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Research Article

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Determinants of Hepatic Function in Liver Cirrhosis in the Rat

Multivariate Analysis

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Abstract

We investigated the determinants of hepatic clearance functions in a rat model of liver cirrhosis induced by phenobarbital/CCl_4. Aminopyrine *N*-demethylation (ABT), galactose elimination (GBT), and serum bile acids (SBA) were determined in vivo. The livers were then characterized hemodynamically: intrahepatic shunting (IHS) was determined by microspheres and sinusoidal capillarization by measuring the extravascular albumin space (EVA) by a multiple indicator dilution technique. The intrinsic clearance was determined by assaying the activity of the rate-limiting enzymes in vitro. Hepatocellular volume (HCV) was measured by morphometry.

ABT and SBA, but not GBT, differentiated cirrhotic from normal liver. IHS ranged from normal to 10%; all cirrhotic livers showed evidence of sinusoidal capillarization (reduced EVA). The cirrhotic livers showed a bimodal distribution of HCV, HCV being decreased in 50% of the cirrhotic livers.

Multivariate analysis showed EVA and portal flow to be the main determinants of microsomal (ABT) and cytosolic (GBT) clearance function; SBA, by contrast, were determined solely by IHS.

We conclude that sinusoidal capillarization is the main determinant of hepatic clearance, while serum bile acids reflect intrahepatic shunting. These findings emphasize the importance of alterations of hepatic nutritional flow to explain reduced clearance function in cirrhosis of the liver.

Introduction

Clearance of endo- and xenobiotics is one of the predominant functions of the liver; it is determined by hepatic perfusion and the so-called intrinsic clearance (1), a measure of the metabolic capacity of the liver. In chronic liver disease, perfusion is altered both quantitatively and qualitatively. The amount of blood passing through the liver can be increased (2) but in end-stage liver disease is most often decreased (3). Among the qualitative alterations the development of porto-systemic shunts (4) and sinusoidal capillarization (5) have been shown to be of prime importance.

Intrinsic clearance is dependent upon the enzymatic composition of the liver as well as on liver cell volume. The hepatic content of different enzymes has been shown to be decreased (6, 7). In contrast, little data are available on liver cell volume. We have recently demonstrated a bimodal distribution of liver cell volume in animals with cirrhosis of the liver, about half having maintained and half having decreased liver cell volume (8).

These changes, then, would one lead to expect a decreased clearance of endo- and xenobiotics in chronic liver disease; this has indeed been demonstrated for a variety of substrates (9–12). Different theories have been forwarded to understand the determinants of clearance function in chronic liver disease. The most encompassing, the so-called "intact cell hypothesis" states that a reduced volume of liver cells with a normal enzymatic makeup (intact cell) is only perfused in part due to the development of portosystemic shunts (13). Measurement of all these variables is necessary, however, to better understand determinants of liver function.

We therefore quantitatively assessed different aspects of hepatic clearance function in a rat model of liver cirrhosis in vivo and correlated these measurements with different aspects of hepatic hemodynamics, enzymatic composition of the liver and morphometrically determined liver cell volume. The functional tests selected for characterization of cirrhosis included determination of serum bile acid levels, a purported measure of shunting (14), aminopyrine *N*-demethylation (ABT),[1] an expression of microsomal metabolic capacity (15) and galactose elimination capacity, a supposed measure of functional liver volume (16).

Methods

Materials. Male Sprague-Dawley rats were purchased from the Süddeutsche Versuchstierfarm, Tuttlingen, FRG, and kept on standard conditions. [^{14}C]Dimethylaminopyrine and 1-[^{14}C]galactose were obtained from Amersham International, Buckinghamshire, UK, and diluted with unlabeled material to the specific activities given previously (17). [^{51}Cr]Chromate sodium was obtained from New England Nuclear, Boston, MA; erythrocytes were labeled as previously described (18). ^{57}Co-microspheres, diameter 15±1 μm and [^{14}C]sucrose (sp act 673 mCi/mmol) were purchased from New England Nuclear. ^{99m}Tc-albumin was prepared by reduction with stannous chloride. Bovine erythrocytes were obtained from the local slaughterhouse and washed five times with physiologic saline solution. BSA was fatty acid poor from Calbiochem-Behring Diagnostics, La Jolla, CA. All other reagents were analytical grade from different commercial sources.

