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An Approach to Stain Actin in Parotid Gland Cells in Paraffin-Embedded Material

Staining by Human Anti-Actin Antibodies Using the Indirect Unlabeled Immunoperoxidase Technique

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Introduction

The indifferent cells in parotid gland tumours have been a point of discussion concerning their histogenetical classification. With regard to the normal gland, one has assumed that the myoepithelial cells may be related to these cells. However, the information about the percentage of myoepithelial cells in parotid tumours varies from 5% (Chisholm et al., 1974; 1975) to 90% (Leifer et al., 1974). Those differences are due to the fact that it is difficult to demonstrate the presence of actin or filamentous structures by routine light microscopic or even electron microscopic methods (Seifert and Donath, 1978).

Therefore, the staining of actin by antibodies could be a method to clarify those problems of classification.

In the last years, several groups have been successful in obtaining anti-actin antibodies and in demonstrating their specifity (Trenchev et al., 1974; Fagraeus et al., 1975; Lazarides, 1975; Owaribe and Hatano, 1975). These antibodies were experimentally produced. A different approach to obtain anti-actin antibodies was undertaken in the last years. Following the observation that antibodies against actin were observed in the course of human diseases, these antibodies were used as a diagnostic tool and also for cell research (Fagraeus and Norberg, 1978). Antibodies against actin are found in patients with chronic aggressive hepatitis (Gabbiani et al., 1973; Lidman et al., 1976; Andersen et al., 1976; Botazzo et al., 1976); they may even be found in patients with mononucleosis (Holborrow et al. 1973). The specifity of these human antibodies has been analysed by immunological methods and it was shown that they were directed against actin (Fagraeus and Norberg, 1978).

At this point, it was possible to use human anti-actin antibodies as a diagnostic tool and to stain actin in smooth muscle cells by the indirect immunofluorescent technique (Fagraeus and Norberg, 1978).

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In this study we want to present the results of staining the rat parotid gland by human anti-actin antibodies using the indirect immunoperoxidase method.

Material and Methods

The parotid glands of male Wistar-rats weighing about 300 g were removed after killing the animals by ether anesthesia.

The glands were prepared by crystate sections, unfixed or fixed in acetone.

Simultaneously, the glands were fixed in neutral buffered formaldehyde of Bouin's fixative and embedded in paraffin.

The sections were stained by the indirect unlabeled immunoperoxidase method (Taylor, 1978; Sternberger, 1979) in the following way:

1. Dehydration of the paraffin sections in alcohol (80%).
2. Blockade of the endogenous peroxidase in methanol-0,3% perhydrol.
3. Incubation with normal goat serum for 30 min (dilution 1:20)
4. Incubation with human anti-actin-antibodies (serum from a case of chronic active hepatitis) for 45 min (dilution 1:20), the serum being inactivated by heating at 56° C for 30 min.
5. Incubation with rabbit antiserum to human immunoglobulin compounds for 45 min (dilution 1:20).
6. Incubation with goat anti-rabbit serum for 45 min (dilution 1:3).
7. Incubation with horseradish peroxidase-antiperoxidase complex of the rabbit for 45 min (dilution 1:10), PAP complex of Dakopatt.
8. Reaction with DAB for 3 min.
9. Counterstaining with hemalaun.

(The sera were obtained from medac, Hamburg, with exception of the anti-actin, a gift from Mrs. Astrid Fagraeus, Stockholm).

Each of the aforementioned steps was followed by rinsing with 0.5 ml Tris-HCl-buffer (pH: 7.6).

The specifity of the staining was controlled by serial sections of the same material which were stained in the following way, using some variations with regard to the incubation steps: Concerning step 4, we incubated the section with normal human serum or omitted step 4. Concerning step 5, we used normal rabbit serum for the incubation.

A control with anti-actin serum absorbed by actin was done as well.

Results

The results we made in the fixed and unfixed cryostate sections as well as in the paraffin-embedded material were similar. However, the intensity of the staining and the preservation of the cellular structures were best in the paraffin-sections which had been fixed in neutral formalin.

According to the presence of actin in the smooth muscle cells of the vascular walls, we observed an intense staining of these cells in the walls of the vessels of the parotid gland (Fig. 1).

A similar intense staining was observed in the duct system of the parotid gland, especially concerning the striated duct system and the excretory duct system. In some parts, the basal striations of the duct cells are still well preserved (Fig. 1).

In the neighbourhood of these cells, some spindle-like cells which were equally stained by the anti-actin were observed. These cells were rare, but could be found in some cases (Fig. 1). Staining characteristics and shape of those cells suggest the presence of the so-called myoepithelial cells.

