with values characteristic of air breathing. Practically no differences were found in the values of the thicknesses studied under these conditions in adult animals and in young rats. In the course of the investigations no change in these parameters could be found in young animals on changing to inhalation of a hyperoxic helium–oxygen mixture ($P > 0.5$).

These investigations thus suggest that helium has a direct effect on ABB of the lungs which is practically independent of the O_2 concentration in the respiratory gas mixture and of the animals' age. It can be postulated on the basis of the electron-microscopic findings that among the various structures of ABB the capillary endothelium is that which is most sensitive to the action of helium–oxygen mixtures.

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EFFECT OF ADMINISTRATION OF LIPOSOMES DIFFERING IN CHOLESTEROL AND PHOSPHATIDYLCHOLINE CONTENT ON ULTRASTRUCTURE OF MYELIN-LIKE PARTICLES IN THE MOUSE LIVER

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KEY WORDS: liposomes; myelin-like particles; cholesterol; liver.

The idea of using liposomes as carriers of drugs and physiologically active compounds *in vivo* in order to correct the cell metabolism of target organs is bound up, as has been discovered, with the solution of more difficult problems than was hitherto considered. The main obstacle and the way to achieving the presumed final effect of liposomes, even if these particles preserve their stability during circulation, is the ingestive function of the liver, leading to rapid removal of lipid vesicles from the blood flow [2, 6], and this is accompanied by accumulation of myelin-like structure in the cells and extracellular spaces of the liver [4, 8, 13]. However, all the electron-microscopic evidence that these myelin-like structures are identical with liposomes is based on their external similarity, and considerable misgivings have been expressed [12].

In recent years, with improvements in methods of culture of the sinusoidal and parenchymatous cells of the liver, evidence has been obtained to suggest that its ingestive function includes not only the primary uptake of large liposomes by Kupffer cells, but also the trans-

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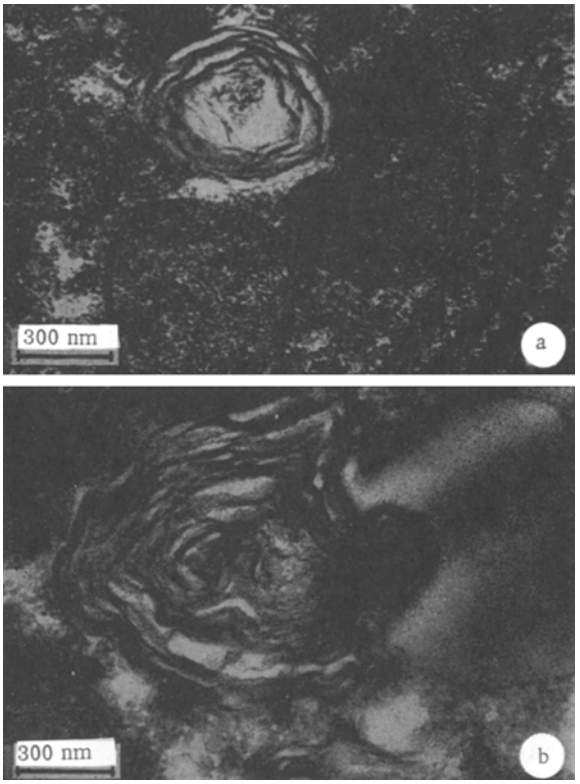

Fig. 1. Myelin-like particles with loosely packed membranes among elements of the endoplasmic reticulum in a hepatocyte. a) 2 min after injection of 100 μl of liposome preparation into portal vein. Ratio of phosphatidylcholine to cholesterol in liposomes 2:1; b) 10 min after injection of 100 μl of liposome preparation into caudal vein. Ratio of phosphatidylcholine to cholesterol in liposomes 7:2.

fer of a certain proportion of the liposomal lipids into hepatocytes [10]. Meanwhile the ability of liposomes to undergo biodegradation after entry into the bloodstream or to exchange their components (phosphatidylcholine and cholesterol) with lipoproteins and cell membranes also is well known [7, 12].

Taking these new aspects of liposome—cell relations into account, it was decided to attempt not only to obtain information on the ultrastructure of the myelin-like particles, but also to compare the distribution of free cholesterol in the microcirculation of the liver, including the sinusoids and Disse's spaces, before and after loading the blood stream with liposome preparations containing cholesterol and phosphatidylcholine in different proportions and in different doses, and administered in different ways (through the portal or caudal vein).

