Clearance by the Liver in Cirrhosis. II. Characterization of Propranolol Uptake with the Multiple-indicator Dilution Technique

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We studied the steady-state hepatic extraction and single-pass hepatic uptake of propranolol in isolated perfused livers from normal rats and compared these values with those of rats with carbon tetrachloride–induced cirrhosis, rats treated with chlorpromazine (an inhibitor of propranolol metabolism) and rats with acute liver injury. The kinetics of propranolol transport in the liver were characterized by means of the multiple-indicator dilution technique, and estimates of cellular influx, efflux and sequestration rate constants were obtained with a computer fit to the model of Goresky. The outflow pattern of propranolol in the hepatic veins was then resolved into throughput material, which had swept past the hepatoyctes along with albumin, and returning material, which had entered the cells but returned in the outflow after escaping metabolic sequestration. The steady-state extraction of propranolol was significantly decreased in the three experimental groups compared with that in controls, but the outflow profile differed within each group. In cirrhotic animals, influx was markedly decreased and the sequestration rate constant remained unchanged; most of the propranolol in the outflow consisted of throughput material. In rats treated with chlorpromazine, the sequestration rate constant was decreased, and propranolol in the outflow was mainly returning material. In rats with acute liver injury, both influx and sequestration rate constants were decreased. Indicator dilution curves for nonsequestered tracers showed a decreased transit time for red blood cells and abnormal diffusion of albumin and sucrose into the space of Disse in cirrhotic rats compared with the other groups. These results demonstrate that the decreased hepatic extraction of propranolol in cirrhotic rats is due to impaired cellular influx rather than to reduced metabolism. We speculate that the limitation of propranolol cellular entry in cirrhosis is related to restriction of diffusion of protein-bound propranolol into the space of Disse, to the presence of small intrahepatic shunts or to both (HEPATOLOGY 1993;18:823-831.)

Cirrhosis leads to impairment of the clearance of several drugs (1, 2). Hepatic clearance involves three sequential steps: delivery of the drug to the liver (i.e., hepatic blood flow), uptake by hepatocytes and irreversible elimination by way of metabolism or biliary secretion. Previous work has indicated that drug delivery to the liver is not rate limiting for clearance in severe cirrhosis and that changes in hepatic blood flow do not account for reduced clearance (3, 4). Several observations also suggest that decreased metabolism cannot fully account for the impaired clearance of drugs with high extraction ratios in cirrhosis (5-8). Taken together, these data suggest that anomalies of hepatic uptake may be present and play a role in explaining reduced clearance in cirrhosis.

Anatomical changes of the hepatic microcirculation, which have been described in liver disease, could progressively limit the uptake of material by hepatocytes. These include capillarization of hepatic sinusoids, collagenization of the space of Disse and intrahepatic shunting (9-13). In a rat model of cirrhosis, the extravascular space accessible to albumin was shown to be decreased (14, 15); consequently, the access of protein-bound substrates to their site of metabolism could be restrained and lead to reduced clearance, irrespective of the metabolic capacity of the hepatocytes.

To evaluate the role of anomalies of uptake in impaired drug disposition, we undertook studies using propranolol as a model drug in rats with CCl_4-induced cirrhosis. The uptake of propranolol was characterized with the multiple-indicator dilution curve (MIDC) technique (16) and related to its steady-state extraction in isolated perfused liver preparations. Anomalies of propranolol disposition in cirrhotic rats were contrasted with those of rats with acute liver injury and rats treated with chlorpromazine, an inhibitor of propranolol metabolism.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Canada Ltd., Saint-Constant, Quebec, Canada) with a starting weight of 250 gm were used throughout the study. Animals were cared for in accordance with the standards and procedures outlined in the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals." All animals were kept under a standard light-dark cycle and fed chow *ad libitum* (Ralston Purina Co., St. Louis, MO). Four groups of rats were studied: (a) control rats, (b) rats with cirrhosis, (c) rats with acute liver injury and (d) control rats exposed to chlorpromazine, an inhibitor of propranolol metabolism. Micronodular cirrhosis was induced with a 12- to 20-week course of CCl_4-phenobarbital as described by Varin and Huet (14). Animals were studied 10 to 20 days after the last dose of CCl_4. Acute liver injury was induced with a single dose of CCl_4 (1.75 ml/kg) administered intragastrically, and animals were studied 24 hr later. In the chlorpromazine group, control animals were used and a bolus of chlorpromazine was added to the reservoir of the perfused liver preparation 5 min before propranolol administration, as described previously (8).

Materials. For MIDC studies, the following substances were used: ^{51}Cr-labeled bovine RBCs prepared with $Na_2{}^{51}CrO_2$ (290 μCi/mg) (Frosst, Kirkland, Quebec, Canada) and [^{14}C]sucrose (3.7 mCi/mmol) and [^{3}H]propranolol (16 mCi/mmol), purchased from Du Pont–New England Nuclear (Boston, MA); ^{99m}Tc-labeled albumin, obtained by adding ^{99m}Tc (St-Luc Hospital) to human serum albumin–stannous chloride complex (Frosst); and ^{141}Ce-labeled microspheres (15.5 ± 0.1 μm diameter; Du Pont–New England Nuclear). Other materials have been described previously (8).

