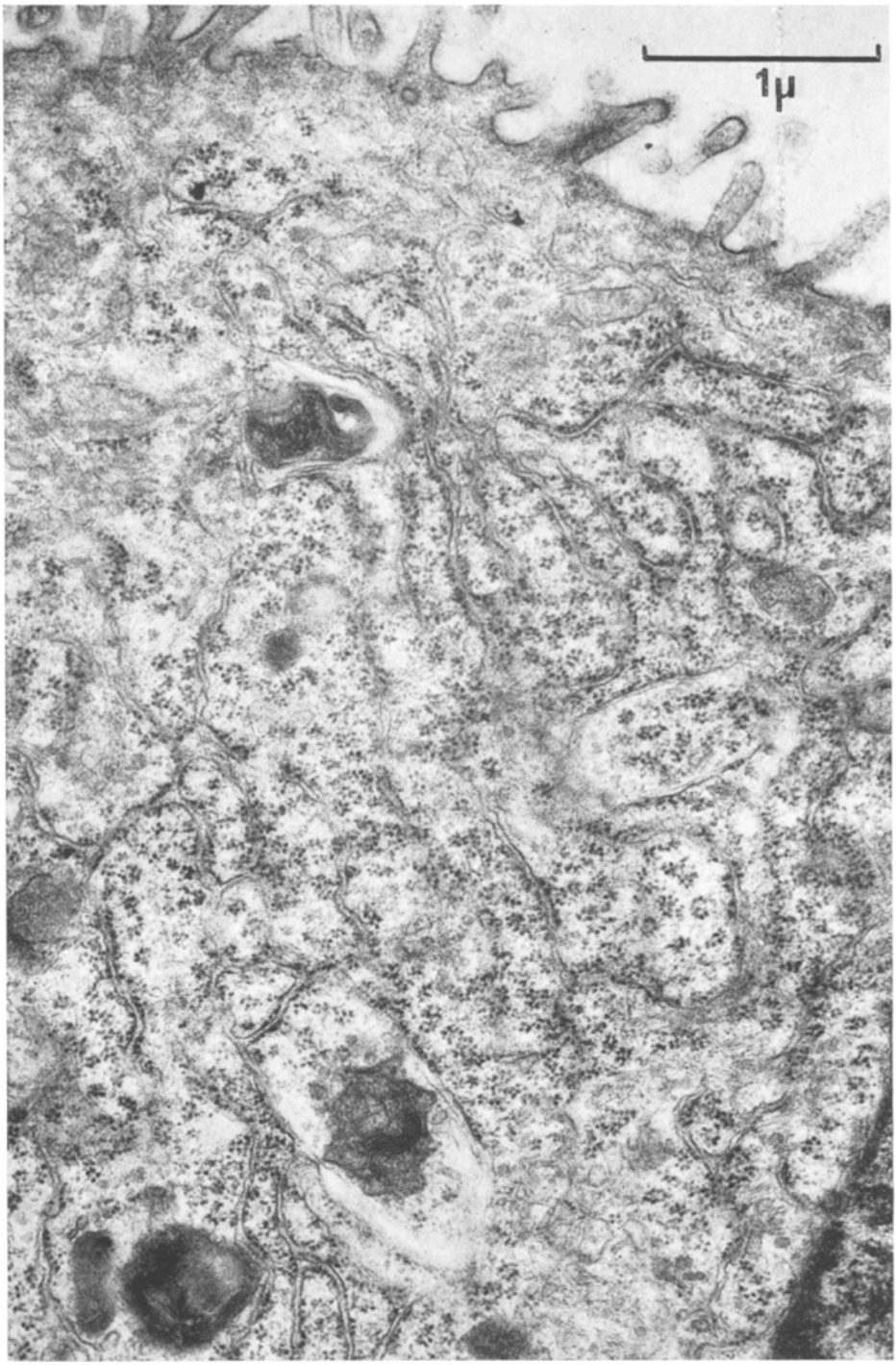

Fig. 6. Detail of a poorly differentiated tumour cell showing the surface microvilli, numerous ribosomes, rough endoplasmic reticulum, and several outlined lamellar osmiophilic bodies. ×30000

