The tracer was observed to accumulate in the channels of RER, which were widely dilated, fragmented, and deprived of ribosomes (Fig. 1c). These phenomena of RER disintegration in the hepatocytes spread to the central zones of the lobules, and hepatocytes with RER fields could no longer be observed; this rules out any possibility of compensation of protein synthesis by some hepatocytes 60 min after injection of CCl_4 into the rats.

As a rule the tracer was found in lysosomes of the hepatocytes (Fig. 1 c, d), evidently in connection with intravital injury to their membranes. Pore formation in the lysosomal membranes opens the door for their enzymes to emerge into the cytosol of the hepatocytes, and in the later stages after exposure to CCl_4 this evidently leads to irreversible changes and death of the centrilobular hepatocytes.

The formation of membrane defects thus precedes the development of ultrastructural changes in the hepatocytes characteristic of CCl_4 poisoning, and the latter process is largely the result of damage to the membranes.

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ANALYSIS OF BINDING AND UPTAKE OF NATIVE AND MODIFIED LOW-DENSITY LIPOPROTEINS BY HUMAN LIVER CELLS IN PRIMARY CULTURE

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The liver plays a key role in regulation of the blood level of circulating low-density lipoproteins (LDL). Not only are LDL precursors synthesized in the liver, but most lipoproteins of this class are broken down in it [6]. The role of the liver cells in LDL elimination is particularly important. Liver cells are known to bind and take up LDL through specific receptors located on their surface [3]. Investigations have shown that all liver cells, including parenchymatous (hepatocytes), have not only receptors for native LDL but also specific receptors for chemically changed or modified LDL [10, 14]. Interest in the latter is due to their possible role in the accumulation of intracellular lipids and the formation of foam cells [4]. It was reported comparatively recently [12] that rat hepatocytes have no receptors for modified LDL. This suggests that some degree of specialization of the cells exists in the

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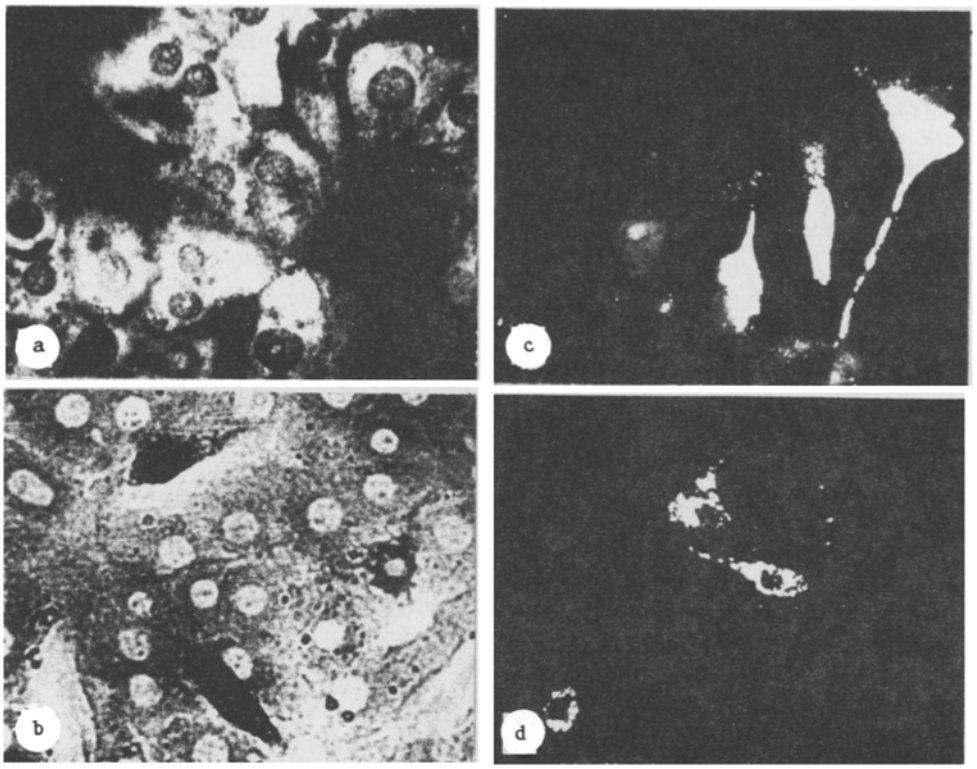

Fig. 1. Identification of cells of a primary culture of human liver. Hepatocytes detected on the basis of the presence of intracellular albumin (a), Kupffer cells — accumulation of phagocytosed fluorescent microspheres (b), stellate cells — adsorption of gold chloride particles on their surface (c), and endothelial cells — uptake of FITC-labeled albumin, treated with formalin (d). Magnification: a, d) 320, b) 250, c) 200 ×.