Experimental Design. Each set of experiments consisted of measuring, in the isolated perfused liver of each animal, (a) steady-state extraction of propranolol, (b) single-pass hepatic uptake of propranolol and liver microcirculation parameters by means of MIDC methodology and (c) presence of intrahepatic shunts, by means of labeled microspheres.

Isolated Perfused Liver and Propranolol Extraction. Rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally, and the liver was isolated and perfused through the portal vein in a closed recycling system. Details of the procedure have been described previously (8, 17). Perfusion flow was set at 20 ml/min. The perfusate consisted of Krebs-Henseleit buffer containing 20% (vol/vol) prewashed bovine RBCs, 1 gm/L glucose, 40 gm/L albumin and 2 gm/L α_1-acid glycoprotein (AAG). The reservoir volume was 250 ml. A bolus of propranolol (25 μg) was added to the reservoir; it was followed by a continuous infusion adjusted to maintain a steady-state propranolol concentration of about 100 ng/ml. After an initial 15- to 20-min equilibration period, blood samples were drawn simultaneously from the inflow and outflow for measurement of propranolol concentration every 3 min for 15 min. Propranolol concentration was determined on HPLC as previously described (8). Steady-state propranolol extraction *(E)* was calculated from the propranolol concentrations measured in the inflow *(Ci)* and outflow *(Co)* as E = (Ci − Co)/Ci. The protein binding of propranolol was measured with equilibrium dialysis of perfusate against buffer (17). The free fraction of propranolol was calculated as propranolol buffer concentration/(perfusate concentration + buffer concentration).

MIDCs. To avoid the bias of non-steady-state conditions, we obtained propranolol dilution curves after injecting tracer doses of [^{3}H]propranolol in the presence of a steady-state concentration of unlabeled propranolol (~100 ng/ml). Once blood samples were drawn for the measurement of propranolol extraction at steady state, the infusion of propranolol was maintained and the hepatic venous outflow was diverted. This created an open circuit for the collection of effluent after injection into the portal vein of a 0.1-ml bolus containing the following indicators: ^{51}Cr-labeled bovine RBCs (4×10^5 dpm), ^{99m}Tc-labeled albumin (6×10^5 dpm), [^{14}C]sucrose (2.5×10^5 dpm) and [^{3}H]propranolol (2×10^6 dpm) in a solution of Krebs-Henseleit buffer adjusted to a hematocrit matched to that of the perfusate and containing albumin (40 gm/L) and AAG (2 gm/L). The total hepatic venous outflow was collected in serial tubes at a rate of one tube per second for 80 sec for the control, acute liver injury and cirrhotic groups. In rats treated with chlorpromazine, additional samples of hepatic venous outflow were obtained at a rate of one tube per 10 sec for up to 15 min.

Three aliquots from each tube were taken: one for the determination of gamma activity (^{99m}Tc, ^{51}Cr), another for determination of ^{14}C activity (sucrose) and a third for the determination of unchanged propranolol after extraction with 1.5% isoamyl alcohol in n-heptane (18). The gamma activities were measured in a Beckman Gamma 8000 counter (Beckman Instruments Inc., Palo Alto, CA), and beta activities were assayed in a Beckman LS-8000 liquid scintillation counter with Ready Solv-HP; Beckman Instruments as the scintillation fluid. We corrected for quenching with an automated external standard method. Manipulation of samples and standards and appropriate crossover corrections for the gamma and beta activities have been described (12). The outflow activity (disintegrations per minute) of each indicator was divided by the amount injected to provide a normalized outflow fraction recovery per milliliter of blood. The dilution curves were corrected for catheter distortion and delay according to the method proposed by Goresky and Silverman (19).

The dilution curves for nonsequestered tracers (RBCs, albumin and sucrose) were analyzed according to Goresky's flow-limited model (16). To calculate the sinusoidal volume, (V_{sin}), we first obtained estimates of the catheter and large vessel transit time delays (t_o) and of the ratio of extravascular to vascular volume of distribution of diffusible tracers (γ) with computerized optimization program (20); t_o and γ were defined as the values yielding the least sum of squares of the deviations between the RBC curve and the diffusible tracer (albumin or sucrose) curve when the latter is transformed linearly to superimpose on the RBC curve. The optimization procedure was programmed in Turbo Pascal (Borland, Scotts Valley, CA) and Lotus 1-2-3 (Lotus Corp., Cambridge, MA) on a Hewlett Packard computer (Vectra 287 series; Hewlett Packard, Toronto, Ontario, Canada). Optimization was continued until least sum of squares of differentiation changed by less than 0.1% from one iteration to the next. We evaluated the result of the nonlinear fitting procedure by visual inspection of the fitted curve (21) and by measuring the coefficients of variation (22) and determination (23) of the fit. The mean transit time of each label was calculated with the method of Meier and Zierler (24), and transit time delay was subtracted from these values to provide estimates of the transit time of the labels through the sinusoids *(t)*. The distribution space for RBC (V_{sin}) was then determined as the product of t and perfusate flow rate, Q:

