tion is their presence just beneath the lowermost keratinized layer, regardless of its keratin type, during the whole sloughing cycle and between the lowermost cells of the outer epidermal generation (i.e. the part of the epidermis which, eventually, will be shed) in the later stages of the cycle[18].

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Ultrastructural evidence for the innervation of human pulmonary alveoli

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Summary. Electron Microscopic observations of the biopsied human pulmonary alveoli showed the occurrence of unmyelinated axons in the interstitium near the type I pneumocytes. These axons very likely have sensory functions.

Previous light microscopic studies of the innervation of the lungs in various animal species presented different results concerning the innervation of the alveoli. Honjin[2] and Spencer and Leof[3] showed nerves in the subpleural alveolar walls; Larsell and Dow[4] found nerve fibres in the alveolar ducts; and Elftman[5] and Hirsch and Kaiser[6] demonstrated nerves to be generally distributed in the alveolar walls; but Gaylor[7] and Fillenz and Woods[8] did not observe sensory nerves beyond the respiratory bronchioles. Despite such discrepancies of the histological studies, there was an increasing physiological evidence suggesting the presence of the sensory nerves in the alveolar walls[9]. In support of the physiological findings, recent ultrastructural investigations have revealed the presence of unmyelinated axons located in the interstitium of the alveolar walls of the rats[10] and mice[11,12]. Axons containing various types of vesicles have also been shown to supply the pulmonary capillaries in the dogs[13]. This paper presents electron microscopic observations of nerves in the human pulmonary alveolar walls.

The human lung tissues were obtained from a lung biopsy taken near a tumor under the pleural surface. They were

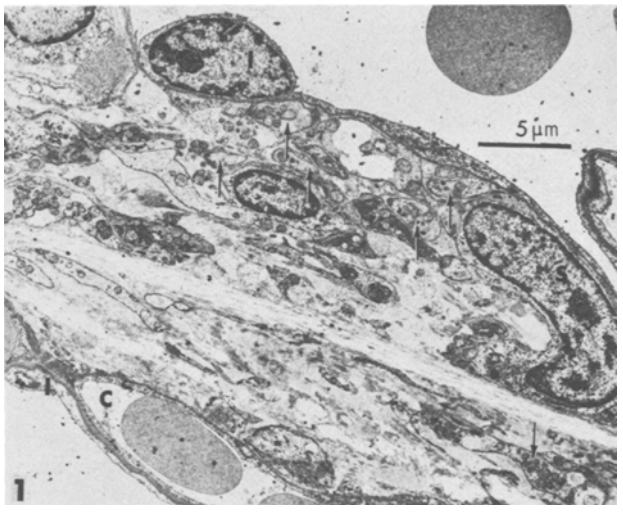

Fig. 1. This is a section through the alveolar septum which is covered on both surfaces by the type I pneumocytes (I) and contains a pulmonary capillary (c). Numerous unmyelinated axons (arrows), partially or completely covered by the Schwann cell (S) are located in the septum, and some of these are immediately under the type I pneumocyte. Calibration: 5 µm.

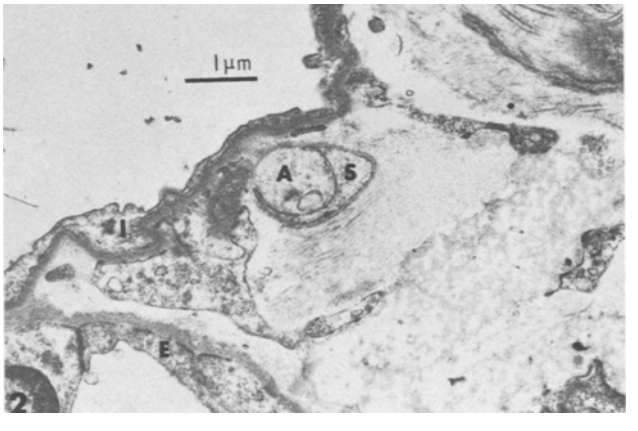

Fig. 2. A single axon (A) partially surrounded by the Schwann cell (S) is near the type I pneumocyte (I). E: Endothelium of the pulmonary capillary. Calibration: 1 µm.

fixed in s-collidine buffered 4% paraformaldehyde (pH 7.2); post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Thin sections of the normal alveolar walls were stained with uranyl acetate and lead citrate and observed under an electron microscope.

