IMMUNOHISTOCHEMICAL DETECTION OF SPECIFIC ANTIGENS IN STAINED SECTIONS OF THE LENS MOUNTED IN BALSAM

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The location of specific proteins in sections through the lens of amphibians (Rana temporaria, Xenopus laevis), stained beforehand with azan and mounted in balsam, was studied by the indirect fluorescent antibodies method. Lenses from normal animals and lenses induced in vitro in the ectoderm of the amphibian gastrula were analyzed. To remove balsam, the lens sections were washed successively in xylol, ethanol, and physiological saline (pH 7.1). They were then treated in accordance with the general principles of immunohistochemical analysis. It is shown that specific antigens can be detected by this method in sections through the lens kept in balsam for 1 year. In sections kept for longer periods (2-3 years) the immunofluorescence test for lens proteins was negative.

KEY WORDS: immunofluorescence; specific antigens; lens of the eye.

Immunohistochemical methods have been successfully used to identify specific proteins (antigens) of developing tissues and organs [1, 2]. A convenient modification of the immunofluorescence test is the technique of Sainte-Marie [3], for which paraffin sections can be used. One limitation of this modification for ontogenetic research is that it does not always permit regions of embryonic tissues required for analysis to be chosen, for the selection must be carried out on unstained paraffin sections. This disadvantage, which can be overcome in most cases during the study of normal embryos, becomes a particularly serious technical handicap during the analysis of differentiations arising in vitro, when the typical morphology of an organ as a rule is disturbed. Immunohistochemical treatment of all serial sections of an embryo in culture is difficult and uneconomical. Moreover, during preparation of material by Sainte-Marie's method the sections are fixed to slides without albumin. During subsequent processing of these sections there is always the risk that they may become separated from the glass.

The difficulties mentioned above were encountered during immunohistochemical analysis of lenses developing in ectodermal explants cultured in vitro. It was accordingly decided to undertake an immunofluorescence analysis of sections through the lens selected after preliminary histological treatment (staining, mounting in balsam).

EXPERIMENTAL METHOD

Experiments were carried out on embryos of Rana temporaria and Xenopus laevis. The following structures were investigated immunohistochemically: 1) lenses from normal embryos, 2) lenses induced in the ectoderm of the frog gastrula during culture in vitro by the action of heterogeneic inducers (saline extracts of the retina and brain of chick embryos at the 7th-13th day of incubation).

The experimental materials (normal embryos, ectodermal explants with lenses) were fixed in Carnoy's or Bouin's solution, taken through alcohols (ethanol, butanol), and embedded in paraffin wax. Serial sections (5μ thick) were glued to slides by means of egg albumin, stained with azan by Heidenhain's method, mounted in Canada balsam, and covered with coverslips. Preparations containing lenticular structures were chosen under the microscope and subjected to immunohistochemical analysis. They were first specially treated to remove Canada balsam. For this purpose the sections were placed in cold xylol for 7-10 h, then washed for 1-2 h in 96° ethanol and in three changes of buffered physiological saline (pH 7.1), for 20-30 min in each. The sections were then treated by the usual methods of immunohistochemical analysis.

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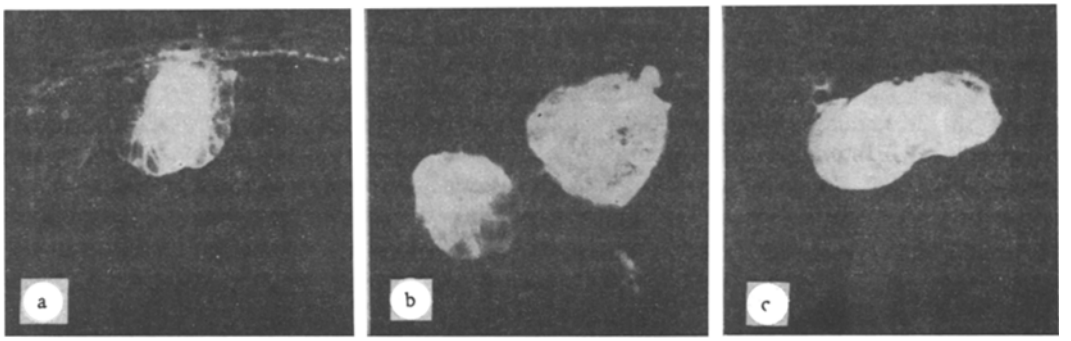

Fig. 1. Immunofluorescence detection of crystallins in sections through lens of X. laevis embryos (a) and in sections of lenses induced in ectodermal explants of R. temporaria (b, c). Sections treated with: a) antiserum against γ-crystallin fraction of X. laevis lens; b, c) antiserum against total spectrum of lens proteins of R. temporaria. Sections kept: a) 1-2 weeks; b) 1-1.5 months; c) 1 year. Objective 20, homal 5.

The indirect fluorescent antibodies method [4] with minor modifications [5-7] was used. Rabbit antisera against the total spectrum of proteins (R. temporaria) or against fractions of proteins (X. laevis) of the lens were used. Antiserum against rabbit serum γ globulins, labeled with fluorescein isothiocyanate (FITC), was from Travenol, USA (dilution 1:9).

EXPERIMENTAL RESULTS

Stained preparations of the lens of normal X. laevis embryos were studied first. After removal of the Canada balsam (see: Experimental Method) the sections were treated successively with antilens and labeled antisera, mounted in glycerol, and examined in the fluorescence microscope. The results of these experiments showed (Fig. 1) that specific fluorescence of the previously stained preparations was indistinguishable in localization and intensity from fluorescence of "native" (paraffin) sections through the lens of early embryos (Fig. 1). Just as normally, the central zone of the lens gave the brightest fluorescence (lenticular fibers); the peripheral, epithelial part of the embryonic lens took up the label less strongly. The essential point was that background fluorescence of tissues surrounding the lens was virtually absent in the stained sections, even though they were glued to the slides with egg albumin. The use of egg albumin in conjunction with the ordinary immunofluorescence technique leads to a sharp increase in the level of nonspecific fluorescence of the sections. In the present experiments, after staining with azan, nonspecific fluorescence of egg albumin was not observed.

Immunohistochemical analysis of lenses developing in eetodermal explants cultured in vitro was carried out by the same method. Stained sections of lenses kept for 1-3 months or 1 and 2 years were investigated. Keeping the stained preparations (at room temperature) for 1 year was found not to affect the character of the immunofluorescence reaction. These lenses bound the immunofluorescent label specifically (Fig. 1). Staining with azan and the presence of egg albumin did not intensify nonspecific fluorescence and did not interfere with the normal manifestation of the immunofluorescence reaction characteristic for lens proteins. No fluorescence specific for lens proteins could be detected in sections from lenses kept 2 years or more.

It can be concluded from the results on the whole that antigenic determinants of specific frog lens proteins are preserved in sections stained and mounted in balsam for 1 year and that they can be detected by the use of appropriate antibodies.

There is no suggestion whatever that these results can be extrapolated to other antigenic systems. Lens proteins are relatively resistant to the harmful action of denaturing agents and do not lose their antigenic properties for a long time. At the same time, the results are evidence that histological treatment (staining, mounting in balsam, the presence of egg albumin) does not increase the level of nonspecific background fluorescence and does not prevent the manifestation of the specific immunofluorescence reaction if the determinants of the antigen studied are still preserved. However, it must be remembered that some dyes used in histological practice (methylene blue, aniline blue, acid and basic fuchsin, Congo red) reduce the intensity of fluorescence [8].

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