ever, counterstaining of the cells in this case is unsatisfactory and requires long exposure of the histological sections to the dyes. As will be evident, this is because of the long time which the nerve tissue undergoes treatment in potassium bichromate solutions.

The method now developed not only enables demyelinization to be detected and the state of the various cells in nerve tissue to be studied in the same preparations, but it also enables the time spent on preparing the material for histological investigation to be greatly reduced (for sealing in celloidin to one month, for embedding in paraffin wax to 1.5 weeks, and for freezing to 5-6 days), i.e., by 2.5 and by more than 10 times, respectively compared with the classical method. In addition, when the suggested method is used it is unnecessary to impregnate the nerve tissue in potassium bichromate, which leads to increased fragility of the material and makes it impossible to obtain sufficiently thin sections. Histological sections obtained in this way (2-3 μ thick) can be examined with immersion magnifications of the light microscope.

The suggested method of simultaneous detection of myelin breakdown products and of cells in demyelinized nerve tissue opens up new prospects for the study of the pathogenesis of demyelinization. It can be used to investigate the process of destruction of the myelin fiber of varied etiology, including for the rapid diagnosis of periaxonal changes in morbid anatomical practice.

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HETEROGENEITY OF BASEMENT MEMBRANES REVEALED IN HUMAN TISSUES BY MONOCLONAL ANTIBODIES TO LAMININ AND ENTACTIN

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KEY WORDS: heterogeneity; laminin; entactin; monoclonal antibodies

The glycoproteins laminin and entactin are components of basement membranes (BM) of varied origin [3, 8, 13]. The presence of these glycoproteins has been demonstrated with the aid of polyclonal antibodies (PA) in virtually all BM of organs studied [5, 7]. The appearance of monoclonal antibodies (MA), with narrow specificity, has demonstrated heterogeneity of BM with respect to several components, including to type IV collagen and laminin [6, 10, 15], i.e., besides MA which, like PA, reveal laminin of BM, there are also others which react with by no means all BM. There is no unanimity at present on whether entactin is a universal component of BM. According to some data [3] entactin was revealed by PA in all rat tissue BM studied. However, in [8], in which the distribution of entactin was studied

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TABLE 1. Distribution of Entactin and Laminin in Various Basement Membranes of Human Tissues Revealed by the Immunohistochemical PAP Method Using Monoclonal Antibodies

Organ	Structures studied	Entactin	Laminin
Kidney	BM of		
	tubules (all types)	+	+
	capsule of glomerulus	+	+
	capillaries of glomeruli	+	—
	endothelium of arteries	+	—
	SMC of arteries	+	—
Liver	Endothelium of sinusoids	+	—
	BM of		
	bile ducts	+	+
	endothelium of arteries		
	veins	+	+
	SMC of arteries	+	—
Heart	BM of		
	cardiomyocytes	+	+
	endothelium of arteries	+	+
	SMC of arteries	+	—
Skin	epidermis	+	+
Spleen	BM of		
	trabeculae	+	+
	endothelium of arteries	+	+
	SMC of arteries	+	—
Ileum	BM of		
	epithelium of crypts	+	+
	capillaries of villi	+	+
	endothelium of arteries, veins, and lymphatics	+	+
	SMC of arteries	+	—
	SMC of veins	+	+
	SMC of muscle coat	+	+

in human tissue with the aid of PA, it was discovered only in BM of endothelium and of muscle cells, but not of epithelial cells. Previously MA was obtained to laminin and entactin isolated from an EHS mouse tumor [11].

The aim of the present investigation was to study specificity of these MA with respect to various BM of human tissues with the aid of the peroxidase-antiperoxidase (PAP) method.