Although electron histochemistry is a highly specific [3] method of detecting free, nonesterified cholesterol in the liver by the cholesterol—digitonin reaction, and has received wide approbation [5, 11, 14], nevertheless this method has not hitherto been used to study the mechanisms of liposome—cell interaction. In turn, the usual method of transmission electron microscopy is unsuitable for this purpose, for the necessary dehydration is accompanied by almost total extraction of cholesterol from the preparation. Accordingly, comparative data on myelin-like structures and the distribution of cholesterol in the liver after liposome adminstration are not to be found in the literature.

EXPERIMENTAL METHOD

Male CBWA albino mice weighing 20-22 g were used. The animals ceased to be fed 24 h before the experiment began. Preparations of large multi-lamellar liposomes, 600-800 nm in diameter and containing 0.01 mg lipids/μl physiological saline, were obtained by the standard method [2] from a mixture of ovolecithin and cholesterol in molar proportions of 2:1 or 7:2, and injected into the portal or caudal vein in doses of 10 and 100 μl. Control animals re-

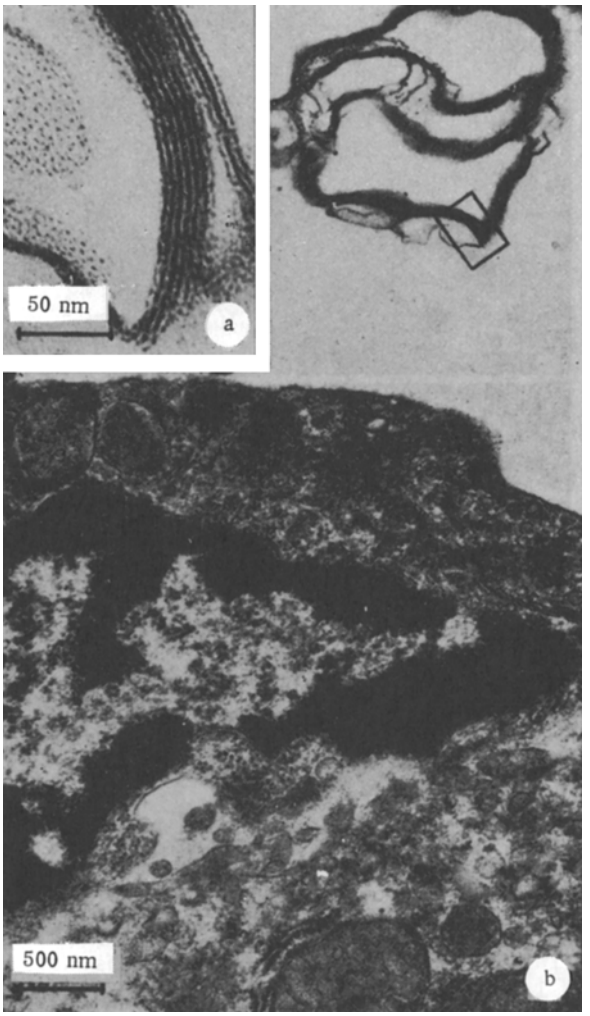

Fig. 2. Myelin-like particle with densely packed membranes in lumen of sinusoid, 2 min after injection of 100 µl of lipsome preparation into portal vein. Ratio of phosphatidylcholine to cholesterol in liposomes 7:2 (b). Photomicrograph in top left hand corner shows fragment of wall of particle under high power (a).

ceived corresponding doses of pure physiological saline by the same route. Samples of liver were taken 1, 2, and 3 min after a single injection of liposomes into the portal vein or 10 and 60 min after their injection into the caudal vein. The order of preparation of the solutions for cholesterol–digitonin incubation, and the sequence and duration of the operations when processing the pieces of liver for electron microscopy, and also the conditions of their embedding in resin were all described previously [1]. Ultrathin sections were cut on an LKB III Ultratome (Sweden) stained with uranyl acetate and lead citrate, and examined in the JEM-100B electron microscope (Jeol, Japan).

EXPERIMENTAL RESULTS

According to data in the literature, liposomes identified in the form of myelin-like structures have been found in sinusoids, Kupffer cells, and hepatocytes [4, 8, 13, 15]. Meanwhile the study of the distribution of liposomes tagged with peroxidase showed [15] that regions of distribution of this marker on the surface of or inside the liver cells do not coincide with the locations of myelin-like structures. Accordingly the first step of the present investigation was to study the state of these structures under control and experimental conditions, but without the use of a histochemical reaction.