Unmyelinated axons occurred in groups (figure 1) or singly (figure 2) in the interstitium surrounded by the connective tissue cells and fibres, and the type I pneumocytes. These axons were covered completely or partially by the Schwann cells. The axoplasm contained mitochondria, vesicles, dense lamellated bodies and microtubules. The axons were usually located closer to the type I pneumocytes than the pulmonary capillary endothelium (figures 1 and 2). Some of the axons were separated from the type I pneumocytes only by a layer of basal lamina.

This paper revealed the ultrastructural evidence of axons in the alveolar walls of the human lung. The axons did not contain characteristic vesicles for adrenergic or cholinergic nerves and therefore these axons very likely had sensory functions. These axons may represent the sensory nerves of the type J receptors which were predicted by Paintal[9] to be located in the interstitium of the alveolar wall.

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Retrospective immunofluorescence of specific antigens in stained and balsam embedded sections of the developing amphibian lens

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Summary. A modification of the indirect immunofluorescent method is proposed for the detection of specific proteins in sections of the developing amphibian lens that were attached using egg albumin, stained and embedded into Canada balsam.

In the present work we tried to identify the specific antigens in previously stained and balsam embedded sections of the developing amphibian lens and to determine the time-period of storage during which these sections can still be used for immunochemical analysis.

Material and methods. Experiments were performed using embryos of *Rana temporaria* and *Xenopus laevis.* The following structures were examined by immunohistochemical techniques: a) lenses of normal embryos; b) lenses induced in the ectoderm of early gastrula of *R. temporaria* in vitro by heterogeneous inducers[2,3].

The experimental material was fixed with Carnoy or Bouin solution, dehydrated with alcohols (ethanol and butanol) and embedded into paraffin. Serial sections (5 µm) were glued to slides using egg albumin, dried in a thermostat for 7 days at 37 °C and stained by azan according to the method of Heidenhain[4]. Thereafter they were embedded in Canada balsam and covered with cover glasses. The preparations were stored at room temperature for periods ranging from 2 weeks to 2 years. The preparations chosen for immunohistochemical analysis were placed into chilled xylene to remove Canada balsam (10 °C, 2–7 h), washed in 96° ethanol (2–3 changes, 1 h each) and in 3 changes of buffered saline (pH 7.1, 30 min each). Thereafter the sections were processed according to an indirect method using fluorescent antibodies[5] with some modifications[6–8]. The following rabbit antisera were used: 1) to the total lens proteins of *Rana temporaria;* 2) to the γ-crystallin of *Xenopus laevis* lens[9, 10]. FITC-labelled antiserum to rabbit γ-globulins (Travenol, USA) was used. The specificity of the fluorescence was compared to that of the controls including antigen-adsorbed antiserum[5,6,8].

Results and discussion. Initially we examined the lens preparations of normal *Xenopus laevis* embryos. After the removal of Canada balsam the sections were sequentially treated with the antilens serum and the labelled antiserum and examined in the fluorescence microscope. These sections were stored no more than 2–3 weeks. The specific fluorescence of stained preparations does not differ in its location and intensity from the fluorescence of unstained sections. The brightest fluorescence is observed in the central area of the lens (lens fibres) just as in the control. Peripheral epithelial part of the lens usually does not bind the immunofluorescent label (figure 1). The background fluorescence of tissues surrounding the lens was virtually absent in stained preparations, although these were glued onto slides using egg protein. The egg albumin is characterized by a high nonspecific adsorbing activity which usually resulted in a drastic increase in the level of nonspecific fluorescence of preparations. Perhaps the azan staining used in our experiments decreased this property of the egg albumin.

A similar technique was used for the immunofluorescent analysis of lenses developing in ectodermal explants cultivated in vitro. We examined the stained sections of lenses stored for 1–3 months or 1–2 years. It has been found that the storage of stained preparations for 1.5 years does not affect the pattern of immunofluorescence. Such lenses specifically bind the immunofluorescent label (figure 2). Azan staining and presence of egg albumin does not