Experimental design. Cirrhosis was induced in rats weighing 150–180 g by chronic exposure to phenobarbital and carbon tetrachloride for 12 wk according to McLean et al. (19) as previously described (18); treatment was withheld 2 wk before the study, a time sufficient for

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1. *Abbreviations used in this paper:* ABT, aminopyrine *N*-demethylation; ALT, alkaline phosphatase; EVA, extravascular albumin space; GBT, galactose elimination; GK, galactokinase activity; HCV, hepatocellular volume; IHS intrahepatic shunting; IVS, intravascular space; PF, portal flow; SBA, serum bile acids.

the phenobarbital effects to disappear (8). Untreated littermates kept under identical conditions served as controls.

Aminopyrine and galactose metabolism were studied by breath tests previously described in detail from this laboratory (8, 17) after injection of [^{14}C]aminopyrine (0.5 μCi/0.3 mg i.p.) or [^{14}C]galactose (0.5 μCi plus 0.75 g/kg unlabeled galactose i.p.) on two consecutive days.

On the third day, the animals were anesthetized with pentobarbital (50 mg/kg i.p.), a blood sample for determination of ALT, alkaline phosphatase and serum bile acids was obtained and portal pressure measured manometrically. Then, the animal was prepared for recirculating in situ liver perfusion as previously described (18); particular care was taken to ligate all potential extrahepatic collaterals. The perfusion medium consisted of washed bovine erythrocytes (20% vol/vol), BSA (2% wt/vol) and dextrose (0.1% wt/vol) in Krebs-Ringer bicarbonate buffer. It was carried out using a pressure-head (18) with pressure set at the portal pressure measured in vivo. ALT and potassium release into the perfusate were measured as criteria of viability (18). All reported experiments met those criteria.

After an equilibration period of 20 min, a set of multiple indicator dilution curves was obtained using ^{51}Cr-labeled erythrocytes (0.5 μCi) as an intravascular and ^{99m}Tc-albumin (10 μCi) and [^{14}C]sucrose (0.5 μCi) as extravascular markers (18). To determine intrahepatic shunting, 40–50,000 microspheres were injected and shunt fraction determined according to Groszman et al. (20).

Single pass extraction of [^{14}C]taurocholate (1 μCi), propranolol (1 mg), and aminopyrine (2.5 mg) was determined by injecting the compound into the portal vein and collecting the ensuing hepatic venous effluent for 2 min.

At the end of the perfusion experiment, the liver and spleen were removed and weighed. Then, the liver was subjected to rigorous systematic random sampling (21) with half of the liver being used for histological analysis and half for biochemical determinations of the rate-limiting enzymes in appropriate subcellular fractions (17).

Microsomal fractions were prepared by differential centrifugation and the first 100,000 *g* supernatant taken as cytosolic fraction (8). Cytochrome P-450 content and the kinetics of ABT were determined by methods referenced previously (8) in homogenate and in the microsomal fraction. Galactokinase activity was determined in the cytosolic fraction by a radiometric assay described by Shin-Buehring et al. (22).

Five pieces of liver of 1 cm^3 were selected at random as previously described (21), fixed in buffered formalin (4% vol/vol), embedded in paraffin, cut and stained with hematoxylin-eosin and elastica-Van Gieson. Morphometry was carried out on the latter sections by the point counting procedure of Weibel (23) using a microscope with a sampling stage as previously described (8, 17). Final magnification was 400 and point to point distance was 860 μm. Each point was classified as overlying either: parenchyma, hepatocytes, nonhepatocytes (Kupffer, endothelial, or Ito cells), and bile ducts; nonparenchyma, vessels, connective tissue (including fibroblasts). It should be pointed out that the use of immersion rather than perfusion fixation may have led to collapse of vessels and that therefore this compartment may have been underestimated in the present study.

Analytical techniques. ^{3}H- and ^{14}C-radioactivity was determined on a Packard TriCarb 2260 liquid scintillation counter using external standardization for quench correction. The gamma-emitters (^{51}Cr, ^{99m}Tc, and ^{57}Co) were counted on a gamma-spectrometer (model 5130; Packard Instruments Co., Downers Grove, IL) with appropriate correction for isotope spillover where necessary.

ALT and alkaline phosphatase activity were determined by commercial kinetic assays on a Cobas centrifuging analyzer (Hoffmann-La Roche Diagnostics, Basel, Switzerland). Serum bile acids were determined by a commercial radioimmunoassay (Becton-Dickinson Co., Orangeburg, NY). Protein in the different subcellular fractions was determined by the Lowry assay using BSA as the standard (24).