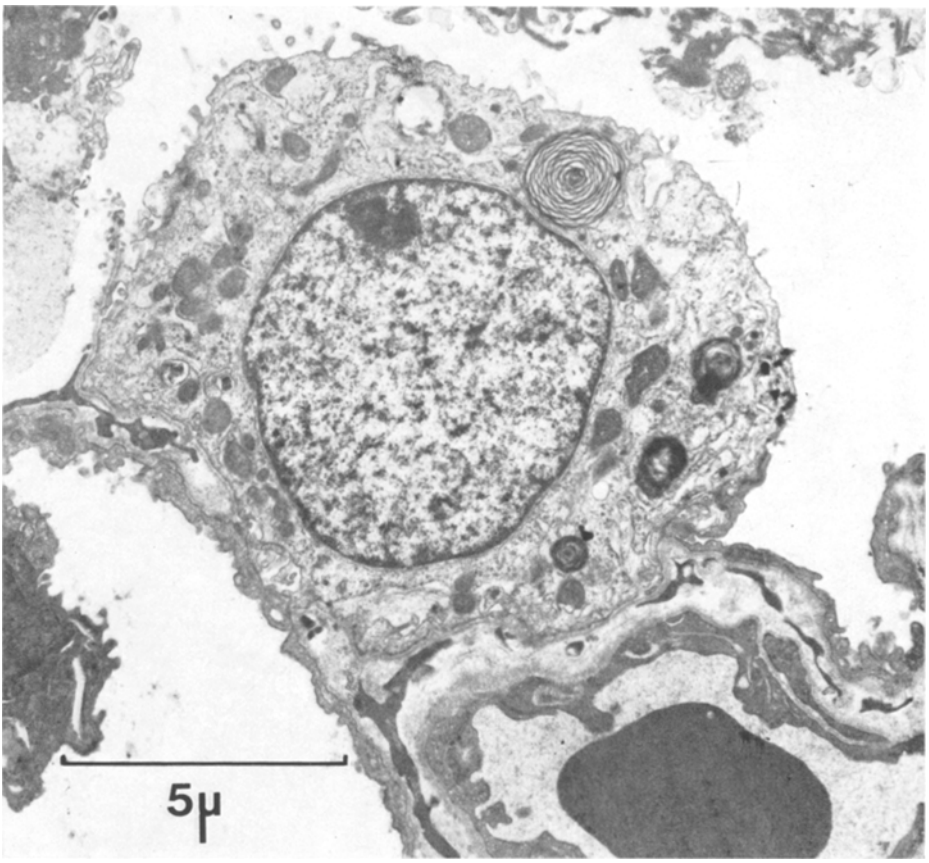

Fig. 7. Isolated tumour cell at the periphery of a tumour nodule, with a well differentiated osmiophilic lamellar body. ×7500

Golgi saccules and vesicles were prominent. Small mitochondria were irregularly distributed.

Variable numbers of lamellar osmiophilic bodies were distributed in the cytoplasm, some of them apparently about to be extruded at the cell surface, especially in cells at the margin of the tumour nodules (Fig. 7). In less differentiated tumour cells, these osmiophilic bodies were less easy to identify, as they were small and sometimes only outlined, but they were nevertheless present (Fig. 6). Junctional complexes, usually of the tight or gap junction type, were often seen between adjacent cells (Fig. 5).

In the first type of nodule, the cells always rested with their basal poles on a continuous basal lamina (Fig. 7). In the second type of nodule, only the deepest cell layer was in contact with a basal lamina (Fig. 10).

Alveolar walls were thickened by bundles of collagen and elastic tissue, but there was no increase in cell population in the alveolar interstitium. Alveolar capillaries were scarce within tumour nodules. Some of them contained tumour cells, tightly packed against endothelial cells (Fig. 9).

