AN INDIRECT IMMUNOELECTRON-MICROSCOPIC METHOD OF LOCATING ALBUMIN IN MOUSE HEPATOCYTES

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The mouse liver was fixed in 12 different kinds of fixative based on glutaraldehyde, paraformaldehyde, picric acid, and lysine and the location of albumin was studied by an indirect immunoperoxidase method. Comparison of the results showed that the best method of fixation is by immersion in 8% paraformaldehyde solution. Albumin was found on ribosomes and nuclear membranes and on the granular endoplasmic reticulum and also in the matrix of the hepatocyte cytoplasm. It is suggested that newly synthesized albumin is secreted directly into the cytosol, from which it enters the extracellular space.

KEY WORDS: hepatocyte; albumin; immunocytochemistry.

One of the main functions of the mammalian liver is to synthesize serum albumin. Immunofluorescence and immunohistochemical investigations have shown that this synthesis is located in the hepatocytes [1, 2, 5, 10, 13] and at the same time, they have raised a number of questions: the distribution and possible cyclic character of the process, and the mechanisms by which albumin leaves the cell. Some help with the study of these problems is provided by the highly sensitive electron-microscopic immunoperoxidase method, which allows the protein under investigation to be tagged selectively and intracellularly. However, the widespread introduction of this method into hepatology has been delayed by the absence of any standard methods of preparation of the tissue for investigation. This is primarily true of the individual fixation of the intracellular protein, without changing its antigenicity or its distribution in the cell. Previous electron-microscopic studies aimed at detecting albumin in hepatocytes have used the direct immunoperoxidase method [6-9]. However, according to Sternberger [15], the indirect variant of the method is more sensitive and specific.

This paper describes an attempt to discover the optimal parameters of fixation of liver tissue for detection of albumin by the indirect electron-microscopic immunoperoxidase method. At the same time the results obtained by the direct [6-9] and indirect variants of the method were compared.

EXPERIMENTAL METHOD

1. Preparation of Pure Antibodies. Monospecific antibodies against mouse albumin were used. The antibodies were prepared from the corresponding rabbit antiserum on a solid adsorbent, namely Sepharose 4B, activated with cyanogen bromide (from Sigma, USA) [3]. Pure goat antibodies against rabbit IgG were isolated from the corresponding antiserum on glutaraldehyde immunosorbent [4]. The goat antibodies were conjugated with horseradish peroxidase (RZ = 3.0; Serva, West Germany) by a two-stage method [15].

2. Indirect Immunoperoxidase Method. Pieces of liver from BALB/C mice were fixed by immersion in 12 different kinds of fixative based on glutaraldehyde (GA), paraformaldehyde (PF), picric acid (PA), and lysine (L): 1) 2.5% GA for 2 h; 2) 2.5% GA + 4% PF for 2 h; 3) 1.25% GA + 2% PF for 2 and 5 h; 4) 0.25% GA + 7% PF for 5 and 16 h; 5) 0.05% GA + 8% PF for 2, 5, and 16 h; 6) 8% PF for 5 and 16 h; 7) 6% PF for 5 and 16 h; 8) 4% for 5 and 16 h; 9) 4% PF + PA for 1, 5, and 16 h [14]; 10) 2% PF + PA for 2 h; 11) 2% PF + L for 2 h [12]; 12) 4% PF + L for 2 h. When fixatives of types 1, 3, 6, and 7 were used, prefixation of the liver by perfusion was carried out in half of the cases. The fixatives were made up in 0.1M phosphate or cacodylate buffer, pH 7.2, with or without the addition of 7% sucrose. Fixation was carried out at 4°C with constant mixing. The pieces of liver were then rinsed for 24 h in physiological saline buffered with 0.1 M phosphate buffer in the ratio of