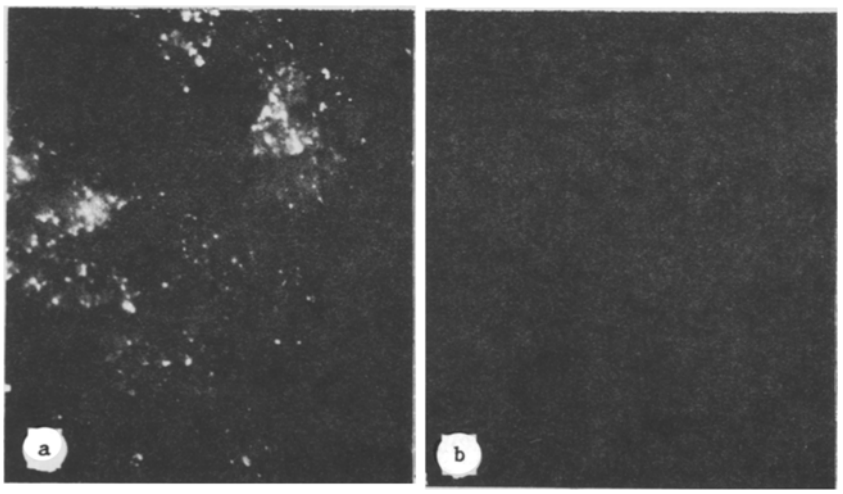

Fig. 2. Visualization of binding sites of native LDL on surface of human liver cells in primary culture. Incubation of cells with (a) and without LDL (b) followed by detection of LDL particles on cell surface. 500 ×.

"antiatherogenic" function of the liver, aimed at removing native and modified LDL from the blood. With the recent development of a method of culture of human hepatocytes [9], it is now possible to test this hypothesis on a simple and convenient model, namely primary cultures of human liver.

Binding and uptake of native and modified LDL by cells of a primary culture of human liver were analyzed visually in the investigation described below. The indirect immuno-