Eventually it was possible to distinguish two categories of myelin-like structure which differed in the packing density of the membrane in the corresponding structures (Fig. la, b; Fig. 2). Myelin-like structures with loosely packed membranes, although rare in the liver

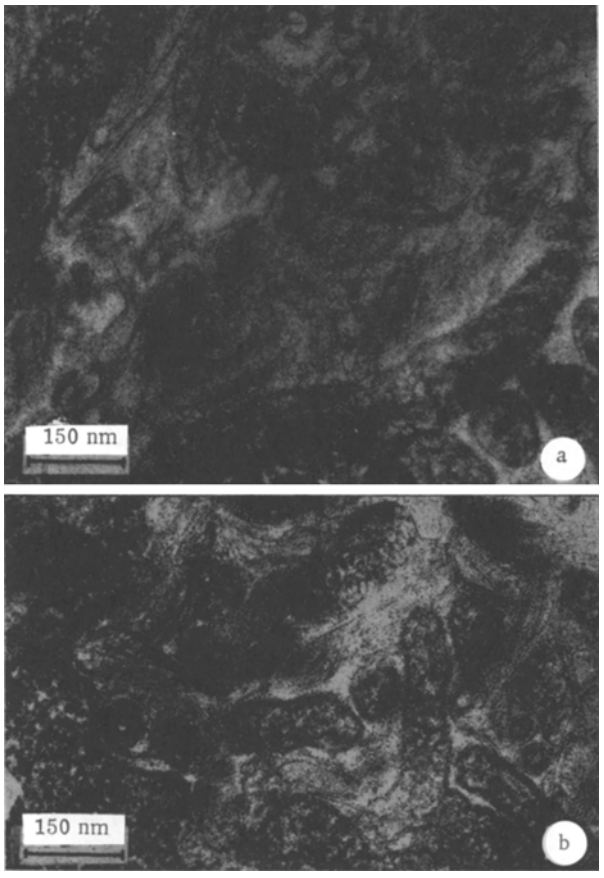

Fig. 3. Accumulation of cholesterol-containing granules in microcirculation of the liver, 2 min after injection of 100 µl of liposome preparation into portal vein. Ratio of phosphatidylcholine to cholesterol in liposomes 2:1. a) Sinusoidal space, b) Disse's space.

of the control animals, were an essential component of the intra- and intercellular spaces, including hepatocytes, sinusoids, and canaliculi.

Loading the bloodstream with liposomes, irrespective of the method of injection, the dose of the preparation, and the length of time the liposomes remained in the blood, and also irrespective of the ratio of phosphatidylcholine to cholesterol in the particles, gave a stable and lasting effect of an increase in the number of myelin-like structures in the hepatocytes (Fig. 1a, b) as early as 2-3 min, and in the canaliculi — 10 min after injection of the liposomes. This effect was accompanied by an increase in the number of lipid inclusions, which were mainly closely associated with the myelin-like structures (Fig. 1b). Meanwhile no evidence of accumulation of myelin-like structures in the microcirculation or surrounding cells (endothelial and Kupffer cells, Ito cells, pit cells, and macrophages) or on the surface of the hepatocytes, could be found.

The data described above must evidently be interpreted as further confirmation of the conclusion drawn by many workers that liposomes, formed from phosphatidylcholine and cholesterol in the ratio of 7:2 or 2:1, undergo total biodegradation immediately on entering the bloodstream. From this point of view it would be reasonable to suggest that the end or intermediate products of destruction of the liposomal membranes are the direct or indirect precursors of lipid drops and of myelin-like structures arising in hepatocytes in response to injection of lipid vesicles.

Another category of multilamellar concentric shapes (Fig. 2), which we described as myelin-like structures with dense packing of their membranes, could perhaps have been liposomes, for they were found in sinusoids for 2-3 min after injection of the preparation. Despite the close apposition of the osmiophobic and osmiophilic layers, all particles to some degree or other were deformed and had regions of separation of the layers or splitting of the

wall. In all cases the initial points and the whole zone of separation ran strictly along the osmiophilic layer (Fig. 2), i.e., along the aqueous phase between neighboring bilayers.

The description given above does not contradict the thesis that liposomes undergo rapid biodegradation on contact with blood, but nevertheless the problem of the degree of degradation of these particles and the fate of the products of such degradation is not yet completely clear.

The results of a study of the distribution of cholesterol, as one component of liposomal membranes, in the microcirculatory system of the liver by means of the cholesterol–digitonin reaction sheds some light on this problem.

The reaction is based on the ability of cholesterol to react with digitonin and to form a cholesterol–digitonin complex, insoluble in dehydrating agents, which was identified in ultrathin sections in the intracellular and extracellular spaces of various organs and tissues, including the liver, aorta, kidney, blood cells, and other objects, where it was found in the form of a few polymorphic granules [1, 3, 5, 11, 14]. Small numbers of similar granules also were found in sinusoids of the liver in mice of the control group in the present investigations. Granules either were not seen in Disse's spaces or they occurred only episodically.