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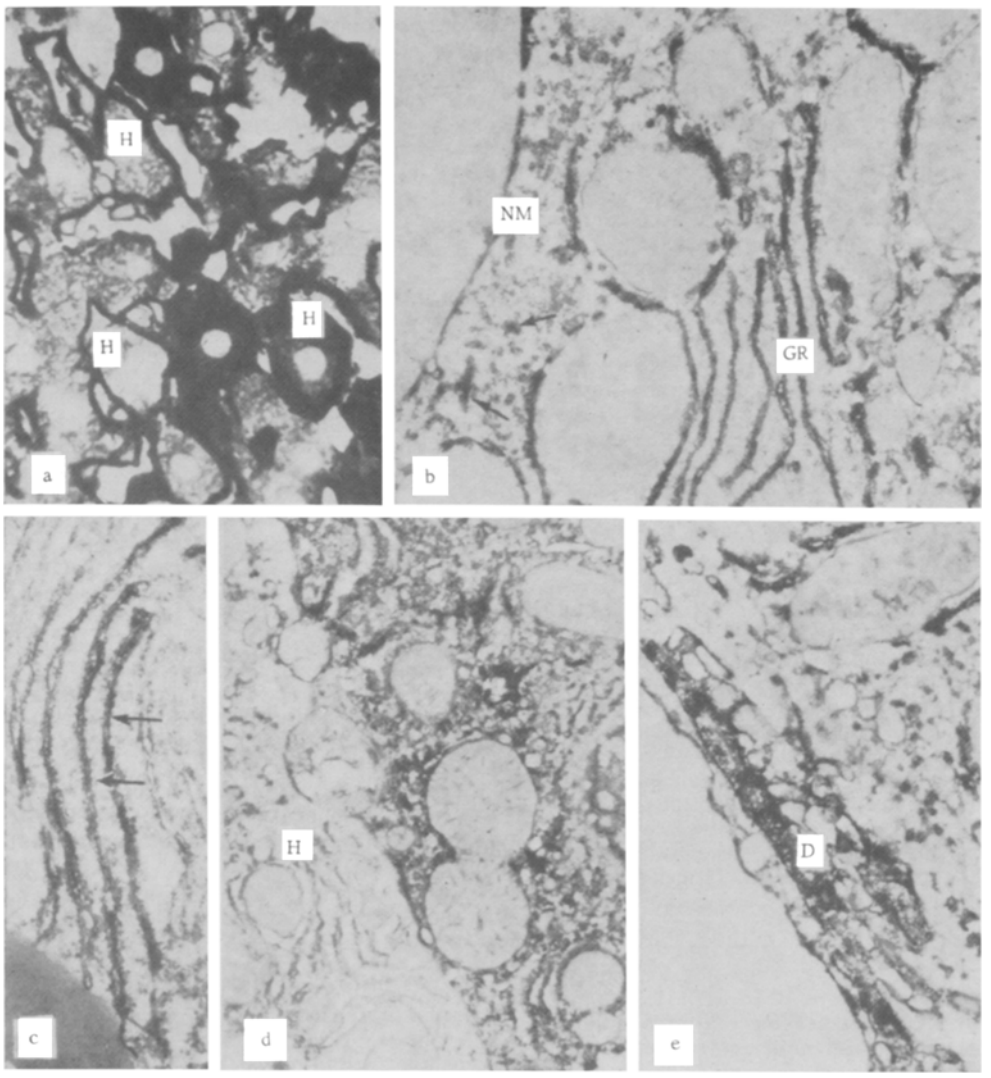

Fig. 1. Immunocytochemical location of albumin in mouse hepatocytes: a) semithin section through liver. Different intensities of staining on hepatocyte (H) cytoplasm, 900×; b) location of reaction product on ribosomes and layers of nuclear membrane (NM), membranes of granular endoplasmic reticulum (GR), and in cytoplasm (arrows). N) Nucleus, 20,000×; c) location of reaction product on membranes and ribosomes of granular endoplasmic reticulum (arrows), 34,000×; d) on right, hepatocyte (H) with diffuse deposition of reaction product in cytoplasm. On left, hepatocyte containing traces of reaction product, 11,000×; e) location of reaction product in Desse's space (D), 15,000×.