EXPERIMENTAL METHOD

Autopsy specimens of liver, spleen, kidneys, heart, skin, and ileum obtained from men aged 48-55 years dying from ischemic heart disease, and taken 3-4 h after death, were used. The samples were frozen in liquid nitrogen, and frozen sections 5 μ thick were cut, fixed with acetone at 4°C for 10 min, dried, and kept at −20°C. Before staining the sections were washed with phosphate-buffered saline (PBS) pH 7.4, incubated for 30 min successively with conditioned media from hybroidomas (LT3.1 and ELM2) producing antibodies to laminin and entactin [11], and then with rabbit antiserum against mouse immunoglobulins (1:20), to which normal human serum was added (1:30) to inhibit the cross reaction with human immunoglobulins, and with monoclonal PAP reagents (provided by A. I. Fairman [1]). After each incubation the sections were washed with PBS for 10 min. Peroxidase activity was revealed by the reaction with diaminobenzidine [4]. The sections were counterstained with Ehrlich's hematoxylin. Sections incubated with PBS instead of MA, and subsequent operations carried out as indicated above, were used as the control.

EXPERIMENTAL RESULTS

The data on staining of BM of varied origin with the aid of MA to laminin and entactin are given in Table 1. They demonstrate the existence of several basement membranes in which laminin is not detectable by LT3.1 MA. Meanwhile, several investigations with the use of PA have conclusively demonstrated the presence of laminin in these structures: BM of capillaries of the renal glomeruli, BM of smooth muscle cells (SMC) of blood vessels, and in the endothelium of the hepatic sinusoids [2, 5, 8].

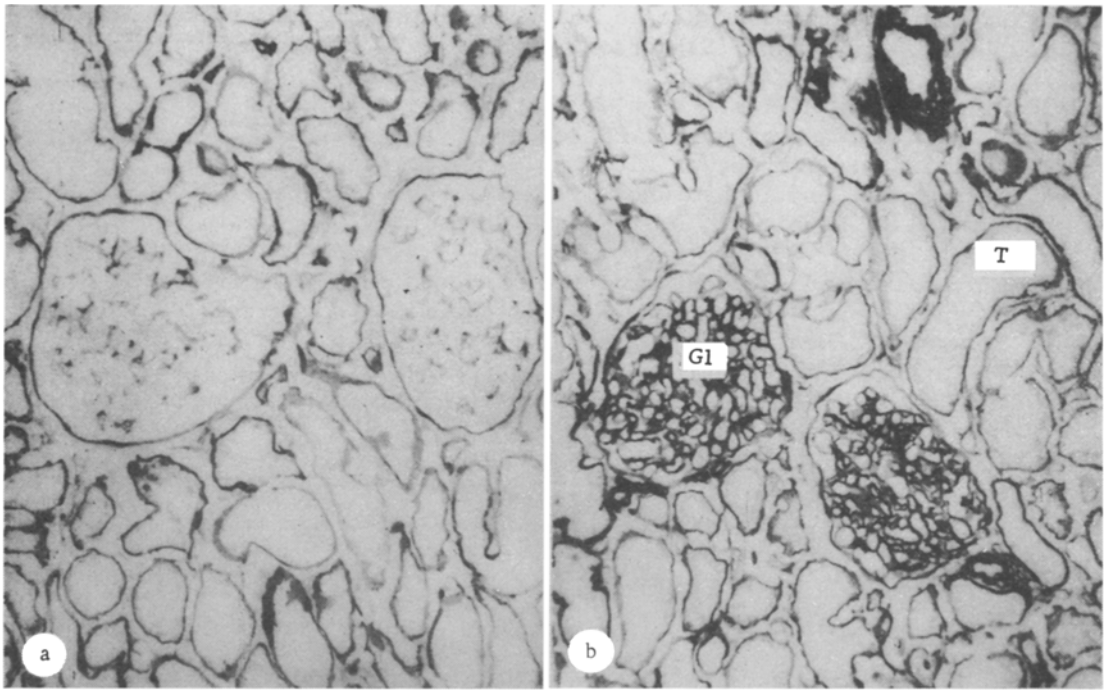

Fig. 1. Localization of laminin and entactin in human kidney revealed with the aid of monoclonal antibodies (MA) and the PAP method on frozen sections. Sections prepared without counterstaining by hematoxylin. a) MA to laminin; b) MA to entactin. Gl) Glomeruli; T) tubules. 125 ×.