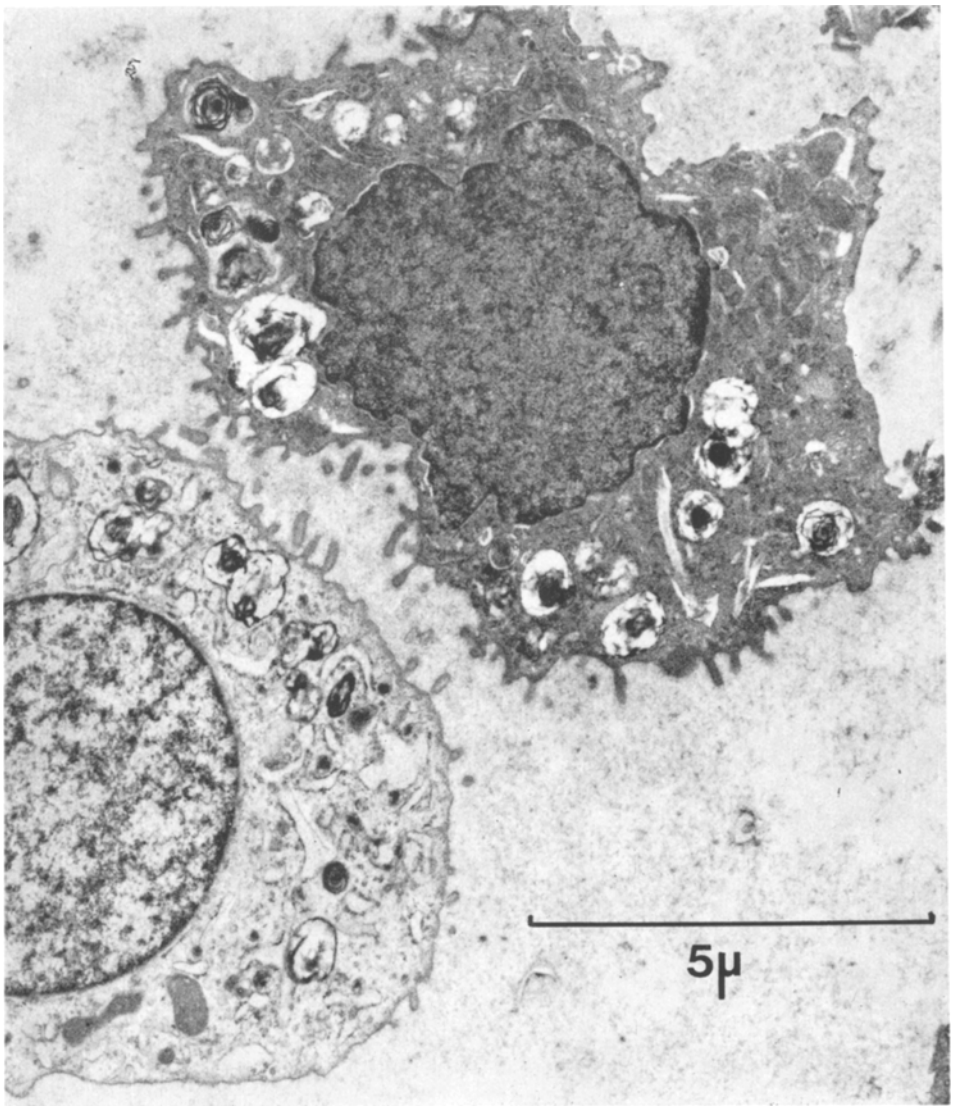

Fig. 8. Two cells lying free in an alveolar lumen: the dark one is probably a desquamated type II pneumocyte, the clear one probably a tumour cell. ×10000

Normal pneumocytes, mostly of the granular type, were sometimes interspersed amongst tumour cells. Normal cells, tumour cells and normal alveolar macrophages were occasionally seen free in the air spaces (Fig. 8).

Interspersed amongst tumour cells of the above types were numerous isolated dentritic cells, easily identifiable at low magnification by their shape, generally translucent cytoplasm and indented nucleus (Figs. 4, 10). At higher magnification, these cells were seen to contain the rod-shaped cytoplasmic organelles charac-