$$V_{sin} = Q \times t_{RBC} \ (ml \cdot gm^{-1} \ liver) \quad (1)$$

The extravascular volume *(EVV)* accessible to diffusible tracers was calculated as a model-independent parameter according to the transit time method (25) as

$$EVV_{dif} = Q \times (1 - Ht) \times (t_{dif} - t_{RBC}) \ (ml \cdot gm^{-1} \ liver) \quad (2)$$

where *Ht* is hematocrit and t_{dif} is the transit time of albumin or sucrose.

We analyzed the kinetic of the transport of unchanged

TABLE 1. Characteristics of *in situ* perfused rat livers

Characteristics	Controls (n = 13)	Cirrhosis (n = 12)	Acute liver injury (n = 11)	Chlorpromazine (n = 6)
Liver weight (gm)	17.1 ± 2.2[a]	18.1 ± 4.5	21.6 ± 2.9[b]	14.3 ± 2.9
Liver weight/body weight	0.030 ± 0.002	0.037 ± 0.008[b]	0.042 ± 0.002[b]	0.033 ± 0.002
Collagen content (μg/mg protein)	23.7 ± 3.2	53.3 ± 13.2[b]	22.6 ± 3.0	24.5 ± 5.7
Perfusate blood flow (ml/min)	20.9 ± 1.3	20.2 ± 0.8	20.3 ± 0.5	20.2 ± 0.3
Portal perfusion pressure (mm Hg)	10.4 ± 1.5	17.6 ± 4.7[b]	13.9 ± 2.8	10.9 ± 1.0
Bile flow (μl/min)	18.5 ± 4.0	11.0 ± 5.3[b]	9.3 ± 5.1[b]	18.8 ± 1.6
Oxygen consumption (μmol/min/gm liver)	1.85 ± 0.32	1.33 ± 0.24[b]	1.54 ± 0.24	1.92 ± 0.26
Intrahepatic shunting (%)	0.8 ± 1.7	1.9 ± 2.5	0.6 ± 0.8	NA

NA = not available.
[a]Data expressed as mean ± S.D.
[b]$p < 0.05$ compared with controls.

propranolol with a model that accounts for tracer diffusion, exchange and sequestration in the hepatic circulation (22, 26, 27). According to this model, the outflow profile of the extracted substance comprises two parts: a throughput component and a returning component. The throughput component represents material leaving in the outflow without having entered the hepatocytes. The returning component is the amount of unchanged propranolol that has entered the hepatocyte and subsequently emerges in the outflow, escaping cellular sequestration and metabolism.

The concentration of propranolol was related to the concentration of its appropriate reference, albumin, with the equation

$$C_{prop}[t] = e^{-k_1\Theta t/[1+\gamma]}C_{alb}[t] + e^{-[k_2+k_3]t} \times \int_0^t e^{-[k_1\Theta/(1+\gamma)-k_2-k_3]t'}C_{alb}[t] \times \sum_{n=1}^{\infty} \frac{[k_1\Theta/(1+\gamma)\,k_2t']^n(t-t')^{n-1}}{n!(n-1)!}dt' \quad (3)$$

where $C_{prop}(t)$ is concentration of transported substance in the effluent at time t, t is elapsed time minus large vessel transit time, $C_{alb}(t)$ is concentration of reference substance (albumin) in effluent at time t, k_1 is influx rate constant, k_2 is efflux rate constant, k_3 is sequestration rate constant and $\Theta/(1+\gamma)$ is the ratio of the propranolol cellular space to its extracellular space.

We assumed that albumin was the ideal reference for propranolol's behavior in the extracellular space. We performed optimization for the best-fit values of the variables $k_1\Theta$, k_2 and k_3 with an iteration program as mentioned earlier. Criteria for goodness of fit remained the same.

Intrahepatic Shunting. The presence of large intrahepatic shunts (>15 μm) was determined with a bolus containing ^{51}Cr-labeled bovine RBCs (4×10^5 dpm), with a hematocrit matched to that of the perfusate, and ^{141}Ce-labeled microspheres (7,000 beads, 7×10^5 dpm). The total hepatic venous outflow was collected in serial tubes at 1-sec intervals for 40 sec. Gamma activity was measured in 0.1-ml aliquots. Because microspheres larger than the diameter of normal sinusoids would be trapped if no intrahepatic shunts formed, large intrahepatic shunts (expressed as percentage) were calculated from microsphere recovery in the outflow as the ratio of the area under the curve *(AUC)* of microspheres *(MS)* over that of the RBCs: $(AUC_{MS}/AUC_{RBC}) \times 100$.

Histological Examination. At the end of the experiment, the liver was flushed with Krebs-Henseleit buffer, and a specimen was taken for liver histological study. All livers were examined under light microscopy after hematoxylin and eosin staining. In addition, the histological specimens were subjected to determination of hepatic collagen content according to a colorimetric method using Sirius red and Fast green (28).

