CARBOHYDRATE COMPONENTS OF RAT LIVER AND HEPATOMA NUCLEAR MATRIX

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Investigations of isolated cell nuclei and the insoluble nuclear skeleton or nuclear matrix (NM) remaining behind after removal of the nuclear sap, chromatin, and membranous components from the nuclei have been directed mainly toward the study of proteins and nucleic acids, and the carbohydrate components have received little study [12, 13]. It was shown previously that nuclei isolated from different cells contain a fairly constant quantity of hexosamines [1]. Data on the content and character of carbohydrates of the cell nucleus published in the literature are heterogeneous but there is no doubt that the chief components are glycoproteins and glycosaminoglycans [11, 12]. In cultures of melanoma and HeLa cells the latter are represented chiefly by chondroitin sulfate [11, 12] and, in the rat liver, by hyaluronic acid [8]. Carbohydrates of the cell nucleus are largely concentrated in the nuclear membrane. For instance isolated rat liver nuclear membranes contain 3-5% of the total carbohydrates and only traces of sialic acid [4, 12, 13]. A more detailed study has shown that the chief carbohydrate residues of the nuclear membrane are mannose and glucosamine [9]. The same conclusion also follows from the results of tests with lectins [15]; in the case of tumor cells, moreover, the reaction with concanavalin A (con A) for mannose is intensified, whereas that with soy agglutinin for galactosamine is depressed [13]. Electrophoresis of proteins in polyacrylamide gel with sodium dodecylsulfate (SDS) show that con A binds mainly with high-molecular-weight proteins of the nuclear membrane [14] and chromatin preparations [10, 11]. In preparations of NM in rat liver 5.5% of total carbohydrates was found but only traces of sialic acid [6]. A fairly high concentration of hexosamines and hexoses also was found in the residual fraction of nuclei obtained by a method similar to that of obtaining NM [8]. Treatment of NM, diluted with alkali or hyaluronidase, i.e., by agents destroying or removing glycosaminoglycans, also leads to liberation of granules 25-30 nm in diameter, which are evidently the basic structure of NM [7].

In this investigation an attempt was made to detect glycoprotein components of NM from the liver and from solid and ascites rat hepatomas by electrophoresis of proteins followed by the reaction with con A — peroxidase and by an electron-microscopic study of binding of con A conjugated with ferritin (for neutral carbohydrates), and staining with ruthenium red (for acid polysaccharides).

EXPERIMENTAL METHOD

Preparations of isolated nuclei and NM were obtained from normal liver, hepatoma 27 (4 weeks after transplantation), and Zajdela's ascites hepatoma (5th day after transplantation) of noninbred male albino rats weighing 150-200 g, obtained from the Central Nursery, Academy of Medical Sciences of the USSR, as described previously [2, 5]. Electrophoresis of NM proteins was carried out in 7.5% polyacrylamide gel containing SDS [2]. Tests for carbohydrate components were carried out on the gels by the con A—peroxidase method [16]. Luft's ruthenium red staining was carried out by the method in [3] and treatment of the electron-microscopic preparations with con A, conjugated with ferritin, by the method in [15]. Preparations for electron microscopy were embedded in Epon-Araldite, and ultrathin sections were examined in the JEM 100B electron microscope (Japan).

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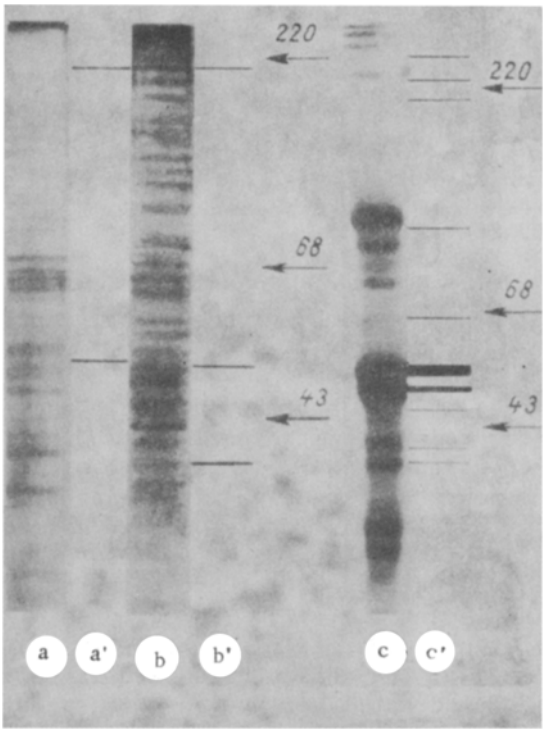

Fig. 1. Electrophoretogram of NM proteins in 7.5% polyacrylamide gel with SDS and scheme of staining for glycoproteins. a, a') Normal liver; b, b') hepatoma 27; c, c') Zajdela's hepatoma; a, b, c) staining with Coomassie R 250; a', b', c') staining by con A–peroxidase reaction. Numbers indicate molecular weights (in kilodaltons).