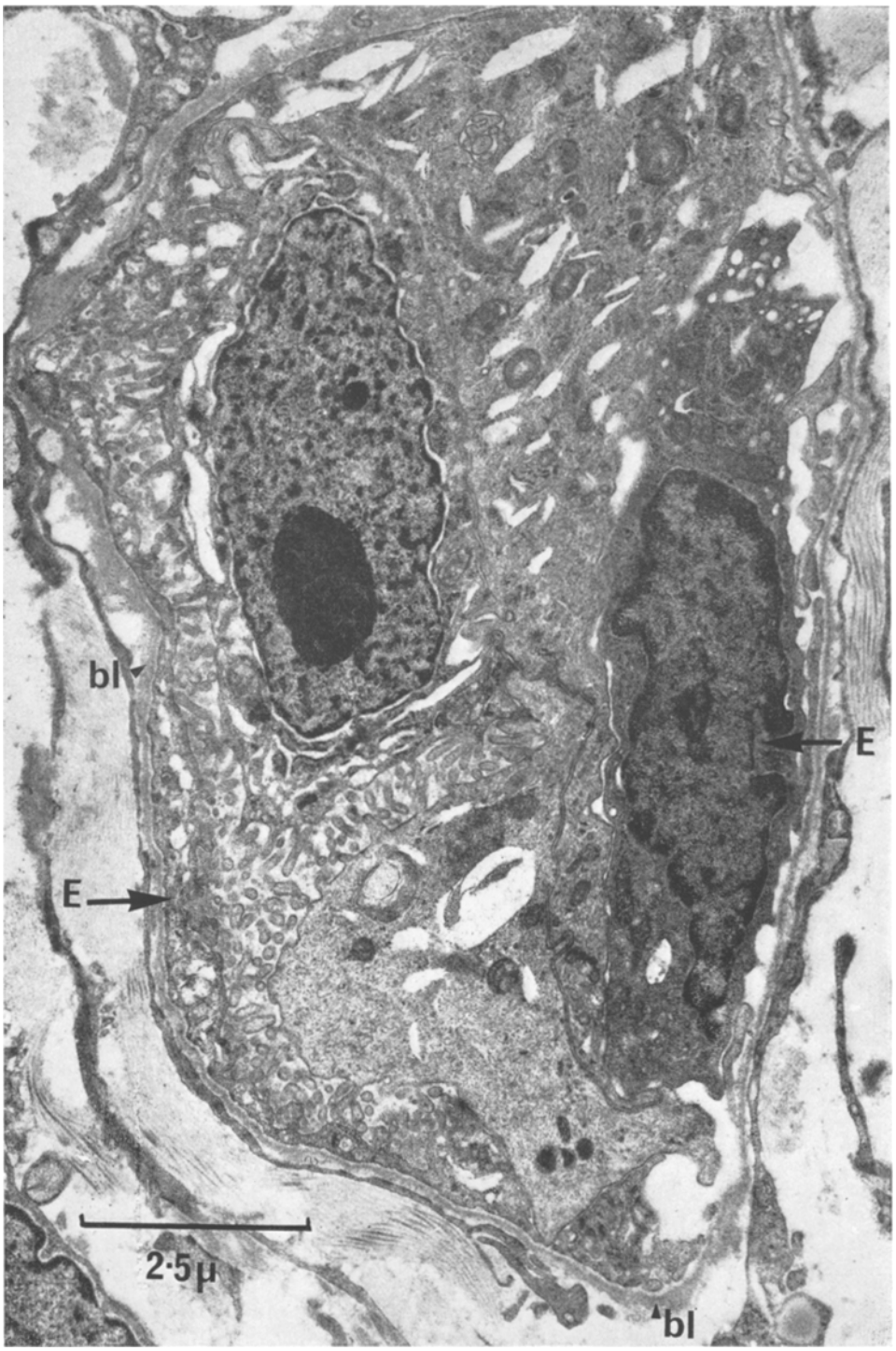

Fig. 9. Capillary lumen filled up with tumour cells. Residual endothelial cells are visible only in some places (*E*). *bl* Endothelial basal lamina. ×12500