Statistical Analysis. Data are presented as mean ± S.D. Means between the different groups were compared with one-way ANOVA for unpaired data, with Scheffé's test for multiple comparisons (29). Linear-regression analysis was performed with the least-squares method; p values less than 0.05 were considered significant.

RESULTS

Characteristics of the Liver Perfusion. During perfusion, the appearance of the livers remained normal; and bile flow, portal pressure and oxygen consumption remained stable throughout each experiment. In rats with cirrhosis, collagen content was increased and portal pressure, bile production and oxygen consumption were decreased compared with control animals (Table 1). Bile flow was also less in rats with acute liver injury.

Propranolol Extraction by the Perfused Liver. Steady-state propranolol extraction and clearance by the isolated perfused livers were significantly reduced in the three experimental groups compared with controls. Representative examples are shown in Figure 1, and results are summarized in Table 2. Under the experimental conditions used (i.e., presence of albumin and AAG), most of the propranolol in the perfusate was protein bound and the free fraction was low (Table 2). In the chlorpromazine group, we noted a twofold increase in the free fraction of propranolol (Table 2) because of competitive inhibition by chlorpromazine for binding sites, as previously reported (8).

MIDCs. Typical sets of MIDCs for nonsequestered tracers are illustrated in Figure 2. The transit times and volumes of distribution of RBCs, albumin and sucrose are summarized in Figure 3. In control animals, labeled RBCs emerged first in the outflow, reached a peak concentration rapidly and declined monoexponentially

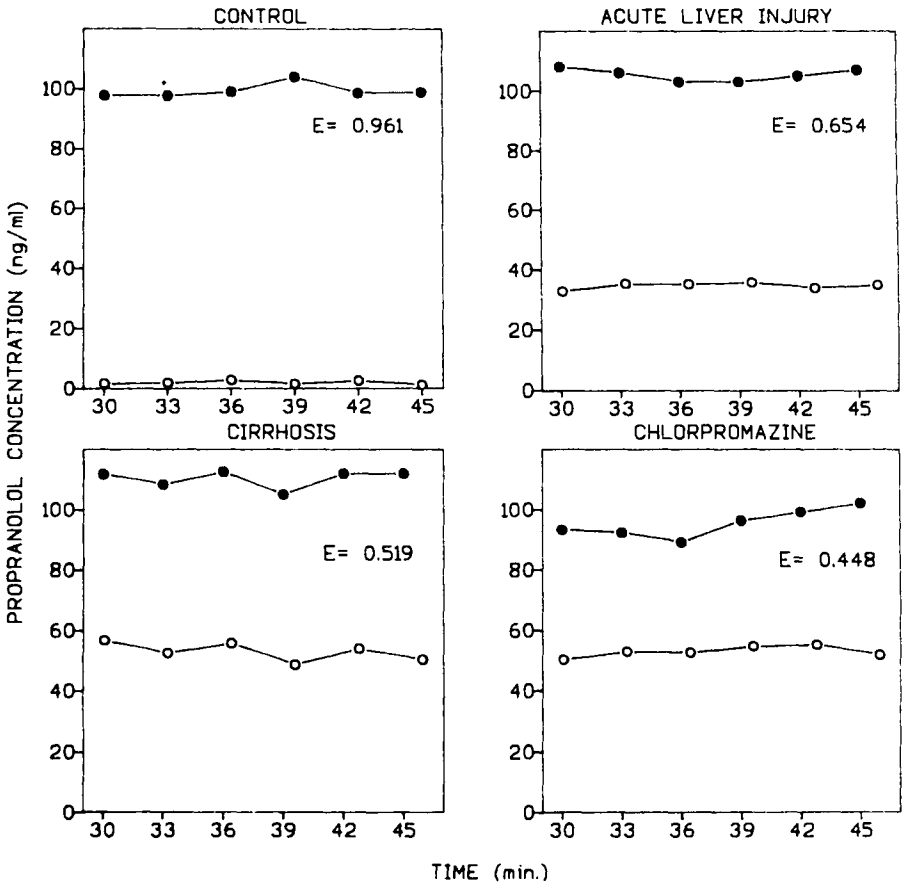

FIG. 1. Representative examples of propranolol extraction (*E*) at steady state by isolated perfused livers. ● = propranolol inflow concentration; ○ = propranolol outflow concentration.

