HISTOCHEMICAL INVESTIGATION OF CELLULAR AND TISSUE GLYCOCONJUGATES WITH LECTINS IN SEMITHIN SECTIONS

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The use of lectins for selective detection of glycoconjugates in cells and tissues under normal conditions and in various forms of pathology is one of the most rapidly developing trends in modern histochemistry [8, 11]. Sets of lectins suitable for histochemical differentiation of glycoconjugates differing in their terminal nonreducing mono- or disaccharide residues (D-mannose, D-galactose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-fructose), which previously was impossible by means of traditional histochemical methods [1], have been suggested. The overwhelming majority of histochemical investigations using lectins at the present time are based on analysis of paraffin or frozen sections [8].

Meanwhile the accuracy and informativeness of investigations conducted at the level of resolving power of the light microscope can be considerably increased by the use of semithin sections. Sections 0.5-1 μ thick, obtained from blocks prepared for electron-microscopy have many advantages over paraffin or frozen sections. The most important of these advantages are the more detailed and clear localization of intracellular structures, the possibility of using identical material for light and electron microscopy simultaneously, and improvement of the quality of sections by the use of ultramicrotomes.

Methods of staining semithin sections with hematoxylin and eosin and with toluidine blue have been described in the literature [5]. An essential condition for the successful use of lectins for processing semithin sections is that the chemical structure of glycoconjugates is preserved after fixation and embedding of the material in resins, and during subsequent processing of the sections in aggressive media (for example, in a saturated for staining semithin sections have been published [12], many technical details of this process, in the writers' view, have been inadequately studied.

The aim of the present investigation was to study the technical conditions for the use of lectins, obtained from raw material of Soviet origin, for selective histochemical localization of glycoconjugates in semithin sections.

EXPERIMENTAL METHODS

The testes, liver blood vessels, and gastric mucosa of noninbred albino rats were used. Pieces of the organs measuring 2×2×2×2 mm were fixed in 2.5% glutaraldehyde in buffered physiological saline (BPS) for 4 h at 4°C (pH 7.4). The material was then washed in BPS, and incubated for 1 h in a 0.2 solution of glycine in BPS to block the remaining aldehyde groups of the fixative [6]. The fragments were then dehydrated and embedded in Epon and Araldite by the usual method.

Sections 0.5-1 μ thick were cut on a UMTP-5 ultramicrotome, glued to slides with a 5% aqueous solution of serum albumin or histone, and dried in an incubator at 42°C for 12-24 h. To remove the resin, the sections were incubated for 5-10 sec in a saturated alcoholic solution of KOH, washed in two portions of 96° ethanol, and to inactivate endogenous peroxidase, they were immersed for 20 min in methanol, containing 0.3% H_2O_2. The sections were then taken through alcohols and washed in two portions of BPS, for 5 min each time.

The semithin sections were treated with soy lectin (specific for N-acetyl-D-galactosamine) and wheat germ agglutinin (specific for N-acetyl-D-glucosamine), obtained by the method in [3, 4]. To compare the results of staining, concanavalin A (con A), from Sigma, USA, which

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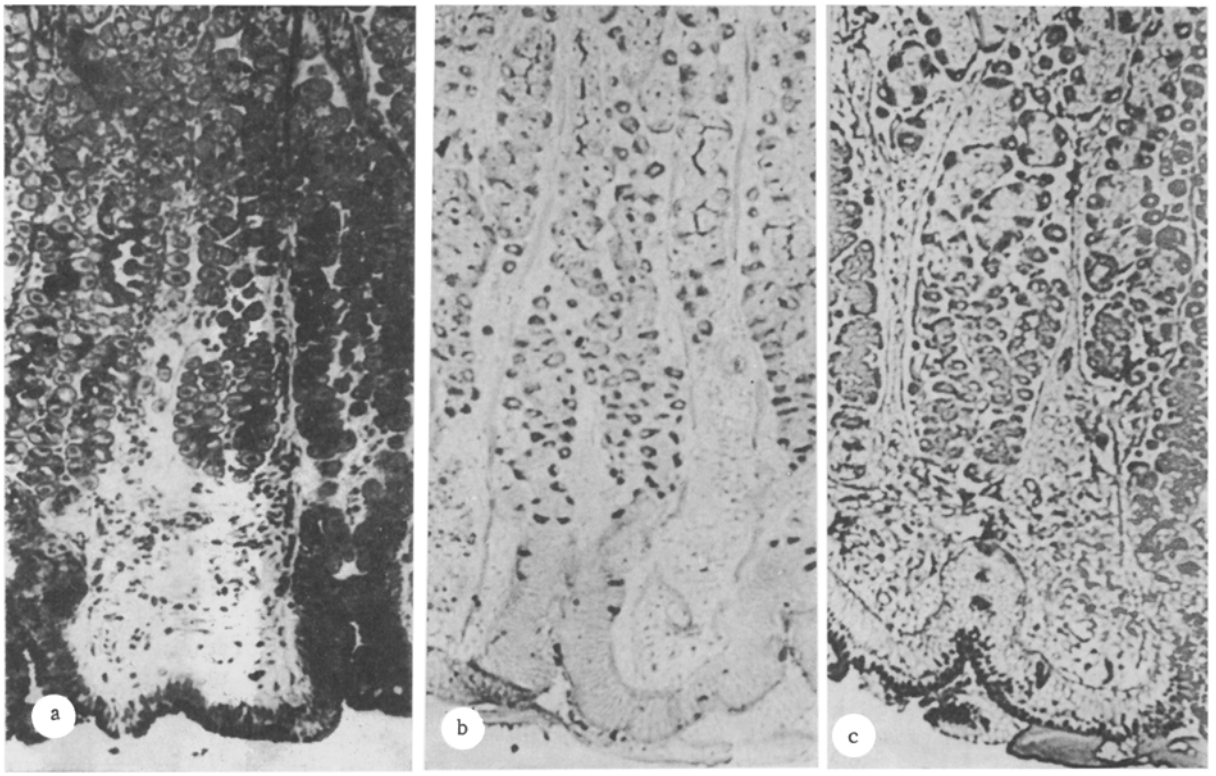

