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Transfer of retinol from parenchymal to stellate cells in liver is mediated by retinol-binding protein

(vitamin A storage/liver cells/perisinusoidal stellate cells/fat-storing cells)

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ABSTRACT **Newly absorbed chylomicron remnant retinyl ester is endocytosed by parenchymal liver cells, and retinol is subsequently transferred to perisinusoidal stellate cells in liver. In the present study we have used several approaches to elucidate the mechanism for the paracrine transfer of retinol between liver parenchymal and stellate cells. In one series of experiments, chylomicrons labeled with [^{3}H]retinyl palmitate or with retinyl [^{3}H]palmitate were injected intravenously into rats. It was shown that the retinol as well as the palmitate moiety were initially taken up in parenchymal liver cells. However, only the retinol moiety was detected in stellate cells, indicating that the retinyl ester is hydrolyzed before retinol is transferred to stellate cells. It is well known that parenchymal liver cells secrete retinol bound to retinol-binding protein (RBP), and we have recently found that stellate cells do have RBP receptors. Here we report that antibodies against RBP completely block the transfer of retinol from parenchymal to stellate cells. These findings indicate that following uptake of chylomicron remnant retinyl ester in parenchymal cells, the retinyl ester is hydrolyzed, and retinol secreted from parenchymal cells on RBP is taken up by stellate cells by means of RBP receptors.**

Following absorption by enterocytes in the intestine, retinyl esters are transported in chylomicrons and their remnants to the parenchymal liver cells. Some years ago, we reported that most of the retinol taken up by parenchymal cells may subsequently be transferred to perisinusoidal stellate cells in liver (1, 2). This transfer of retinol from parenchymal to stellate cells in liver has recently been confirmed in two additional laboratories (3, 4).

The transfer of newly absorbed retinol from parenchymal to stellate cells in liver seems to be specific. Neither cholesterol (1) nor vitamin D_3 (5), both of which are taken up by liver parenchymal cells from chylomicron remnants, is transferred to any of the nonparenchymal liver cells.

In a series of experiments (reviewed in ref. 6) Wake used morphological techniques to study the distribution of retinol in liver cells. His work clearly showed that lipid droplets in stellate cells have the ability to store large amounts of retinol. The same conclusion is also drawn from recent experiments in which the various liver cells were isolated from rats storing large amounts of retinol in the liver (7). It has, however, been questioned whether stellate cells store retinol when it is administered in a physiological form, and it has also been speculated that retinol enters the stellate cells only when intake of the vitamin is excessive (8, 9).

Recent evidence has, however, documented that stellate cells also are actively involved in retinol storage under normal conditions. By separating the various liver cells from normal rats with several methods yielding high recoveries of cells, we have recently found that stellate cells contained 28–34 nmol of retinol per 10^6 cells and that parenchymal cells contained 0.5–0.8 nmol of retinol per 10^6 cells (10). Endothelial cells and Kupffer cells contained negligible amounts of retinol. When converting these values to whole liver, it follows that as much as 80% or more of the total retinol in liver is present in the stellate cells (10).

This storage of retinyl esters in stellate cells may be due to the observed transfer of newly absorbed retinol from parenchymal to stellate cells. The aim of the present work was to study the mechanism for this transfer of retinol from parenchymal cells to stellate cells in liver.

MATERIALS AND METHODS

Materials. Antibodies raised in sheep against rat retinol-binding protein (RBP) (11) were kindly provided by DeWitt S. Goodman (Columbia University, College of Physicians and Surgeons, New York). Collagenase (type 1) and all-*trans*-retinol were from Sigma. [11,12(n)-^{3}H]Retinol (all-*trans*) (43 Ci/mmol; 1 Ci = 37 GBq) and [1(n)-^{3}H]palmitoyl chloride (10 mCi/mmol) were obtained from Amersham International, Buckinghamshire, U.K. Retinyl [^{3}H]palmitate was synthesized according to Huang and Goodman (12).

Lymph Collection. The operation was performed as described (13). One hundred microcuries of [^{3}H]retinol (600 international units) dissolved in 0.6 ml of intralipid was given in some experiments by gastric intubation to rats whose cysterna chyli had been cannulated. When labeled lymph was ultracentrifuged, >90% of the radioactivity was recovered in the chylomicron fraction ($\rho < 1.006$ g/ml) as retinyl palmitate.