TABLE 2. Propranolol elimination by isolated perfused liver

Characteristics	Controls (n = 13)	Cirrhosis (n = 12)	Acute liver injury (n = 11)	Chlorpromazine (n = 6)
Propranolol clearance (ml/min)	20.3 ± 1.1[a]	11.4 ± 3.6[b]	16.9 ± 1.1[b]	6.6 ± 1.7[b]
Propranolol extraction	0.972 ± 0.015	0.564 ± 0.174[b]	0.831 ± 0.051[b]	0.329 ± 0.087[b]
Perfusate blood flow (ml/min)	20.9 ± 1.3	20.2 ± 0.8	20.3 ± 0.5	20.2 ± 0.3
Free fraction of propranolol in perfusate	0.043 ± 0.003	0.043 ± 0.003	0.041 ± 0.003	0.096 ± 0.019[b]

[a]Data expressed as mean ± S.D.
[b]$p < 0.05$ compared with controls.

with time. Curves for labeled albumin and sucrose showed lower peaks and appeared later in the outflow. A few microspheres were recovered in the outflow (Table 1). In rats with cirrhosis, the transit time of RBCs was faster than in the control group, and V_{sin} was reduced. The EVV accessible to albumin was decreased, and albumin appeared earlier in the outflow curve and tended to move toward and superimpose itself on the labeled-RBC curve (Fig. 2). The transit time of sucrose was prolonged; sucrose EVV was increased. The sucrose curves could not be transformed to be superimposed on the RBC curves, indicating that the diffusion of sucrose into its extravascular space was no longer flow limited. No intrahepatic shunts were present in this model of cirrhosis, as assessed with the microsphere method (Table 1). In rats with acute liver injury, visual inspection of the dilution curves revealed no significant modifications of the hepatic microcirculation (Fig. 2), except for reduction of sinusoidal volume. In some rats (3 of 11), the transit time of RBCs was shortened and the transit time of sucrose was increased without being significant for the entire group. The transit time of albumin was unchanged (Fig. 3). In rats exposed to chlorpromazine, the characteristics of the dilution

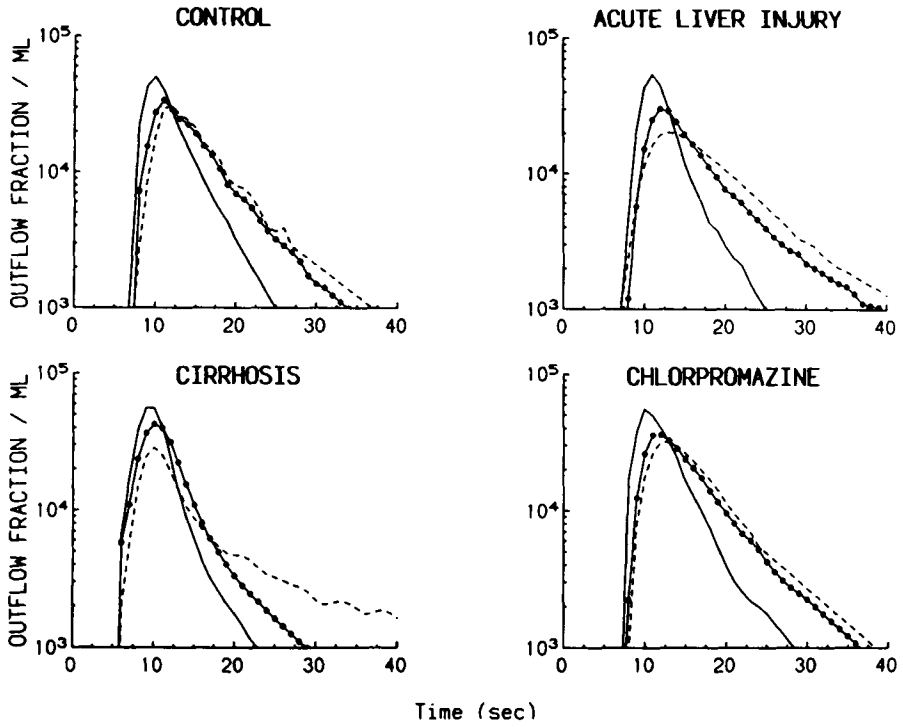

FIG. 2. Representative examples of multiple-indicator outflow curves for ^{51}Cr-labeled RBCs (—), ^{99m}Tc-labeled albumin (●-●-●) and [^{14}C]-sucrose (----) in the four groups of animals studied.