Heterogeneity of BM revealed by LT3.1 antibodies can be explained by several causes such as the different organization of laminin in BM from different organs. In this case the determinant recognized by our MA evidently differs in accessibility for them. Another possibility is that this determinant in some BM is hidden (masked) by other proteins, as has been shown for type V collagen [7]. The character of staining of kidney sections (Fig. 1a) by our MA to laminin was the same as that described in [10] for 4E10 MA. However, 4E10 MA did not stain BM of the endothelium of all the intramural arteries of the urogenital system. According to our results, MA to laminin did not reveal BM of the endothelium of the intramural arteries only in the kidneys, and in all other organs these BM were antigen-positive (Table 1). Our own data on the distribution of entactin differed strongly from the results described by Jaffe et al. [9]. We observed staining of BM of the renal tubules (Fig. 1b), epidermis, and bile ducts, whereas in [9] staining was restricted to BM of the endothelium and muscle cells in all the organs investigated. This difference may be partly explained by the fact that the authors cited used PA to entactin from a different source (a mouse entodermal cell line). They also denatured the entactin first in sodium dodecylsulfate, whereas our ELM2 MA were obtained to native protein. The PA which Jaffe and co-workers used evidently cross-reacted only with certain antigenic determinants of human entactin, which were exposed in only a limited number of BM. Thus the results obtained by Jäffe and co-workers point to the existence of heterogeneity of BM in relation to entactin also, although our own MA did not reveal any such heterogeneity. The problem of heterogeneity of BM with respect to other components likewise is under discussion at the present time [12].

The structural and biochemical heterogeneity of BM is thus evidently linked with differences in the origin and functional status of the cells producing them. MA to different components of BM provide a promising tool with which to investigate the molecular principles of this heterogeneity.

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STRUCTURAL TRANSFORMATIONS IN HEPATOCYTES FOLLOWING ADMINISTRATION OF RHEOPOLYGLUCIN TO MICE AND SUBSEQUENT EXPOSURE TO STRESS

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The introduction of various substances which are metabolized in the liver into the animal body is one way of increasing the function load on that organ. This gives rise to intensification of plastic processes in the hepatocytes [9, 11], to the creation of a structural basis for increased liver function, and intensification of repair processes in the damaged organ [2, 3, 9]. Administration of the lysosomatropic agent rheopolyglucin to mice accelerated resorption of necrotic foci and restoration of the parenchyma of the liver after its damage by carbon tetrachloride [6].

The aim of this investigation was to study structural changes taking place in hepatocytes after administration of rheopolyglucin, which is metabolized in the liver, and the particular features of the response of its parenchyma to stress.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 2 months and weighing 19-21 g. The animals in group 1 (control) were superficially anesthetized with ether and given an injection of 0.9% NaCl solution into the caudal vein in a dose of 1 ml/100 g body weight. Mice of group 2 received a similar injection of rheopolyglucin (10% solution of partially hydro-

TABLE 1. Results of Morphometry of Hepatocytes (M ± m)

Parameter studied	Control	Experiment	
		rheopolyglucin	rheopolyglucin + stress
Nucleus of hepatocyte ($\bar{V}$)	237,0±7,58	337,4±10,8*	349,4±9,68*
Cytoplasm of hepatocyte ($\bar{V}$)	2398,4±239,0	3510,4±314,0*	3469,5±125,4*
Hepatocyte ($\bar{V}$)	2635,4±240,0	3847,8±316,4*	3818,9±125,8*
Ratio of number of binuclear hepatocytes to total number of hepatocytes	0,041±0,007	0,062±0,009	0,080±0,012*

Legend. $\bar{V}$) Volume (in μ^3). Here and in Table 2: *p < 0.05 compared with control.

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