EXPERIMENTAL RESULTS

Electrophoretic fractionation of NM proteins in polyacrylamide gel containing SDS showed that the tumors contained much more of the high-molecular-weight polypeptides than normal liver (Fig. 1a-c). The protein profile of NM of Zajdela's ascites hepatoma differed significantly from that not only of normal liver, but also of solid hepatoma 27. In both hepatomas a band was found with molecular weight (mol. wt.) of about 220 kilodaltons (kd), but proteins with mol. wt. of about 100 and 40 kd were prominent in the ascites hepatoma. This result thus confirms once again the predominance of high-molecular-weight components in tumor NM discovered previously [2].

The reaction with con A–peroxidase revealed two bands in rat liver NM in the region of mol. wt. 200 and 50 kd, and in the case of hepatoma 27, bands in the region of mol. wt. 200, 50, and 38 kd. MN of Zajdela's ascites hepatoma was particularly rich in glycoproteins. Here two bands were clearly distinguished in the region of mol. wt. 54 and 50 kd, and polypeptides with mol. wt. higher than 200 kd and about 105 and 38 kd also were observed (Fig. 1a'-c').

Glycoproteins with mol. wt. of about 200 and 50 kd are thus present in NM of the liver and of both hepatomas. A band in the region of mol. wt. about 38 kd also is present in both tumors but not in the liver, and a number of additional bands are found in Zajdela's ascites hepatoma. The results can be compared only with the results of investigation of the nuclear membrane and chromatin, for there is very little information in the literature about carbohydrates of NM [6]. Determination of carbohydrate components on the basis of incorporation of labeled glucosamine and fucose revealed several bands in the chromatin of HeLa cells, among which there are undoubtedly bands corresponding to mol. wt. of 200 and 50 kd [11]. Rather different values for bands stained with con A after electrophoresis of rat liver chromatin, namely 135, 125, and 50 kd, are given by Rizzo and Bustin [10], but bands of higher molecular weight — about 200 kd or more — can be seen on their densitogram. In isolated