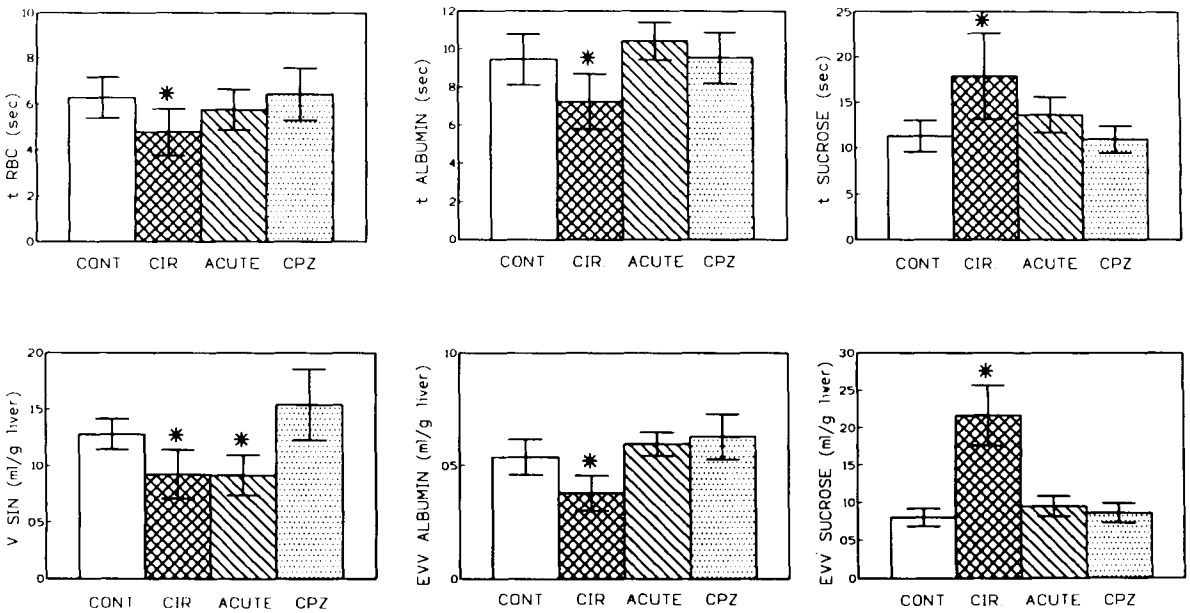

FIG. 3. Transit times and distribution spaces of nonsequestered tracers in isolated perfused livers. *CONT* = control rats; *CIR* = cirrhotic rats; *ACUTE* = acute liver injury; *CPZ* = chlorpromazine; *p < 0.05 compared with controls.

curves for diffusible tracers and RBCs were comparable to those of control animals (Figs. 2 and 3).

Propranolol Curve Analysis. Propranolol indicator dilution curves were obtained in the presence of a steady-state concentration of unlabeled propranolol, with albumin as an extracellular reference. The outflow profile of propranolol in the hepatic vein after injection of a tracer dose of labeled propranolol in the portal vein is shown in Figure 4. Calculated throughput and returning components for each group of animals are shown in Figure 5, and rate constants for propranolol uptake are summarized in Table 3. In control rats, the

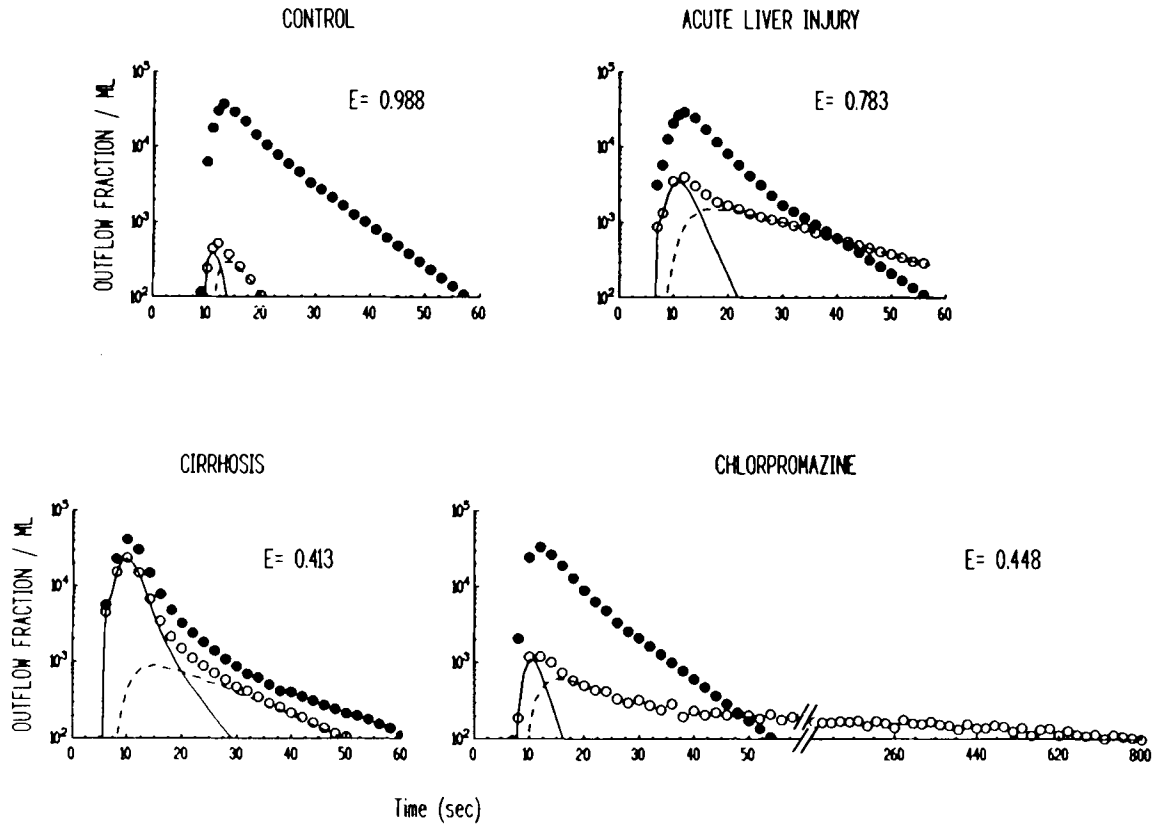

FIG. 4. Resolution of the propranolol outflow curve into its throughput and returning components in the four groups of animals studied. ●●● = Labeled albumin; ○○○ = labeled propranolol; — = computed throughput component; ---- = computed returning component.