Fig. 1. Gastric gland of a rat: a) stained with toluidine blue; b) treated with soy lectin, labeled with horseradish peroxidase; c) treated with wheat germ lectin, labeled with horseradish peroxidase. 63 ×.

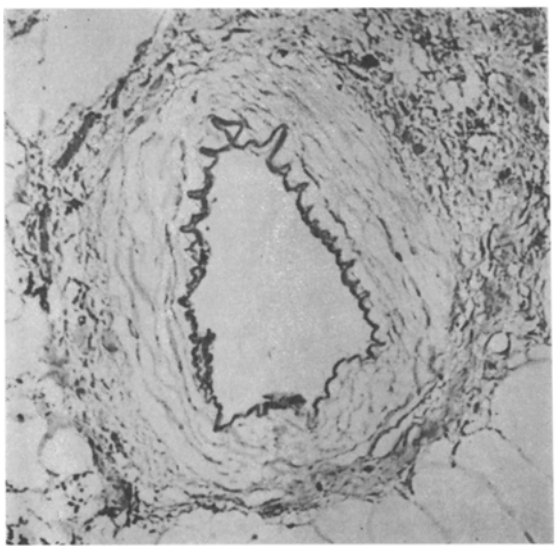

Fig. 2

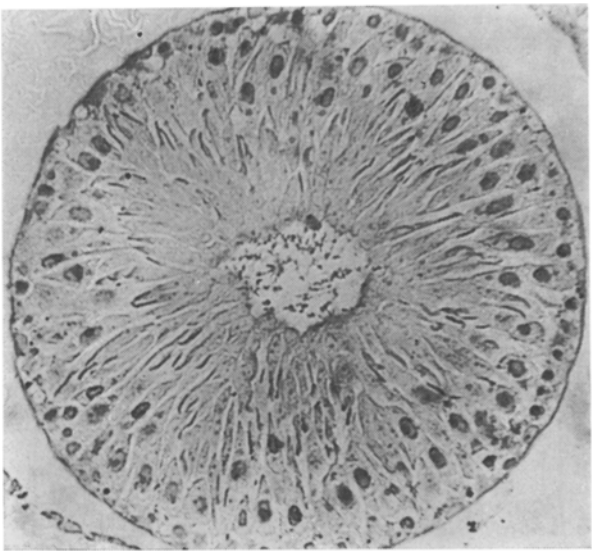

Fig. 3

Fig. 2. Artery of muscular type. Treated with soy lectin, labeled with horseradish peroxidase. 70 ×.

Fig. 3. Convoluted tubule of rat testis. Indirect localization of carbohydrate determinants with con A. 112 ×.

is specific for D-aminosaccharide residues, also was used. Soy lectin and wheat germ agglutinin were labeled with horseradish peroxidase by the method [9]. Con A was detected by an indirect method, using horseradish peroxidase from Reanal, Hungary, additionally purified by ion-exchange chromatography.

To detect glycoconjugates specific for the lectins, a 0.005% solution of the labeled soy lectin and wheat germ in BPS was applied to semithin sections, which were incubated for 30 min at room temperature. The lectin-binding sites were visualized by treating the sections with a 0.05% solution of 3,3'-diamino-benzidine hydrochloride in BPS in the presence of 0.015% H_2O_2. The reaction of development of diaminobenzine was carried out for 3-10 min under the control of an operating microscope and was stopped by immersing the sections in water. The sections were washed for 5 min each time in two portions of water, taken through alcohols of increasing strength, cleared in xylol, and mounted in balsom. Brown deposits of oxidized diaminobenzidine were formed at the sites occupied by glycoconjugates specific for the lectin used.

To detect glycoconjugates specific for con A, a 0.003% solution of con A in BPS was applied to the sections, which were washed after 30 min in two portions of BPS, for 5 min each time, after which a 0.001% solution of peroxidase was applied to the sections, which were incubated for 30 min. Subsequent treatment of the sections was the same as for soy lectin and wheat germ agglutinin.