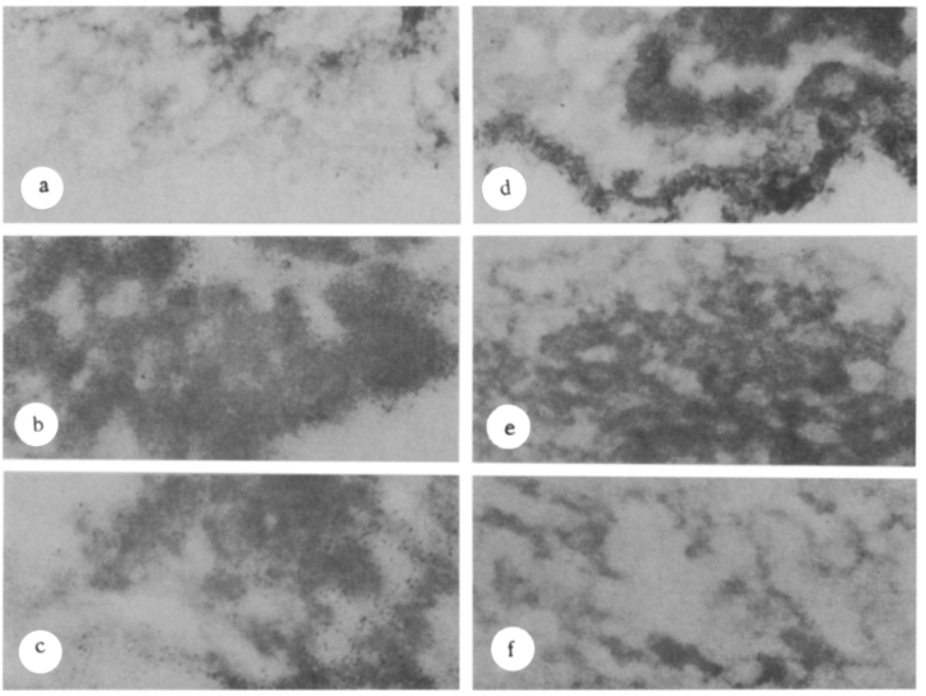

Fig. 2. Electron microscopy of rat liver and hepatoma NM. a-c) Staining with con A conjugated with ferritin for neutral carbohydrate residues (45,000 ×); d-f) staining with ruthenium red for acid polysaccharides (60,000 ×); a, d) normal liver; b, e) hepatoma 27; c, f) Zajdela's hepatoma.

nuclear membranes of rat liver con A binds mainly with glycoproteins with mol. wt. of about 180 and 34 kd, but bands in the 50 kd region also are found [14]. Considering, however, that preparations of both chromatin [10] and nuclear membrane [14] contain components of NM, and that molecular weights can be determined only very approximately, it can be reliably concluded that glycoproteins with mol. wt. of about 200 and 50 kd are characteristic of liver NM.

Electron-microscopic investigation of NM stained with con A–ferritin revealed numerous binding points, mainly at the periphery of NM (Fig. 2a-c). The reaction was appreciably stronger in NM of hepatoma 27 and, in particular, Zajdela's hepatoma than in NM of the liver. This result is in good agreement with the stronger agglutination by con A of nuclei isolated from tumors than from normal liver [13], and on binding of con A by rat liver nuclear membranes predominantly on their surface facing the perinuclear space [15]. Carbohydrate residues binding con A, i.e., mannose and, possibly, glucose residues, are still present in NM deprived of its membrane material.

Staining with ruthenium red by Luft's method reveals mainly acid polysaccharides or glycosaminoglycans [3]. It will be clear from Fig. 2d-f that mainly the periphery of NM is stained, and by contrast with staining by con A–ferritin, its intensity is much greater in normal liver than in tumors.

It can thus be concluded that NM of liver and hepatomas contains both glycoproteins that react with con A and glycosaminoglycans staining with ruthenium red. In agreement with data in the literature [11-13] preparations obtained from tumors are relatively richer in neutral glycoproteins but poorer in glycosaminoglycans. Differences of a similar kind also were observed in the regenerating liver compared with normal liver [8]. The results of a study of nuclear matrix structures treated with hyaluronidase and dilute alkali suggest that glycosaminoglycans determine association of the globular structures of NM [7].

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ACTION OF HIGH-DENSITY LIPOPROTEINS ON CHOLESTEROL BIOSYNTHESIS IN THE RAT LIVER

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High-density lipoproteins (HDL) have a protective action and prevent the development of atherosclerosis. The attention of research workers has been drawn to the ability of HDL to accept excess cholesterol in the intima and media of the vascular wall [14]. HDL loaded with cholesterol are transported to the liver where some of them give up their excess of cholesterol esters and make good the deficiency of phospholipids, whereas some are assimilated by the liver cells and undergo catabolism [14].

The liver metabolizes not more than 5-8% of the total HDL pool [15], but nevertheless it is the principal site of HDL biosynthesis. The liver also plays a special role in cholesterol catabolism. Interaction of HDL with liver cells both *in vivo* and *in vitro* is accordingly interesting. Data in the literature on this question are contradictory. It has been shown that HDL activate cholesterol biosynthesis in the hepatocytes [5] but do not change cholesterol synthesis *in vitro* [11] or even inhibit it [4]. In an attempt to discover the cause of these contradictions, a method of conducting experiments *in vivo* to study the effect of HDL on cholesterol biosynthesis in the liver was devised.

The object of this investigation was to study cholesterol biosynthesis in the rat liver *in vivo* during a temporary but considerable rise in the blood HDL level.

EXPERIMENTAL METHOD

Male Wistar rats weighing 230-250 g were used. The experimental and control groups each contained eight animals. Sixteen rats were used as donors. The control and experimental animals were lightly anesthetized with pentobarbital two weeks before the experiment and a polyethylene catheter 0.3 mm in diameter was introduced into the femoral vein and immediately filled with a 6% aqueous solution of polyvinylpyrrolidone. The catheter was fixed by ligatures. The free end of the catheter was sealed with hot forceps and brought out through the skin at the base of the tail, where it was secured with a tantalum clip. The point of emer-

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