single-pass extraction of propranolol was almost complete. The influx of propranolol ($k_1\Theta/1 + \gamma$) was considerably decreased in cirrhotic animals, resulting in a large throughput component. In rats treated with chlorpromazine, influx was normal but the elimination rate constant was decreased, resulting in a large amount of returning material. In rats with acute liver injury, both uptake and elimination were decreased compared with controls.

In rats with cirrhosis, the proportion of propranolol consisting of throughput material in the outflow increased as the extraction ratio decreased (Fig. 6), suggesting that the limitation of propranolol cellular entry is a progressive phenomenon that develops with increasing severity of cirrhosis. We found no correlation between these parameters in the other three groups of animals.

DISCUSSION

In this study, we examined liver microcirculation and propranolol disposition by the perfused liver in three different pathological conditions: cirrhosis, acute liver injury following a single dose of CCl_4 and exposure to chlorpromazine, an inhibitor of propranolol metabolism. Steady-state extraction of propranolol was decreased in all three experimental groups compared with controls, but we noted major differences in the hepatic handling of propranolol as assessed with the MIDC method.

In control animals, propranolol was efficiently removed by the perfused liver despite extensive protein binding, in agreement with previous reports (17, 30-32). MIDC showed almost complete single-pass extraction of propranolol, in accord with measurements of steady-state extraction. Assessment of hepatic microcirculation in the control group showed values of sinusoidal and albumin and sucrose spaces similar to those reported by other investigators (14, 33, 34).

Chlorpromazine is a potent inhibitor of ring oxidation, the major pathway of propranolol metabolism in the rat (8, 35-37). Rats treated with chlorpromazine provide a model in which reduced enzyme activity is the only factor responsible for the decrease in propranolol elimination. Steady-state extraction was reduced by 66% compared with controls. MIDC showed a normal influx rate constant for propranolol, with a decrease in the elimination rate constant resulting in slow release of unchanged propranolol in the hepatic outflow for as long as 15 min after injection of the tracer. Thus, in rats exposed to chlorpromazine, propranolol entered the hepatocytes freely, as in control animals, but it could not be metabolized because of reduced enzyme activity and it subsequently returned in the emerging venous blood.

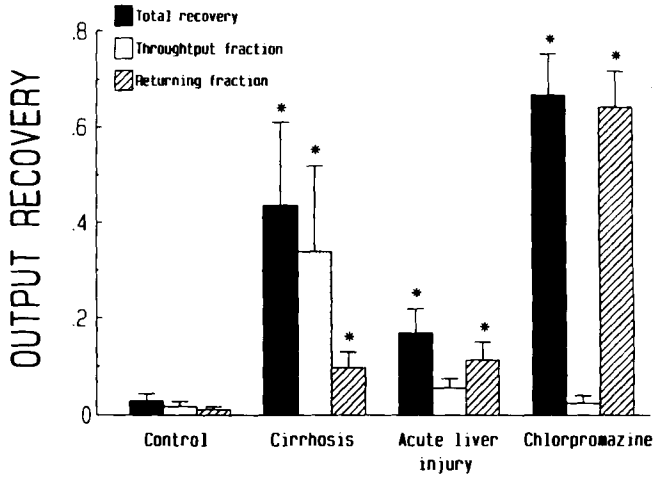

FIG. 5. Recovery of unchanged propranolol in the outflow, expressed as fraction of the dose injected. *$p < 0.05$ compared with controls.

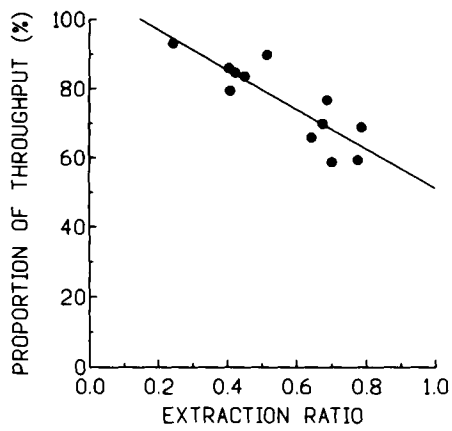

FIG. 6. Relationship between proportion of propranolol in outflow consisting of throughput material (% of total amount of propranolol in the outflow) and hepatic propranolol extraction ratio in cirrhotic rats ($r = -0.860$, $p < 0.05$).