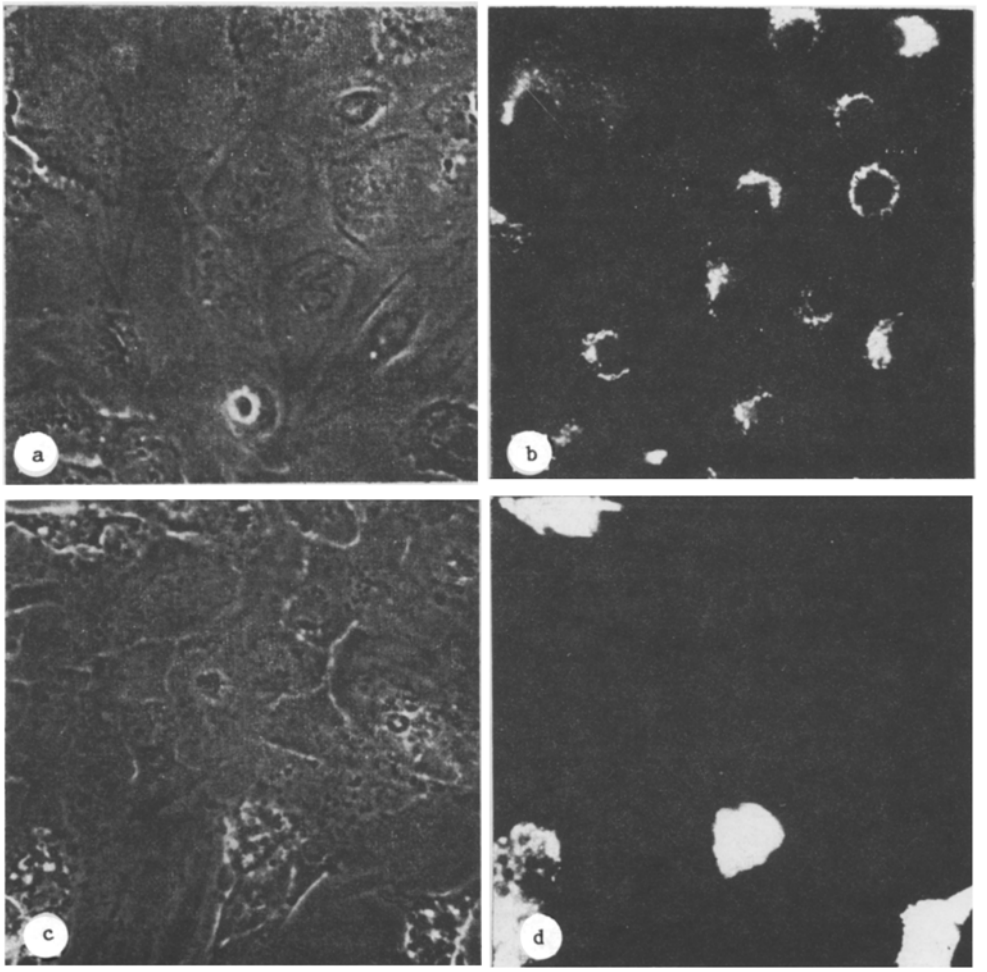

Fig. 3. Uptake of native (a, b) and modified LDL (c, d), labeled with fluorescent dye Di-1, by cells of primary culture of human liver. Phase contrast (a, c) and fluorescence microscopy of same parts of field of vision. 250 ×.

fluorescence method and lipoproteins labeled with the fluorescent dye 3',3'-dioctadecylindocarbocyanine (Di-1) were used for this purpose.

EXPERIMENTAL METHOD

Autopsy material obtained during urgent autopsy within 1 h after death on subjects aged 1-5 years was used. Liver cells were isolated by perfusion [9], seeded on coverslips with a density of 3×10^4 cells/cm^2, and cultured in medium RPMI-1640 containing 10% fetal calf serum, 10^{-3} mM insulin, 10^{-5} mM hydrocortisone, and 0.1 μg/ml kanamycin ("Sigma," West Germany). More than 30-50% of the cells adhered to the substrate and were viable in the trypan blue test. The number of hepatocytes in culture was determined by the indirect immunofluorescence method, using rabbit antiserum to human albumin ("Behring Diagnostics Corp.") and sheep's antibodies to rabbit IgG, labeled with fluorescein isothiocyanate (FITC) by the formula in [13]. Kupffer cells were detected on the basis of their active phagocytosis of fluorescent carboxylated microspheres 1.5 μ in diameter ("Polysciences," USA), which were incubated with the cells for 1 h at 37°C. Stellate or vitamin A-containing cells were deleted by staining the culture with gold chloride [15], while endothelial liver cells were detected according to their ability to absorb FITC-labeled human albumin, treated with formalin according to the prescription of [2]. Visual analysis of lipoprotein binding with the cells was carried out by the method in [1]. For this purpose the cells were transferred into medium containing delipidized serum (DLS; d>1.25 g/ml) and 24 h later they were incubated for 3 h at 37°C with 15 μg/ml of human LDL (d = 1.019-1.050 g/ml) or LDL modified by malonic dialdehyde (MDA) as in [5]. After fixation, cell-bound lipoproteins were revealed by the indirect fluorescent method and rabbit antibodies to LDL, characterized previously [8]. Parallel studies were made of uptake of lipoproteins labeled with Di-1 by the method in [11]. The cells were incubated in medium with

DLS containing 20 μg/ml of Di-1-LDL or Di-1-MDA-LDL for 4-5 h at 37°C, and then for 10 min with unlabeled lipoproteins.

EXPERIMENTAL RESULTS

In view of the particular features of a primary culture, notably the variability of its cell composition, methods of identifying all the main types of human liver cells in culture were developed as a first step. Analysis of the cell composition of primary cultures (n = 5) of a child's liver showed that most (about 80%) cells were hepatocytes, hexagonal in shape, and containing intracellular albumin (Fig. 1a). From 3 to 12% of Kupffer cells, distinguished by their large size and active phagocytosis of fluorescent microspheres (Fig. 1b), also were found in the culture. About 3-9% of the culture consisted of stellate cells, which stained intensively with gold chloride (Fig. 1c). The endothelial cells (1-9%) of the liver were relatively small and elongated, and they could be identified on the basis of selective uptake of FITC-labeled modified albumin (Fig. 1d). They often lay side by side and formed a cell chain.