Labeling of Chylomicrons with Retinyl [^{3}H]Palmitate. Chylomicrons were labeled with retinyl [^{3}H]palmitate as described for cholesteryl oleate (14). Two portions of 50 μl (0.42 μmol) of [^{3}H]retinyl palmitate in acetone were added to 0.5 ml of a human plasma fraction ($\rho > 1.24$ g/ml) containing the cholesteryl ester transfer protein. Following evaporation of the acetone with N_2, the solution was incubated at 37°C for 10 min. Then, chylomicrons (500 μg of protein) were added, and this mixture was incubated for 6–7 hr at 37°C. The lipoproteins were reisolated by ultracentrifugation (15).

Isolation of Liver Cells. Livers of male Wistar rats (250–300 g) were perfused by a collagenase technique (16), and parenchymal liver cells were prepared from the total liver cell suspension by differential centrifugation (17). To get rid of contaminating nonparenchymal cells, the parenchymal cell suspension was furthered purified by centrifugal elutriation (2) at 1200 rpm. Cells were introduced into the chamber at a flow rate of 20 ml/min, and the parenchymal cells were washed out at 45 ml/min. The final parenchymal cell suspension was >99% pure, as judged by light and fluorescence microscopy (2).

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Abbreviation: RBP, retinol-binding protein.

Nonparenchymal liver cells were isolated by differential centrifugation of the total cell suspension as described (17). The nonparenchymal cells were washed free of cell debris by centrifugation in 20% Nycodens (18). The nonparenchymal cell fraction was devoid of parenchymal cells, as judged by light microscopy.

***In Situ* Perfusions of Livers.** Prior to the *in situ* perfusion, parenchymal liver cells were loaded *in vivo* with radioactive retinoid. Ten minutes after injection of chylomicron [^{3}H]retinyl esters into the right femoral vein, the hepatic vein was cannulated and the liver was perfused *in situ*. The perfusion buffer consisted of 8.0 g of NaCl, 0.4 g of KCl, 0.06 g of $Na_2HPO_4 \cdot 2H_2O$, 0.047 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2.05 g of $NaHCO_3$, and 0.15 g of $CaCl_2 \cdot 2H_2O$ dissolved in H_2O to a final volume of 1000 ml. The perfusion buffer was gassed with 95% O_2/5% CO_2 during perfusion. After different periods of time, a liver lobe was tied off, and parenchymal and nonparenchymal cells were prepared as described above. When converting radioactivity in aliquots of cell suspension to total liver values, we assumed that the liver contains 125 × 10^6 parenchymal cells per g of wet weight and 65 × 10^6 nonparenchymal cells per g of wet weight (19). The total weight of liver was assumed to be 4.1% of total body weight (1).

RESULTS

Transfer of Retinol from Parenchymal to Stellate Cells. Chylomicron retinyl esters labeled in the retinol moiety were injected intravenously into five rats, and the radioactivity in total liver, parenchymal, and nonparenchymal liver cells was determined after different periods of times (Fig. 1*A*). In accordance with previous observations (1), we observed an initial uptake of radioactivity in parenchymal cells and a subsequent transfer of radioactivity to nonparenchymal cells.

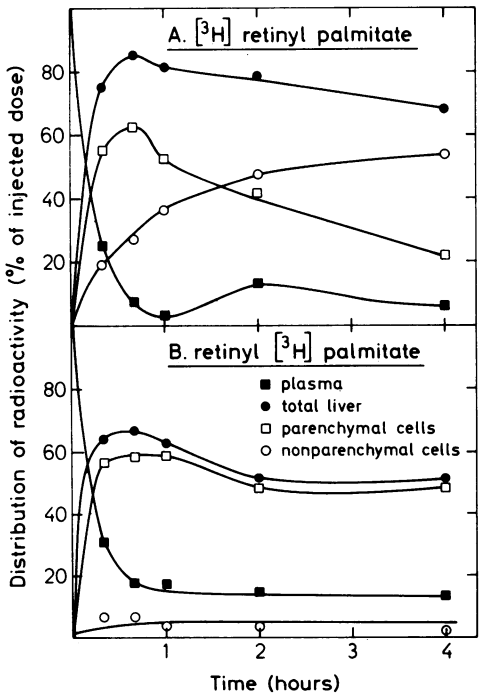

FIG. 1. Transfer of retinyl ester between parenchymal and nonparenchymal liver cells. Chylomicrons labeled with [^{3}H]retinyl palmitate (*A*) or retinyl [^{3}H]palmitate (*B*) (1–2 μCi, 1–5 mg of triacylglycerol) were injected intravenously into five rats each, and the radioactivity in plasma (■), total liver (●), parenchymal liver cells (□), and nonparenchymal (○) liver cells was determined after different periods of time.