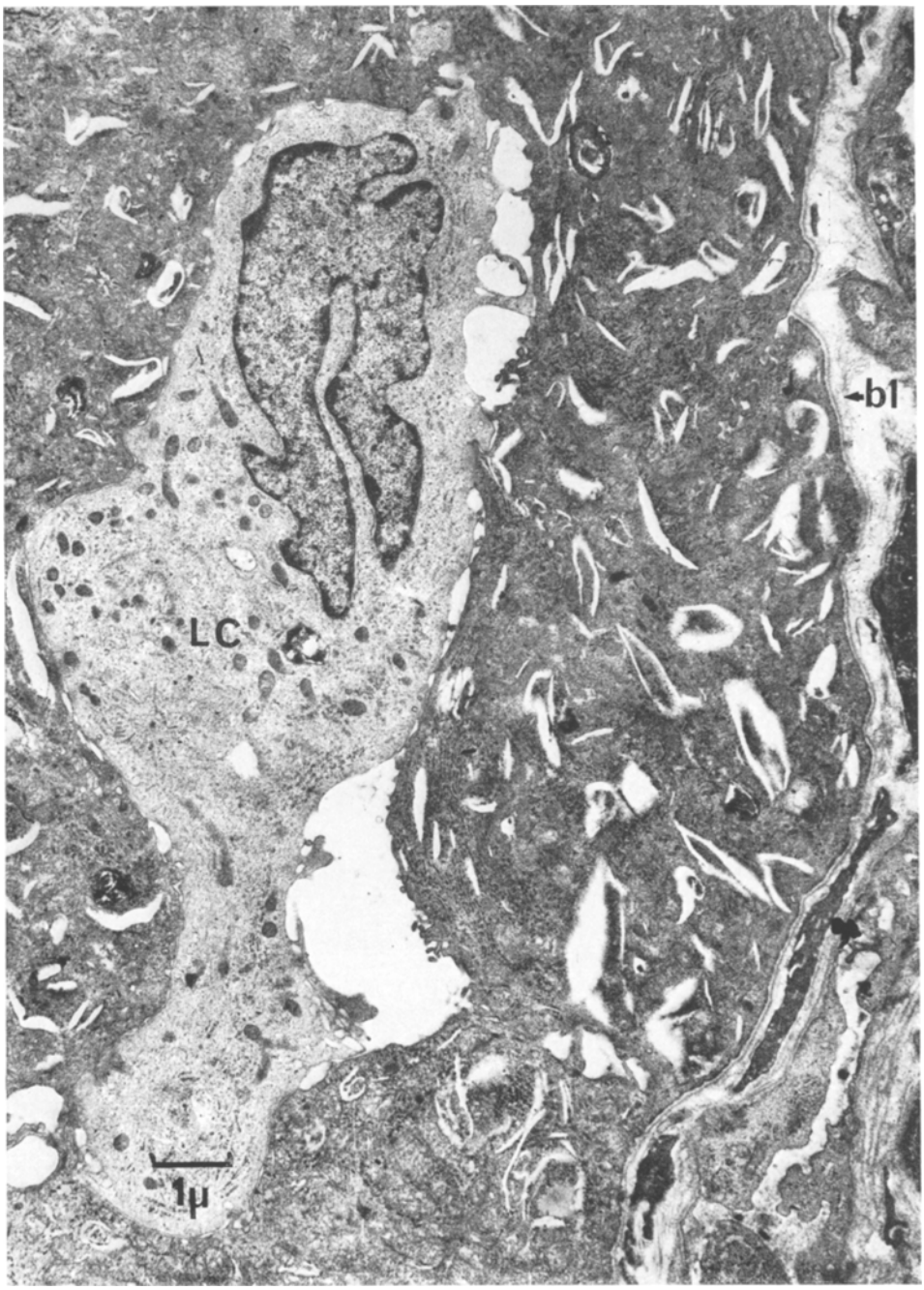

Fig. 10. "Langerhans cell" (*LC*) situated between tumour cells, the deepest of which lies on the epithelial basal lamina (*bl*). ×10000

