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

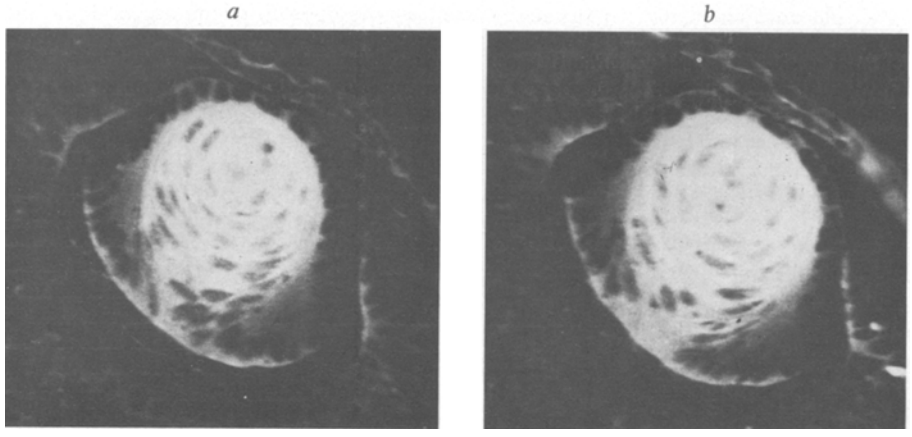

Fig. 1. Immunofluorescent detection of proteins in *X. laevis* embryonal lens sections. *a* Immunofluorescence of the unstained section, *b* immunofluorescence of the prestained section. Immunofluorescent staining: monospecific antiserum to *X. laevis*^{ap} γ-crystallin + FITC-labelled antiserum to rabbit serum γ-globulins.