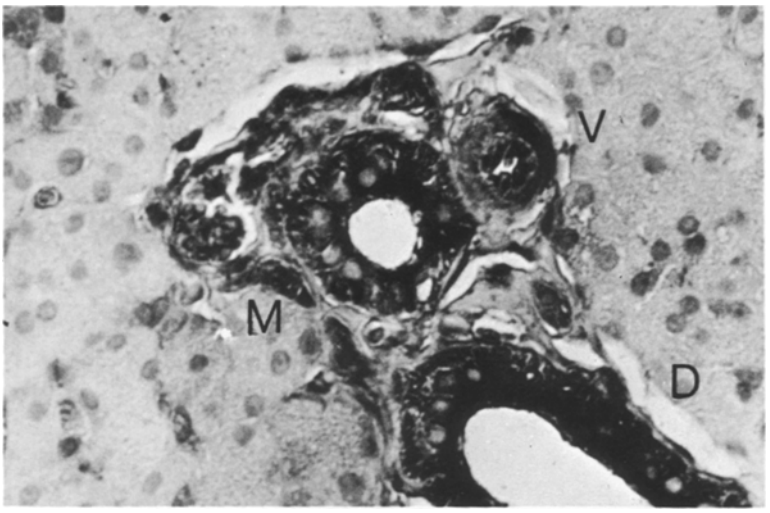

Fig. 1. Rat parotid gland. Immunoperoxidase technique with antiactin. In the middle and in the lower intensely stained duct cells (*D*). On the right one blood vessel (*V*). On the left two spindle-shaped intensely stained cells (*M*) adjacent to the duct (myoepithelial cells). Magnification × 480

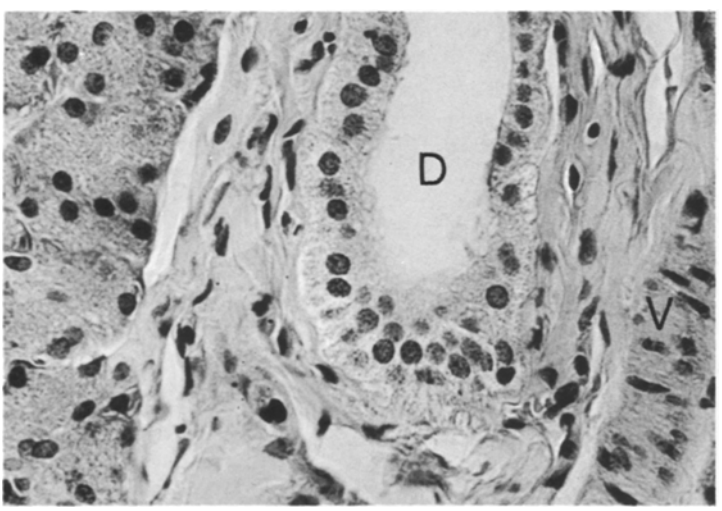

Fig. 2. Rat parotid gland. Immunoperoxidase technique. Incubation with normal rabbit serum as control. In the middle a non-stained duct (*D*); adjacent on the right a non-stained vessel (*V*). Magnification × 480

The staining of the aforementioned cells in the rat parotid gland was not obtained if normal human serum of normal rabbit serum was used instead of anti-actin serum (Fig. 2).

The intense staining of actin was not present when absorbed antisera were used.

Discussion

Actin plays an important role in the cells of the human and the animal body. It is a major component of the striated and smooth muscle cells, but also of other cells like the myoepithelial ones (Hamperl, 1970; Gabbiani, 1979). Studies of the presence of actin in various cells have been undertaken mainly by immunofluorescence (Archer and Kao, 1968; Line and Archer, 1972; Fagraeus and Norberg, 1978). After broad use of experimentally produced anti-actin antibodies, human anti-actin serum has been introduced as a diagnostic tool in the last years (Fagraeus and Norberg, 1978). Thus, the presence of actin was demonstrated in the smooth muscle cells of the gastrointestinal tract and of the blood vessels. It could be seen in other cells as well, like the brush borders of the proximal renal tubules and of the basal parts of the tubular cells of the rat kidney (Fagraeus and Norberg, 1978).

In this work, human anti-actin serum was used in the indirect immunoperoxidase technique (Taylor, 1978; Sternberger, 1979). This method was introduced to study the rat parotid gland with special attention to those cells which generally showed a certain amount in actin like the myoepithelial cells.

In agreement with the former works using the immunofluorescent technique (Archer and Kao, 1968), the presence of actin could be clearly demonstrated in the smooth muscle cells of the vessels in the gland. Additionally, a strongly positive staining was seen in the duct cells and in some spindle-shaped cells. The staining of the duct cells, expecially the striated duct cells, seems to correspond to the observation that the presence of actin could be demonstrated in the tubular system of the kidney (Fagraeus and Norberg, 1978). Perhaps, this finding reflects some structural and physiological similarities between these systems.

The other cell type which was stained by anti-actin was a spindle-shaped cell. Those cells were sometimes observed in the neighbourhood of the ducts. They seem to correspond to the myoepithelial cells. This observation is comparable to the staining of myoepithelial cells in the rat salivary gland by immunofluorescence technique (Line and Archer, 1972). The immunoperoxidase technique in combination with human anti-actin antibodies seems to be a valuable tool to mark the presence of actin in various populations of cells. Since actin is not only present in one kind of cell, but represents an important part of the cytoskeleton of cells, it is interesting to notice that this constitutent could be observed in the myoepithelial as well as in the excretory duct cells.

However, it should be remarked that generally actin is damaged by standard fixation procedures. The staining of actin in formalin-embedded material by the indirect peroxidase technique may be due to the higher sensibility of this method in comparison to the immunofluorescence method (Sternberger, 1979).

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