9 : 1 (BPS). After slow freezing of the fragments in a cryostat at −20°C, sections 15 μm thick were cut. Sections of the experimental series were incubated in a solution of pure antibodies against mouse albumin (0.05 mg/ml) at 4°C with constant mixing for 2 h. Sections of the control series were treated with a solution of normal rabbit IgG. After rinsing in BPS for 12-16 h the sections of the experimental and control series were treated with a conjugate of pure goat antibodies against rabbit IgG (0.3 mg/ml) at 4°C with gentle mixing for 4 h. The sections were rinsed for 14-16 h in cold BPS. The sections were rinsed with deionized water, after which the reaction for detection of peroxidase activity with 3,3-diaminobenzidine was carried out. After three washings with deionized water the sections were fixed in 1.33% osmium tetroxide solution in 0.1 M phosphate buffer, pH 7.4. The sections were quickly dehydrated in acetones of increasing concentration and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB III Ultrotome. Ultrathin sections were examined without additional staining with the JEM-100 S electron microscope.

EXPERIMENTAL RESULTS

1. Evaluation of Fixation. The suitability of the fixative was assessed by preservation of the ultrastructure of the hepatocytes and the intensity and reproducibility of the immunologic reaction. The results of these tests showed that the most suitable method of fixation is by immersion in 8% PF for 16 h. Poor preservation of the tissue was obtained by the use of lower concentrations of PF or a shorter period of fixation. The addition of even small amounts of GA to the fixative caused a marked decrease in the strength of the immunologic reaction. Fixation with PF+ PA or PF+ L mixtures gave poor preservation of the ultrastructure and completely suppressed the immunologic reaction. The type of buffer and the presence of sucrose had no significant effect on these parameters, but better results were obtained by the use of cacodylate buffer without the addition of sucrose.

2. Determination of the Location of Albumin. In unstained semithin sections of the liver a positive intracellular reaction for albumin appeared as deposits of dark brown reaction product. In the control series no such deposits were found. In the experimental series most hepatocytes contained reaction product, but its amount and, consequently, the intensity of staining of the cytoplasm varied considerably. Besides hepatocytes with weak or patchy staining there were also a few cells with intensively and diffusely stained cytoplasm (Fig. 1a). As a result of the irregular distribution of hepatocytes containing albumin in the tissue and the weak positive reaction in some hepatocytes it was impossible to estimate the number of positively stained cells quantitatively.

Electron-microscopic examination of material from the control series revealed weak nonspecific deposits of reaction product on the membranes of the hepatocytes and, in particular, in the region of the microvilli. Definite endogenous peroxidase activity was found in the cytoplasm of the erythrocytes and on membranes of the granular endoplasmic reticulum of the Kupffer cells and certain endothelial cells of the sinusoids.

In the experimental series, an electron-dense precipitate was located in most hepatocytes on the various layers and ribosomes of the nuclear membrane, on membranes of the granular endoplasmic reticulum, and as speckled deposits actually in the matrix of the cytoplasm (Fig. 1b, c). The number of tagged cisterns of the ergastoplasm and the size of the speckled foci in the matrix varied from one cell to another. In some hepatocytes massive deposits of reaction product occupied the whole matrix of the cytoplasm (Fig. 1d). Trimming the blocks to a point showed that these cells corresponded to diffusely and intensively stained hepatocytes in semithin sections. A very small quantity of precipitate could be observed sometimes in the lumen of structures of the granular and smooth endoplasmic reticulum and Golgi complex. As regards the extracellular distribution of reaction product, it was almost constantly present in the Desse's space (Fig. 1e).