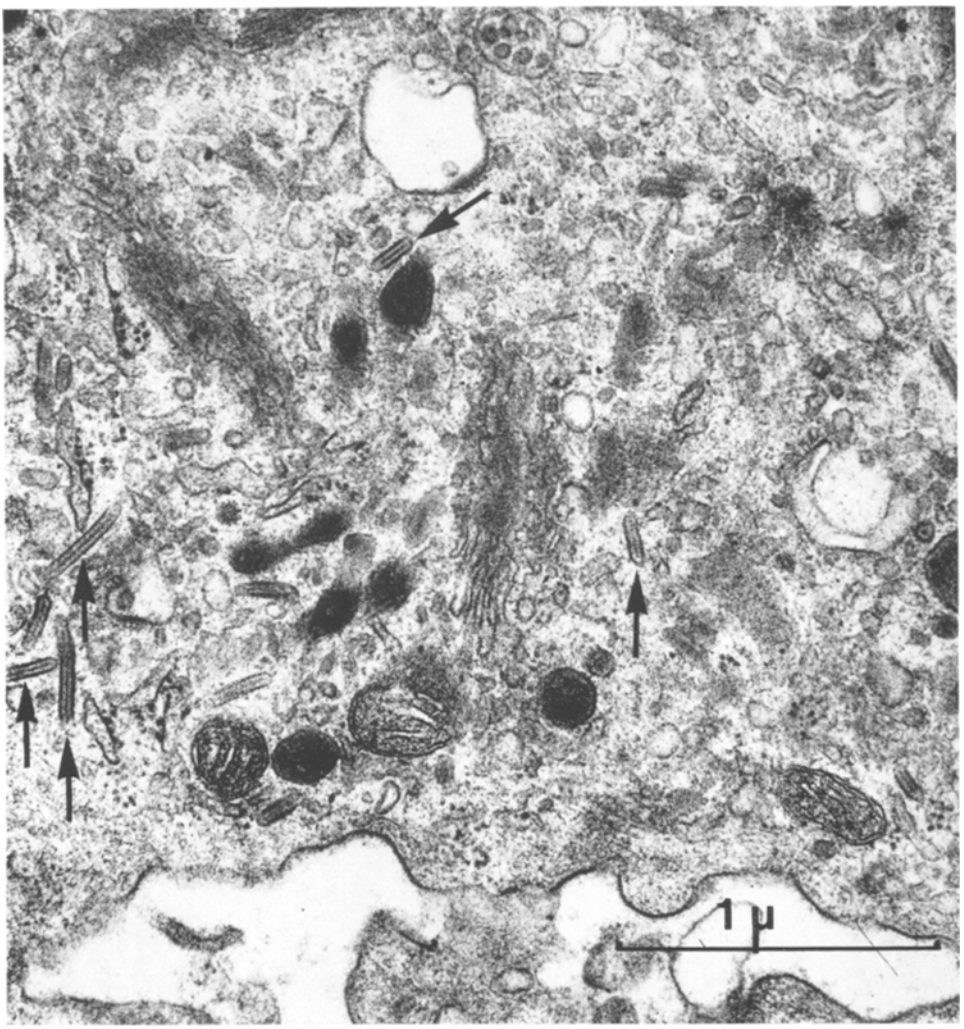

Fig. 11. Detail of the cytoplasm of a "Langerhans cell" from the tumour showing the characteristic rod-shaped granules (arrows). ×41 000

teristic of both normal epidermal Langerhans cells, and H-X cells of lesions of Histiocytosis-X (Fig. 11). With regard to their isolated situation within the epithelium, their dendritic shape, the relatively low concentration of characteristic cytoplasmic organelles, the absence of such organelles from the nucleus (Gianotti *et al.*, 1968; Wolff, 1972), and the absence of "worm-like bodies" from the cytoplasm (Basset *et al.*, 1972) the cells resembled much more epidermal Langerhans cells, than H-X cells.

Discussion

Before discussing the main point of interest in this case, i.e. the occurrence of dendritic Langerhans-type cells, it is desirable to specify the nature of the

tumour which contained them. A firm diagnosis of bronchiolar-alveolar tumour may be difficult to justify on the basis of pure light microscopic examination, as there elways remains the possibility of confusion with a metastasizing tumour of the glandular-papillary type, especially as the latter occurs more frequently. Primary and secondary tumours exhibit many common features, and even the finding of widespread lymphatic and blood vessel invasion by tumour cells can no longer be considered diagnostic of a secondary tumour (Liebow, 1960). Electron microscopy, however, can contribute to clarifying the diagnosis. In the present instance, the fact that the tumour cells were related to a continuous basement membrane, suggests its primary nature, and this suggestion is reinforced by the observation that the cells exhibited certain characteristics similar to those of type II pneumocytes. Amongst these features were the presence of microvilli at the apical pole, tight or gap junctions between adjacent cells, cytoplasmic "splits", and in particular, osmiophilic lamellar bodies, which even the least differentiated tumour cells contained. These similarities, taken together with the finding of normal type II pneumocytes and tumour cells in juxtaposition, strongly suggest that the tumour was, in fact, derived from alveolar epithelium. The absence of cilia and of evidence of mucous secretion by the tumour cells would seem to exclude the possibility of a bronchiolar origin in this case. Previous authors (Adamson *et al.*, 1969; Coalson *et al.*, 1970; Nash *et al.*, 1972; Mollo *et al.*, 1973) have produced convincing evidence that human bronchiolar-alveolar tumours are generally of alveolar cell origin, and the same would appear to apply to similar tumours in animals, both spontaneous and experimentally induced (Brooks, 1968; Nisbet *et al.*, 1971; Perk *et al.*, 1971; Straks and Feron, 1973). Kuhn (1972) however maintains that the origin of bronchiolar-alveolar tumour cells from type II pneumocytes exclusively, remains to be finally proven, and Geller and Toker (1969) give a similar opinion.

