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A procedure to obtain long-lasting fluorescence in formaldehyde fixed tissues

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Summary. The authors made a study of the small intestine by immunofluorescence in 5% phosphate-buffered formaldehyde fixed tissues, using the Nairn (1976) technique associated with Evans blue (Fry and Wilkinson 1963). The ability of the sections to fluoresce has been maintained after a storage time of more than 4 years. Similar very good results were obtained when skin biopsies from pemphigus foliaceus patients were fixed in formaldehyde for two h and afterwards washed in 30% sucrose solution for 12 to 18 h.

Introduction

The rapid fading of fluorescence during microscopic studies and the necessity of fixation at low temperatures, are the two great limitations of some histoimmunofluorescence techniques. In 1969, Eidelman and Berschauer introduced a method for immunofluorescence in digestive tract mucosa after fixation in formaldehyde. They fixed intestinal mucosa fragments in a solution of 10% isotonic phosphate-buffered formaldehyde, pH 7.0 at 4° C for 4 h. Afterwards the material was placed in 30% sucrose solution and left overnight. After being sectioned in cryostat, the biopsy material was processed for immunofluorescence, by the Nairn (1976) technique.

In previous works (Moreira 1984 and Moreira and Barbieri 1985), we used a slightly modified Eidelman and Berschauer (1969) technique to study IgA, IgM and IgG producing cells in the small intestine mucosa. After a prolonged exposure to ultraviolet light and/or a long storage time, we noted that the loss of fluorescence was almost negligible.

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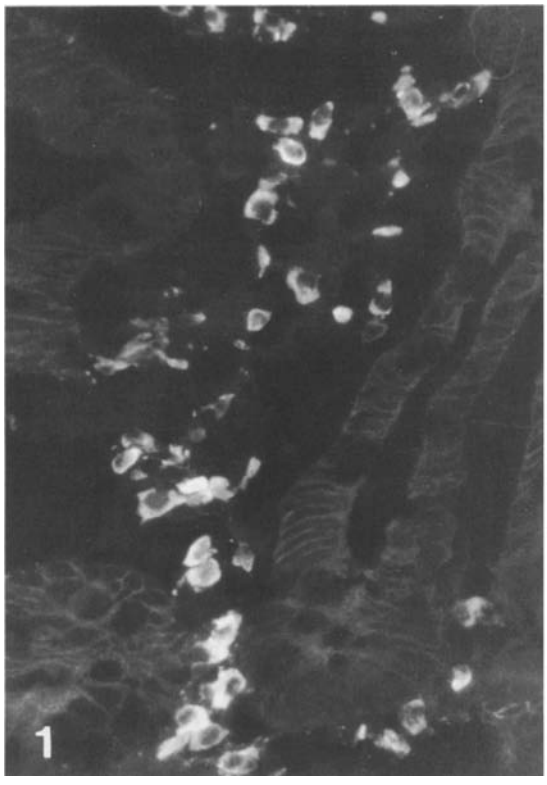
Material and method

Intestinal mucosa biopsies were fixed for 4 h and skin biopsies for 2 h in 5% phosphate-buffered, pH 7.0 at 4° C. Afterwards the material was left in 30% sucrose, also at 4° C, for 12 to 18 h. It was then carefully oriented on a piece of cork and cut in a cryostat at −20° C. Sections of tissue were put on slides which had been boiled in water with detergent and washed in a 50/50 solution of alcohol and ether. After obtaining the sections the slides were left to dry at room temperature for 1 h prior to being stored at −20° C. Before the immunofluorescent staining the slides were left to dry for 15 min. Later they were washed in 3 baths of PBS for 10 min each. The sections were circled by red nail varnish and covered with antibodies anti-human IgA, IgM and IgG (Behringwerke, AG; Marburg W. Germany – Behring Institut), diluted in Evans blue at 1%, in a concentration of 1/50, 1/20 and 1/16, respectively. After incubation for 30 min at room temperature, the slides were washed again in 3 baths of PBS for 10 min each. After being completely dried, the slides were mounted in alkaline glycerine, pH 8.5 and carefully sealed with clear nail varnish to prevent the redrying of the glycerine. After studying in ultraviolet light, the slides were stored in a freezer at −20° C.

Results

In our studies the same section of intestinal mucosa was exposed to ultraviolet light for one h, with continuous field exposures of 30 s on the average, without loss of fluorescence intensity.

After morphometry, the material was stored at −20° C for later analysis, including photographic documentation. The fluorescence in slides already analysed and stocked for 18 months was maintained and it has been possible to obtain good micrographies from such slides. After continuous field exposure of up to 4 min, for micrographic purposes, the material was perfectly adequate for further analysis and more photographs. These



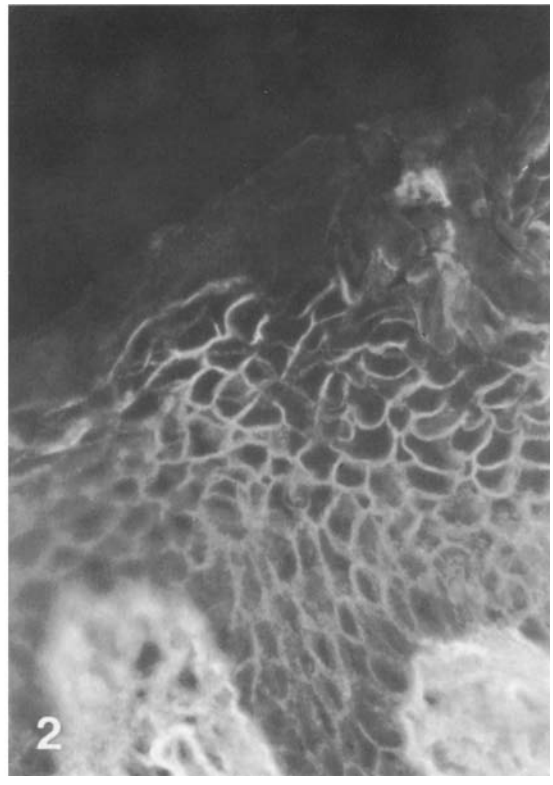


Fig. 1. Small intestine – IgA – sections processed by immunofluorescence 4 years and 6 months before

Fig. 2. Skin – pemphigus foliaceus disease – IgG – sections fixed in formaldehyde and processed by immunofluorescence 2 months before

Fig. 3. Skin – pemphigus foliaceus disease – IgG – sections fixed and processed by immunofluorescence 15 days before

slides are still stored at −20° C in a freezer with their original sealing. Fluorescence fading after 4 years of storage is only just perceptible (Fig. 1).

It seems to us that the preservation of fluorescence must be in part due to the fixation of the material and the care taken in drying and sealing recommended by Nairn (1976), because he himself refers to the preservation of his material for several years. It seems however, that the introduction of Evans blue somehow favoured the preservation of fluorescence in our biopsies of intestinal mucosa. Slides processes without Evans blue became faint after some relatively short storage time. Similar results were obtained for us with sections of oral mucosa. We have obtained excellent results with skin biopsies of pemphigus foliaceus patients (Figs. 2 e 3), when the time fixation in phosphate-buffered formaldehyde was 2 h, followed by washing in 30% sucrose for 12 to 18 h. More prolonged times of fixation impaired the sectioning of the material and also made it difficult to ensure the adherence of the sections to the slide.

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