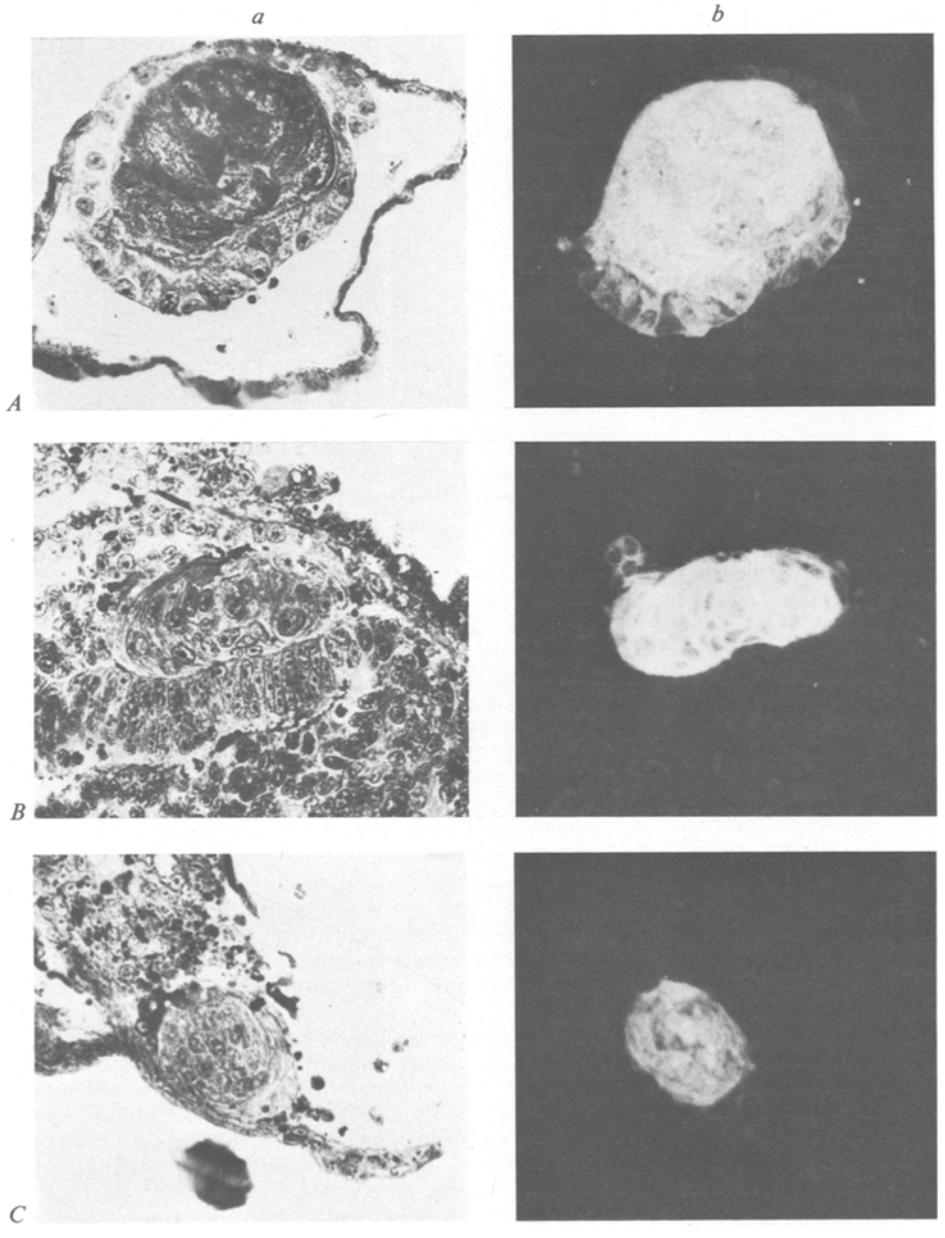

Fig. 2. Immunofluorescent detection of specific proteins in the sections of lenses induced in the ectodermal explants of *R. temporaria*. Retrospective immunofluorescence. The sections were stored for: *A* 2 weeks, *B* 1 year, *C* 1.5 years. *a* Azan staining according to Heidenhain, *b* immunofluorescent staining: antiserum to the total lens proteins of *R. temporaria* + FITC-labelled antiserum to rabbit serum γ-globulins.

increase the unspecific fluorescence and does not interfere with the normal immunofluorescent reaction characteristic for amphibian lens proteins. The specific fluorescence was not observed in the sections that were stored for 2 years or more. Thus, antigenic determinants of specific lens proteins of amphibian embryos persist in stained sections at room temperature for 1–1.5 years. These antigen determinants can be identified using conventional techniques of immunofluorescence.

Attempting to extrapolate this data to other antigenic systems[14] one has to take in account that certain histological stains such as methylene or aniline blue, acidic or basic fuchsine, Congo-red, hematoxyline-eosin, and others[11–13] lead to a decrease of the immunofluorescence.

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Bovine parathyroid catecholamines: A chemical and histochemical study

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Summary. Bovine parathyroid glands contain large amounts of dopamine (3.4–13.9 pg/μg), but very little norepinephrine. Fluorescent histochemistry demonstrates only rare adrenergic nerve terminals on vasculature. Single dopamine-containing cells, most likely mast cells, are scattered in large numbers throughout the connective tissue stroma.

Calcium is generally thought to be the principal physiologic regulator of parathyroid function[1]. Recently, however, a number of other agents have been shown to alter parathyroid hormone release in vivo and in vitro. These are: β-adrenergic[2–4], α-adrenergic[5] and dopaminergic[6] catecholamines, prostaglandin E_2[7] and secretin[8]. The presence of receptors for catecholamines on dispersed bovine parathyroid cells suggests the possibility of direct innervation of the bovine parathyroid gland. Although nerve endings, apparently terminating on parathyroid cells, have been demonstrated by light microscopy[9,10], electron microscopy[11] and fluorescence histochemistry[12], there is relatively little information on the innervation of the bovine parathyroid gland[13]. In the present report, we have directly measured catecholamine levels in bovine parathyroid tissue and employed fluorescence histochemistry to investigate the possibility of catecholamine-containing nerve endings in this species.

Methods. Bovine parathyroid tissue was obtained from a local abattoir within 5–10 min of death. Glands were cut into slices (about 2×4×4 mm) with a scalpel and placed immediately on dry ice for determination of catecholamines or in liquid nitrogen for fluorescence studies. Catecholamines were assayed according to modifications[14] of the method of Coyle and Henry[15]. The frozen tissue was processed for fluorescent histochemistry[16]. The tissue was then freeze-dried at −30 °C for 4 days and exposed to dry paraformaldehyde gas at 80 °C for 1 h, vacuum embedded in paraffin for 20 min at 60 °C and sectioned at 14 μm.

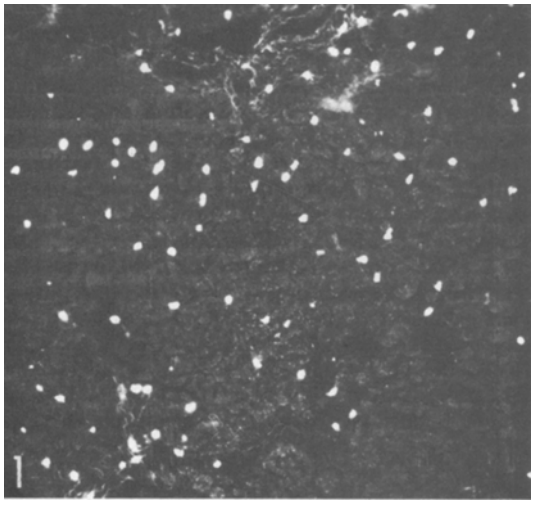

Fig. 1. An abundant number of fluorescent cells scattered throughout the connective tissue stroma of the parathyroid gland. × 100.

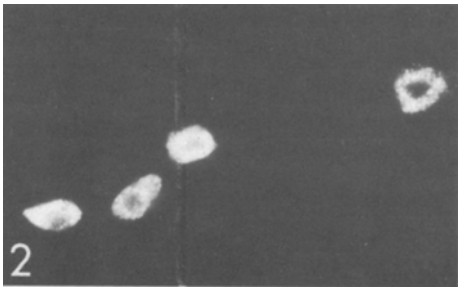

Fig. 2. High power of fluorescent cells, probably mast cells, whose nuclei are unstained. × 655.