Langerhans cells have not so far been reported as being present in epithelium lining respiratory bronchioles, alveolar ducts, alveolar sacs or alveoli of normal lung. Neither have they been observed in pulmonary connective tissue. Considering the number of published studies dealing with lung ultrastructure, it might seem justified therefore to state categorically that Langerhans cells are not constituent elements of normal lung. However, it may be pointed out that despite detailed studies by a number of investigators extending over almost a decade, the presence of these cells in normal human dermis was not reported. It is now established however that they do occur in small numbers in this situation (Zelickson, 1965; Breathnach, 1971). Increasingly, reports are beginning to appear of their discovery in other tissues such as lymph nodes, thymus and connective tissue (Böck, 1973; van Haelst, 1969; Hoshino *et al.*, 1970; Jimbow *et al.*, 1969; Kondo, 1969; Olah *et al.*, 1968; Shamoto *et al.*, 1971) where their presence was not previously documented or suspected. If one may judge from experience with other tissues therefore, it would not be entirely surprising if, fairly soon, the discovery of Langerhans cells in normal lung were to be reported. The nowadays generally accepted view (Wolff, 1972) that the cells are of mesenchymal origin suggests they would more likely be discovered in the pulmonary connective tissue, rather than interspersed among the epithelial cells. Certainly, studies aimed directly at settling this point seem desirable.

Should the presence of Langerhans cells in normal lung eventually be established, their occurrence in fairly large numbers within the present tumour could be accounted for on the basis of a stimulating or irritative effect of the malignant process, leading to an increase in their numbers. Alternatively, their presence might be regarded as part of a defense process, since their occurrence in lymph nodes, thymus, and spleen could suggest they may have some function in this connection. Whatever view might be taken, their presence within this tumour provides some indirect circumstantial evidence for their occurrence in normal lung. It will be interesting to see if further reports of Langerhans cells within other types of pulmonary tumour will appear in the near future.

If Langerhans cells are eventually discovered in normal lung, the difficulty concerning pulmonary lesions of Histiocytosis-X will be partly resolved. The H-X cells of these lesions could then be regarded as derived from a resident population of Langerhans cells, as has been suggested for cutaneous lesions. But, major problems relating to lesions in all situations would still remain, e.g., what is the aetiological factor responsible for causing proliferation and modification of the Langerhans cells to give rise to numerous H-X cells? Also what is the significance of the undoubted differences—of degree rather than of kind (Nezelof *et al.*, 1973)—between the two? In this connection it is of considerable interest to note that the cells observed within the present tumour resemble much more "normal" Langerhans cells, than H-X cells of Histiocytosis-X.

One further point concerning this case merits some discussion. Although it is not possible to be certain, the patient's occupational history suggested the possibility of exposure to radiation over a prolonged period, and this might be considered of aetiological importance, In one of a series of cases of bronchiolar-alveolar tumour reported recently by Galy and Marcq (1973) there was a definite history of exposure to radiation. The association between exposure to radiation and the development of lung and bronchial carcinoma, especially the oat-cell form, is well known (Archer *et al.*, 1973; Saccomano *et al.*, 1964; Spencer *et al.*, 1968) but in the case of the uranium miner series, the level of radiation was certainly very high. It is possible that a lower dose, and a different type of radiation over a prolonged period might well produce another histological type of tumour, i.e., as in this case, a bronchiolar-alveolar tumour. Similar tumours have recently been produced experimentally in rats by the use of Radon (Perraud *et al.*, 1972).

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