If these results are compared with those obtained by the direct immunoperoxidase method [6-9] it can be concluded that, besides its usual situation on the membranes and ribosomes of the granular endoplasmic reticulum, albumin is also located on the layers of the nuclear membrane and in the cytoplasmic matrix of the hepatocytes, where it had not previously been described. Selective labeling of the ergastoplasm and nuclear membrane is evidence that albumin is synthesized at this level, like other proteins. Meanwhile, the low intensity of labeling of structures of the smooth endoplasmic reticulum and Golgi complex despite the presence of albumin in the cytoplasmic matrix suggests that hepatocytes can secrete albumin without the participation of those organelles. This conclusion confirms the views of Lin and Chang [11], according to whom albumin molecules synthesized on polysomal aggregates of membranes of the granular endoplasmic reticulum and nuclear membrane pass directly into the cytosol, from which they enter the extracellular space. The present investigation, however, does not completely rule out the possibility that the location of albumin in the matrix of the hepatocytes could be the result of its diffusion from the site of its synthesis or transport during the fixation process.

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POLYFUNCTIONAL ROLE OF THE ALVEOLAR BRUSH CELLS IN THE RAT LUNG

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An investigation by scanning and transmission electron microscopy revealed an increase in the number of vacuoles in the apical part of the cytoplasm in the alveolar brush cells of the regenerating rat lung, hyperplasia of the lamellar complex, and activation of the protein-synthesizing apparatus. Immature surfactant material and secretory granules with an osmiophilic center were found in the cytoplasm of the brush cells. Colchicine, injected intramuscularly into rats six times in the course of the 24 h before sacrifice in a dose of 0.1 mg/100 g body weight, affected neither the number, topography, nor the structure of the bundles of microfibrils present in large numbers in the brush cells. Meanwhile, under the influence of colchicine, some of the microvilli of the alveolar brush cells undergo destruction and resorption. These data on the ultrastructural organization of these cells indicate that they can perform several functions: absorptive, contractile, and secretory.

KEY WORDS: regeneration; surfactant; lung chemoreceptors.

Interest in the study of chemoreceptors of the lung has increased considerably in recent years. According to some observations [7], single Kulchitsky cells or concentrations of them (neuroepithelial bodies), capable of reacting to a change in the gaseous medium through increased secretion of granules containing biogenic amines, are present in the epithelial layer of the bronchi of the mammalian and human lung. No Kulchitsky cells are found in the respiratory bronchioles, alveolar passages or alveoli. Meanwhile, cells described by Meyrick and Reid as "brush cells" [8] are found in the epithelial lining of the air passages and also of the alveoli. The particular features of the ultrastructural organization of these cells, located in the alveoli, or alveolar brush cells (for instance, the presence of numerous vacuoles in the apical cytoplasm, and of microvilli on the cell surface facing the lumen of the alveolus) suggest that these cells, which perform an absorptive function, are alveolar chemoreceptors [3, 6, 8].

The writer has previously postulated principles of autonomous regulation of surface tension within the "surfacton*, the functional analog of the acinus of the lungs, in which the alveolar brush cells play a leading role. This hypothesis is based on the assumption that all cells of the surfacton, including alveolar brush cells, "surfacton,"* the functional with a rich network of feedback [3]. The alveolar brush cells analyze the composition of the hypophase and surfactant and respond primarily to a change in surface tension of the alveoli. The ultrastructure of the alveolar brush cells of the lungs of intact animals has been described several times [3, 6, 8]. However, none of the authors cited states that these cells absorb surfactant material. Yet this phenomenon is evidently the first stage that is essential for the role of alveolar brush cells in the regulation of the intensity of surfactant secretion.

The objects of the present investigation were accordingly: 1) to discover whether alveolar brush cells can absorb surfactant and, in particular, at the time of its active secretion; 2) to study the nature of the microfibrillary apparatus of these cells.

*The term "surfacton" was suggested originally in the Russian literature [3].

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