Visual analysis of LDL binding with the cells showed that nearly all types of cells in culture, including hepatocytes, had binding sites for native LDL, revealed in the form of small granules discretely distributed over the surface of the cells (Fig. 2a). In the control test, in which the stage of treatment with LDL or antibodies to LDL was omitted, this staining was never observed (Fig. 2b). On incubation of the cells with MDA-LDL, single granules of specific fluorescence were found only in solitary cells of the culture (less than 5% of the total number).

Visual analysis of uptake of lipoproteins by the cells showed that hepatocytes, like the other cells of the culture, actively took up Di-1-labeled native LDL (Fig. 3a, b). The cells thereby acquired bright specific fluorescence, observed in the form of round inclusions, located in the perinuclear zone of the cytoplasm (Fig. 3b). Uptake of Di-1-LDL was specific, and was completely inhibited in the case of combined incubation of Di-1-LDL with a 20-fold excess of unlabeled LDL. No such effect was observed when an excess of MDA-LDL was added to the medium.

Unlike native LDL, Di-1-MDA-LDL was taken up only by some of the cells of the culture (Fig. 3c, d). These cells differed from hepatocytes in shape and size and were either large and irregularly shaped or small and elongated. Testing for specificity of uptake of Di-1-MDA-LDL showed that a 20-fold excess of unlabeled MDA-LDL completely prevented the appearance of the stain. A similar effect was observed when an excess of modified albumin, but not native LDL, was added to the medium.

The results are thus evidence that receptors for native LDL are expressed and function actively on the surface of human liver cells in primary culture. The results of visual analysis of binding and uptake of LDL are in agreement with the results of other investigations conducted on rat liver cells [10, 14] and adult human liver cells [9], using radioactively labeled LDL. If the particular features of the distribution of LDL labeled with a radioactive isotope in patients and healthy subjects are taken into consideration [7], it can be concluded that a primary culture of human liver cells is an objective model with which to study expression of receptors for LDL.

The use of visual analysis helped to explain the role of hepatocytes in the utilization of modified LDL. Unlike in earlier investigations [10, 14], we found no evidence that hepatocytes are involved in the uptake of MDA-LDL. Monitoring the shape and size of cells accumulating Di-1-MDA-LDL enabled hepatocytes to be excluded from the list of cells containing "scavenger" receptors, and recognizing modified LDL. Among the most likely claimants for the role of cells responsible for MDA-LDL utilization, the Kupffer cells and endothelial cells of the liver must be mentioned [4, 10, 14]. The results suggest that receptors for MDA-LDL also are diffusely distributed over the surface of endothelial cells and are not concentrated into groups. During light microscopy, therefore, binding sites of MDA-LDL are not visualized on endothelial cells. A combination of fluorescent microspheres of FITC-labeled modified albumin with Di-1-MDA-LDL confirms the validity of this hypothesis.

In our view visual control is an essential condition for the study of the properties of cells in primary cultures, in which contamination with other cells is difficult to rule out.

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MORPHOLOGICAL ANALYSIS OF ACTIVITY OF MUCUS-PRODUCING STRUCTURES OF THE DUODENUM OF RATS FED WHEAT BRAN OF DIFFERENT PARTICLE SIZE

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It was shown previously that an increase in the cellulose content, in the form of addition of wheat bran, in the diet of laboratory rats for a sufficiently long period leads to an increase in the degree of development of the duodenal glands [3]. These experimental conditons also were shown to stimulate secretory activity of the duodenal glands and also of the goblet cells of the intestinal mucosa [4]. These changes correlated with a change in pH of the chyme toward the acid side. It was suggested that this state of affairs could be one cause of morphological and functional changes discovered. Meanwhile the role of the mechanical factor in the realization of the secretory response of the mucus-producing cells due to the passage of chyme containing fairly coarse bran particles along the intestine, cannot be ruled out. The duodenal glands are known to secrete not only in response to the introduction of hydrochloric acid solutions into the intestinal lumen, but also in response to direct mechanical stimulation of its inner surface [6]. The aim of this investigation was to assess the possible effect of the mechanical stimulation of the duodenal mucosa factor on the morphological and functional state of mucus-producing structures contained in it.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 62.7 g were used. For 3 months the animals, divided into three groups, with five in each group, received a diet identical in nitrogen content (10% protein by calorific value), identical in calorific value (468 kcal/100 g diet), and balanced with regard to mineral and vitamin composition, in unrestricted amounts; the main

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