Is the Palmitate Moiety Transferred to Stellate Cells? To test whether the chylomicron retinyl esters are hydrolyzed before the retinoid is transferred to stellate cells, we injected chylomicron retinyl esters labeled in the palmitate moiety intravenously into five rats and determined the radioactivity in total liver, parenchymal, and nonparenchymal liver cells after different periods of time (Fig. 1*B*). After 20–60 min, about two-thirds of the injected dose was recovered in the liver. More than 50% of the tracer was still recovered in the liver when rats were sacrificed at 2 and 4 hr. At all times studied, >85% of the total liver radioactivity was recovered in the parenchymal cell fraction, and only small amounts of fatty acid radioactivity were observed in nonparenchymal liver cells. As only negligible amounts of labeled palmitate were transferred to the stellate cells, this experiment suggests that the chylomicron retinyl esters are hydrolyzed before they leave the parenchymal cells.

Transfer of Retinol to Stellate Cells in Livers Perfused *in Situ*. To obtain more insight into the mechanism for transfer of retinol between liver cells, we also investigated whether retinol was transferred to nonparenchymal stellate cells when the liver was perfused *in situ*.

The parenchymal cells were loaded *in vivo* with radioactive retinoid as described in *Materials and Methods*. Livers were then perfused (noncirculating) *in situ*, and liver cells were isolated after various periods. The result, presented in Fig. 2, shows that a redistribution of radioactivity occurred in the liver cells during the *in situ* perfusion. The radioactivity decreased in parenchymal cells and increased in nonparenchymal cells. At the start of the *in situ* perfusion, about 25% of the recovered dose was found in nonparenchymal cells, whereas after 60 min of perfusion, about 60% of the recovered radioactivity was found in nonparenchymal cells. This result shows that retinol taken up by parenchymal cells in chylomicron remnants is transferred to stellate cells when the liver is perfused *in situ*, suggesting that the transfer *in vivo* does not involve the general circulation.

Antibodies Against RBP Inhibit the Transfer of Retinol to Stellate Cells. It has been clearly shown that parenchymal liver cells synthesize and secrete RBP (20). Furthermore, we have recently shown that stellate cells take up the retinol–RBP complex when injected into rats (21, 22). To test the hypothesis (23, 24) that RBP mediates the transfer of retinol

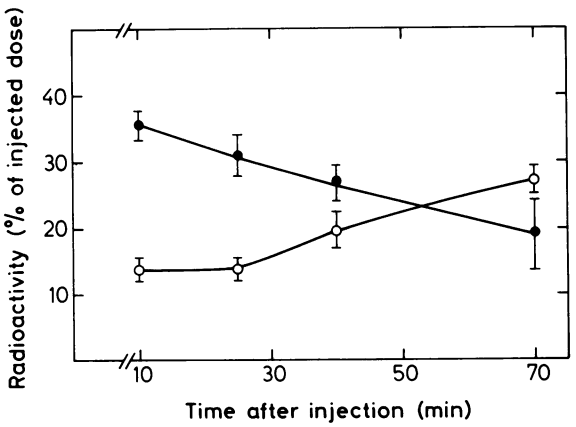

FIG. 2. Transfer of retinol from parenchymal cells to nonparenchymal cells in perfused liver. Ten minutes prior to the *in situ* perfusion, parenchymal liver cells were loaded *in vivo* by injection of chylomicron [^{3}H]retinyl esters (1–2 μCi, 1–5 mg of triacylglycerol) into the right femoral vein. The liver was perfused *in situ* with a perfusion buffer containing 1 mM $CaCl_2$. After different periods of time (three rats at each time point), a liver lobe was tied off for determination of total liver radioactivity, and parenchymal (●) and nonparenchymal (○) cells were prepared from the rest of the liver by collagenase perfusion.

from parenchymal cells to stellate cells, we studied the ability of polyclonal antibodies against RBP to inhibit the transfer of retinol.

Following loading of parenchymal cells *in vivo*, the liver was perfused *in situ* (recirculated) with a buffer containing an excess of antibodies against RBP. Fifty milliliters of perfusion buffer contained 1 ml of antiserum against RBP. One milliliter of antiserum will precipitate about 500 μg of rat RBP (11). Hence, the amount of antibodies present in the perfusate was more than adequate to precipitate all of the RBP found in the total liver (25). Another reason to use this high concentration of antibodies was to reduce any potential uptake of retinol–RBP–IgG by means of Fc receptors on Kupffer cells (26).