To monitor the specificity of the reaction the sections were treated by the scheme described above, but leaving out the lectin; sections with the lectin were incubated in the presence of a 10% solution of the mono- or disaccharide specific for the lectin, or treated with unlabeled lectin and with native peroxidase, and the glyconconjugate was oxidized with 10% HIO_4 for 30 min. In all these cases the sections remained unstained.

EXPERIMENTAL RESULTS

In sections treated by the method described above specific binding of the lectins with glycoconjugates could be clearly seen. Comparison of semithin sections treated with toluidine blue, soy lectin, and wheat germ agglutinin (Fig. 1) showed differences in the localization of structural components of the gastric glands, with affinity for the lectins used. Soy lectin was bound mainly with membranes and cytoplasmic inclusions of goblet cells and the apical surfaces of the gastric epitheliocytes. Wheat germ lectin stained the cytoplasm of the cervical mucocytes, and the membranes and secretory inclusions of the parietal cells intensively; characteristically, binding was more intensive with the contents of the apical part of the gastric epitheliocytes. On staining a blood vessel of muscular type with soy lectin intensive outlining of the inner elastic membrane, the elastic fibers in the composition of the muscular coat of the artery, and membranes of lipocytes and fibrous structures in the adventitia was found (Fig. 2). On treatment of sections through the convoluted tubules of the testis with con A intensive staining of the basement membrane, nuclear membranes, and karyoplasm of primary and secondary spermatocytes and of the nuclear membranes of the sustentacular cells was observed (Fig. 3).

The study of the control sections showed that by treatment with lectins in the presence of the specific mono- or disaccharide it was not always possible to achieve a negative result of staining, because the affinity of some lectins (soy and peanut, in particular) for glycoconjugates may be higher than for the corresponding mono- or disaccharide. By using the method described above there is no need to increase exposure of the sections in KOH solution, which may cause the tissue structures to dissolve completely. These observations differ sharply from the results of investigations [5] in which it is recommended that the sections be incubated in KOH solution for 20 min. In this case we observed loss not only of glycoconjugates, but also of the general structure of the tissue. Evidently the optimal time for removal of the resin must be chosen for each type of embedding.

Under no circumstances must the fixative contain any OsO_4, which gives rise to a nonspecific reaction of diaminobenzidine development. To remove epoxide resin from the sections some workers recommend the use of potassium ethoxide [10] or sodium methoxide in methanol—benzene [7], but we found no significant improvement in staining by so doing. If after fixation in glutaraldehyde the tissue fragments were not rinsed in glycine solution, the sections may be rinsed with this solution during the 5 min immediately before application of the lectin. If such neutralization of the remaining aldehyde groups is not carried out, intensive nonspecific adsorption of the lectin by all tissue structures takes place [6]. When certain lectins are used (wheat germ agglutinin and con A, in particular), only serum albumin should be used to glue the sections to the slides, for ovalbumin causes intensive nonspecific staining of the sections.

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CHANGES IN AREA OF INJURY OF THE NORMAL, NONISCHEMIC MYOCARDIUM ADJACENT TO THE INFARCTION "RISK ZONE" DURING PERMANENT AND TRANSIENT CORONARY OCCLUSION

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A so-called "risk zone" of development of a myocardial infarct (IRZ) has been described in the literature [5, 6, 10], and it menans an area of myocardium, identifiable soon after coronary occlusion, which undergoes primary ischemia (i.e., its arterial system is nonfunctioning). The ratio of the size of IRZ to that of the zone of real damage (ZRD) can be used as a measure of involvement of the normal nonischemic myocardium, adjacent to IRZ, in the pathological process. The zone of normal myocardium around IRZ, corresponds most closely to the concept of a peri-infraction zone (PIZ). The area of the latter can be calculated by subtracting the area of IRZ from the area of ZRD.

There is evidence that in the comparatively early stages of development of infarction (3-6 h after coronary occlusion) ZED is no larger than IRZ and, consequently, there is no PIZ [5, 6, 10]. Meanwhile some workers [3, 4] have given morphological evidence in support of the presence of PIZ.

EXPERIMENTAL METHOD

Permanent and transient coronary occlusion ischemia was produced in noninbred male rats by ligation of the left coronary artery 3-4 mm below the left angle of the base of the infundibulum, by the methods described in [1, 2]. The group of animals was killed by inhalation of ether 10 min after coronary occlusion and the initial area of myocardial ischemia, corresponding to IRZ, was determined. For this purpose, the coronary vessels were injected with a suspension of latex microspheres (LM), stained blue with a 1% solution of trypan blue, post mortem through a cannula introduced through the right carotid artery into the aorta. The diameter of LM as a rule was 4.35-8 μ and their concentration 2.5×10^6-3.1×10^6/mm^3. After the vessels had been injected the heart was removed, cut into segments 2 mm thick starting from the apex, and subjected to segmental morphometry by the technique described for the reaction with nitro-blue tetrazolium (nitro-BT [2]). IRZ, unlike the normal myocardium, did

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