TABLE 3. Rate constants for hepatic uptake of propranolol by isolated perfused liver

Rate constants	Controls (n = 13)	Cirrhosis (n = 12)	Acute liver injury (n = 11)	Chlorpromazine (n = 6)
$k_1\Theta/1 + \gamma$ (sec^{-1})	0.746 ± 0.101	0.237 ± 0.127[b]	0.589 ± 0.127[b]	0.915 ± 0.157
k_2 (sec^{-1})	0.088 ± 0.041	0.070 ± 0.032	0.077 ± 0.025	0.091 ± 0.031
k_3 (sec^{-1})	0.216 ± 0.128	0.134 ± 0.065	0.066 ± 0.014[b]	0.065 ± 0.031[b]

[a]Data expressed as mean ± S.D.
[b]$p < 0.05$ compared with controls.

In cirrhotic animals, the steady-state extraction of propranolol was also decreased, but the outflow curve pattern for unchanged drug was completely different. The hepatic influx rate constant was decreased, whereas the elimination rate constant was not significantly different from that of controls. Most of the propranolol coming out in the hepatic vein was throughput material (i.e., propranolol that traveled along with albumin and did not enter the hepatocytes). In addition, the proportion of propranolol in the outflow consisting of throughput increased as the extraction ratio decreased, suggesting that the restriction of propranolol uptake progressed with increasing severity of cirrhosis.

Two anomalies could account for the impaired uptake of propranolol: capillarization of the hepatic sinusoids and intrahepatic shunting. Analysis of MIDCs for nonsequestered tracers in cirrhotic animals showed discrepancies compatible with both possibilities. The transit time of RBCs was faster than that of controls. This could be because of the presence of "fast-flowing" sinusoids, as suggested by Sherman et al. (38), or intrahepatic shunting. Anatomic "large" intrahepatic shunts, defined on the basis of the fraction of 15-μm microspheres passing through the liver, were absent, but the presence of "small" (< 15 μm) shunts has been postulated in the rat CCl_4 model of cirrhosis (6, 14). These shunts could account for the decreased influx of propranolol, but they cannot be quantitated with the microsphere technique if one assumes that their diameter is comparable to that of normal sinusoids.

"Capillarization" refers to the transformation of sinusoids into capillaries with continuous defenestrated, overlapping endothelium (10, 12, 13, 39). It tends to occur at the periphery of regenerative nodules, whereas the centrilobular area retains a more normal appearance (40). This transformation creates a diffusion barrier, hindering normal macromolecular exchange. In this study, this transformation was evidenced by a decrease in the extravascular space accessible to albumin. Labeled albumin tended to remain in the vascular compartment along with the RBCs and did not diffuse normally into the space of Disse. The diffusion of sucrose into its extravascular space was also abnormal: the transit time of sucrose was increased, and the downslope of the labeled sucrose curves showed a biexponential profile. These changes are compatible with those predicted when the permeability of a limiting exchange barrier is progressively decreased (41, 42).

Recently, Martinez-Hernandez and Martinez (43) provided ultrastructural evidence that the cirrhotic capillary basement membrane acts as a filtration barrier and prevents the hepatic uptake of ferritin in rats with CCl_4-induced cirrhosis. Unlike ferritin, propranolol is a small lipophilic molecule that readily penetrates tight capillary barriers such as that in the brain (44). It is unlikely that capillarization could act as a barrier to its

diffusion as it does for ferritin. Propranolol, however, is extensively bound to albumin and AAG, and it is possible that restricted diffusion of protein-bound propranolol in the space of Disse contributes to decreased uptake by hepatocytes. In isolated hepatocytes, we have shown that albumin facilitates uptake of propranolol by cells (7, 17); restricted diffusion of albumin-bound propranolol at the cell surface could result in a lower uptake rate in cirrhosis.

In rats with acute liver injury, the steady-state extraction of propranolol was also decreased compared with that in controls, and the outflow profile of propranolol dilution curves was intermediate between those of the cirrhotic group and the chlorpromazine group. The rate constant for propranolol influx and the elimination rate constant were both decreased. About a third of the unchanged propranolol coming out in the outflow was throughput material; the other two thirds was returning material. The MIDC for nonsequestered tracers were comparable to those of controls except for a decrease in the V_{sin} (possibly because of cell swelling after a single dose of CCl_4). The anatomical changes responsible for the decrease in propranolol uptake in this setting remain unclear.

In summary, our data indicate that the decrease in propranolol extraction in rats with cirrhosis is mainly due to impaired hepatic uptake of the drug, in contrast to animals with acute liver injury or those treated with an inhibitor of propranolol metabolism, in which impaired hepatic elimination is the major anomaly. These results are in agreement with our previous observations indicating that decreased microsomal enzyme activity could not account for the observed decrease in propranolol extraction in cirrhosis (7, 8). In rats with CCl_4-induced cirrhosis, two anomalies of the liver microcirculation could be responsible for this decreased uptake: (a) restriction in the diffusion of albumin-bound propranolol in the space of Disse to its site of uptake at the surface of the hepatocyte or (b) presence of small (< 15 μm) intrahepatic shunts or fast-flowing sinusoids.

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