On the other hand, extensive aggregations of granules of cholesterol–digitonin complex were found (Fig. 3a) only 1-2 min after injection of the liposomes, and irrespective of their cholesterol content, in many sinusoids, especially in those located in the zone of terminal branches of the portal venules (zone 1 or the acinus, according to Rappoport's terminology [9]). Within the confines of the sinusoid, accumulation of cholesterol was maximal in areas of the fenestrated endothelium, and in some cases passage of the granules through pores (fenestrae) into Disse's space could be observed. After 2-3 min aggregations of granules were found not only in the sinusoids, but also in Disse's space (Fig. 3b), where these particles had a unified structure and were in close contact with the microvillus membrane of the hepatocyte. As was stated above, during this period a parallel process of infiltration of hepatocytes with lipid drops and myelin-like structures took place. Unlike in hepatocytes, in the Kupffer cells or on their surface the presence of myelin-like structures could not be detected, and the cells themselves were spatially separated, with regions of accumulation of cholesterol-containing granules. It must be emphasized that the effect of accumulation of cholesterol-containing granules in the sinusoids and Disse's space was more or less stable during the first 3 min after injection of the preparation, whereas infiltration of the hepatocytes was more prolonged and was still maintained at a high level 60 min after the beginning of the epxeriment. These characteristics were pronounced regardless of the dose of liposome preparation injected or the ratio of cholesterol to phosphatidylcholine in the liposome.

The data described above show that among the heterogeneous population of liver cells the hepatocytes play a direct and principal role in regulation of the liposomal lipid level and, in particular, of the blood cholesterol level. That is why attempts to create long-living liposomes purely by reducing the diameter of the lipid vesicles down to that of the fenestrae and increasing the cholesterol content of the liposomal membranes are doomed to failure.

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VESICULAR PEROXIDASE TRANSPORT BY EPITHELIAL CELLS OF THE ADULT RAT SMALL INTESTINE

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KEY WORDS: ultrastructures; absorption; protein; enterocyte; pinocytosis.

The ability of proteins or their fragments to penetrate through the epithelium of the mucosa of the small intestine has been discussed for a long time. There is now no doubt that in the early postnatal period in mammals proteins, in the native form and, in particular, immunoglobulins can pass through the apical membrane of enterocytes in the small intestine, by entering an epithelial cell by pinocytosis and being transported through it in the composition of vesicles [4, 8, 14]. With the development of luminal digestion, on the transition to definitive feeding the transport mechanism is considered to be reorganized. Instead of pinocytosis, a system of transmembrane transfer of nutrients, which are subsequently transported through the cytosol of the enterocyte, comes into action. Meanwhile the mucosa of the small intestine acquires the property of not allowing proteins to pass through in the unsplit form [1].

It is generally considered that in adult individuals whole protein molecules are not absorbed under normal conditions. However, this does not rule out the possibility that an extremely small amount of unsplit protein may enter the bloodstream on account of a disturbance of the digestive system or some other defect of the absorption mechanism. Very often this is accompanied by an allergic reaction as a result of the entry of a foreign protein into the body [9, 11]. Nevertheless, numerous investigations, mainly physiological, have shown that in adult life native food proteins can pass through the epithelial barrier of the small intestine [10, 13]. The mechanism by which they enter the bloodstream is not yet clear, but it has been suggested that vesicular transport may be that mechanism [5]. The hypothesis has been put forward that not only proteins, but also nutrients of other nature (lipids, carbohydrates, pharmacological substances, and so on) are absorbed in the small intestine by pinocytosis [2].

The aim of the present investigation was an electron-microscopic study of protein (peroxidase) transport in the mucosa of different parts of the small intestine in adult rats within comparatively short time intervals after introduction of the protein into the gastrointestinal tract.

EXPERIMENTAL METHOD

Two groups of sexually mature male Wistar rats weighing 250-300 g were used (the animals were kept on the ordinary animal house diet, and before the experiment they were starved for 24 h but allowed water *ad lib.*). Animals of group 1 received 1 mg peroxidase and 20 mg bovine serum albumin in 2 ml of physiological saline by means of a gastric tube (horseradish peroxidase RZ-1.62 was from Sigma, USA). The animals of group 2 received an injection of about 0.5 ml of a solution of peroxidase (0.5 mg/ml) in Hanks' buffer, pH 7.4, into a 5-cm segment of jejunum, isolated between ligatures under superficial ether anesthesia. At intervals of 20 and 40 min after injection of peroxidase into the gastrointestinal tract, in the animals of group 1 segments of the duodenum and proximal part of the ileum were resected (the rats were anesthetized with hexobarbital 10 min before laparotomy), and in the animals of group

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