The content of radioactivity (mean ± SD) recovered in parenchymal and nonparenchymal cells after 10 min of *in vivo* loading was 352 ± 30 cpm per 10^6 cells (n = 3) and 89 ± 23 cpm per 10^6 cells (n = 3), respectively (Fig. 3*A*). Following 40 min of recirculated perfusion *in situ*, the radioactivity (mean ± SD) recovered in parenchymal cells was reduced to 184 ± 36 cpm per 10^6 cells (n = 3), whereas the radioactivity (mean ± SD) in nonparenchymal cells increased to 199 ± 64 cpm per 10^6 cells (n = 3) (Fig. 3*A*), showing a transfer of retinol to nonparenchymal cells during the perfusion. When antibodies against RBP were included during the *in situ* perfusion (Fig. 3*B*), no increase in radioactivity was observed in nonparenchymal cells. On the contrary, the radioactivity (mean ± SD) in nonparenchymal cells was reduced to 48 ± 22 cpm per 10^6 cells (n = 3). A corresponding increase in radioactivity was instead observed in the perfusion buffer from 118,900 cpm in controls to 231,650 cpm when antibodies against RBP were included (mean of three experiments). As the transfer of retinol from parenchymal to nonparenchymal stellate cells was completely blocked in the presence of antibodies against RBP, we suggest that the transfer of retinol is mediated by RBP.

In control experiments (three rats), we included 1 ml of preimmune rabbit serum in the perfusion buffer. The nonspecific antiserum had no effect on the transfer of retinol from parenchymal to nonparenchymal stellate cells.

DISCUSSION

In this study we have injected chylomicron retinyl esters labeled either in the retinol or the palmitate moiety intravenously into rats and determined radioactivity in liver parenchymal and nonparenchymal cells after different periods of time. These experiments showed that little of the labeled palmitate was transferred to the stellate cells, suggesting that the chylomicron retinyl esters are hydrolyzed before they leave the parenchymal cells. This result is in agreement with a recent report from Blaner *et al.* (5), in which a nondegradable retinyl ether analog was injected into the rats (in association with chylomicrons). They did not detect any increase in radioactivity from the ether analog in stellate cells and concluded that hydrolysis is a prerequisite for transfer of retinol to stellate cells.

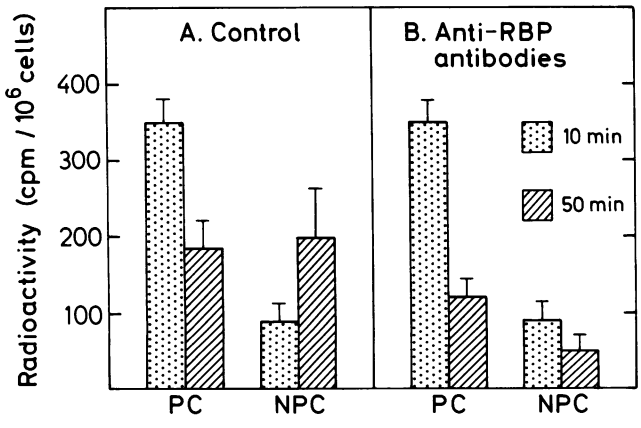

FIG. 3. Effect of anti-RBP antibodies on transfer of retinol from parenchymal cells (PC) to nonparenchymal cells (NPC). Following loading of parenchymal cells *in vivo* by intravenous injection of [^{3}H]retinyl ester-labeled chylomicrons (1–2 μCi, 1–5 mg of triacylglycerol), the liver was perfused *in situ* (recirculated) for 10 or 50 min with a control buffer (*A*) or a buffer containing an excess of antibodies against RBP (*B*). Fifty milliliters of perfusion buffer contained 1 ml of antiserum against RBP. Each value represents the mean ± SD of three liver perfusions.

The structure of RBP and the synthesis and secretion of RBP from the parenchymal cells have been studied in great detail in the laboratories of P. A. Peterson (Uppsala) and D. S. Goodman (New York) (reviewed by Goodman in ref. 20). Recent results from research in our laboratory (21, 22) have shown that stellate cells in liver accumulate retinol and RBP from the retinol–RBP complex in plasma. Based on these results, we speculated (23, 24) that RBP mediated the paracrine transfer of retinol from parenchymal to stellate cells (1–4, 27). This hypothesis was tested directly in the present study by determining the ability of antibodies against RBP to reduce the transfer of newly absorbed retinol from parenchymal to stellate cells. When we included antibodies against RBP in the perfusate, the transfer of retinol to stellate cells was completely blocked, showing that RBP mediates the paracrine transfer of retinol from parenchymal to stellate cells.

These findings shed some light on the hepatic handling of newly absorbed retinol: following uptake and hydrolysis of the retinyl esters in parenchymal cells, retinol bound to RBP is secreted from parenchymal cells and taken up by stellate cells by means of RBP receptors.

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