Aminopyrine was determined by high-pressure liquid chromatography using [^{14}C]aminopyrine as an internal standard as previously described (25). Propranolol was determined by a modification of the high-pressure liquid chromatographic method of Lo et al. (26) using 4-methylpropranolol (a kind gift from ICI, Macclesfield, UK) as an internal standard.

Data and statistical analysis. The aminopyrine breath test was evaluated by determining the peak exhalation rate and the area under the curve of radioactivity excreted from 0 to 2 h (25); since both values yielded comparable results as to severity, only the area under the curve will be reported as ABT_{AUC}. The galactose breath test was evaluated as previously described by determining the slope of the initial increase of the radioactivity vs. time curve; this will be reported as GBT-k (17).

The multiple indicator dilution curves were expressed as frequency function, h(t); mean transit times ($\bar{t}$) were calculated by classical indicator dilution theory (27). Intravascular space (IVS) was calculated as the product of the mean transit times of the erythrocytes ($\bar{t}_{RBC}$) and flow (Q) as

$$IVS = \bar{t}_{RBC} \cdot Q \quad (1)$$

and the extravascular albumin space (EVA) as

$$EVA = PF \cdot (\bar{t}_{ALB} - \bar{t}_{RBC}) \quad (2)$$

where PF stands for plasma flow (PF = (1 − Hct) · Q; 18). Extravascular sucrose space (EVS) was calculated in an analogous fashion by substituting $\bar{t}_{SUC}$ for $\bar{t}_{ALB}$ in equation (2). Extractions (E) were calculated as

$$E = [(D - R)/D] \cdot 100 \quad (3)$$

where D and R stand for the amount given and recovered in the venous effluent, respectively. Bile acid clearance was calculated as the product of extraction and portal flow. Portal resistance (R) was calculated as

$$R = \Delta p/F \quad (4)$$

where F stands for portal flow and Δp for the pressure gradient across the liver; this was set equal to portal pressure since hepatic vein pressure was zero (18). The morphometric data were expressed as volume fractions (V_v) according to Weibel (23). The volumes of the different compartments were obtained by multiplying V_v with liver weight.

The kinetics of ABT in vitro were fitted to the Michaelis-Menten equation by nonlinear least-squares fitting as previously referenced (8). Linear regression analysis was performed by the method of least squares (28). Multiple regression analysis was performed using a logistic model with both forward inclusion and backward exclusion, using an F test to differentiate between different models (29). Means of two groups were compared by t test after testing the equality of variances by a F test (28). All results are reported as mean±1 SD. $P < 0.05$ was considered statistically significant.

Results

16 of 20 animals survived the 12-wk treatment period. Body weight was significantly smaller in cirrhotics, averaging 669±45 and 549±68 g ($P < 0.005$) in controls and cirrhotics, respectively. The organ weights are reported in Table I. While liver weights did not differ between the two groups, spleen weights were increased by 60% in the cirrhotics. All treated animals had cirrhosis by macroscopic inspection and 11/16 had ascites.

The liver function tests in vivo, given also in Table I, showed serum ALT, alkaline phosphatase and bile acids to be elevated in the cirrhotic group. Galactose metabolism was not significantly altered while aminopyrine N-demethylation was markedly impaired (Table I).

The hemodynamic measurements are reported in Table II. All except one of the cirrhotic animals had portal hypertension (portal pressure > 10 cm H_2O) as measured in vivo. The livers were perfused in situ at the portal pressure measured in vivo;

Table I. Organ Weights and Liver Function Tests In Vivo. Means±1 SD Are Given

	Controls	Cirrhotics
n =	10	16
Liver weight (*g*)	20.7±1.4	18.3±4.5
Spleen weight (*g*)	1.0±0.2	1.6±0.6*
Serum ALT (*IU/liter*)	35±4	51±13‡
Serum alkaline phosphatase (*IU/liter*)	185±20	341±160§
Serum bile acids (*μmol/liter*)	1.0±0.8	10.2±9.4‡
Galactose elimination‖	0.060±0.006	0.054±0.018
Aminopyrine *N*-demethylation¶	30.1±5.4	21.6±7.1‡

Differences between the two groups were evaluated by Student's *t* test. * $P < 0.05$; § $P < 0.01$; ‡ $P < 0.005$; ‖ GBT-k (%dose · h^{-2}); ¶ $AUC_{0-2\ h}$.

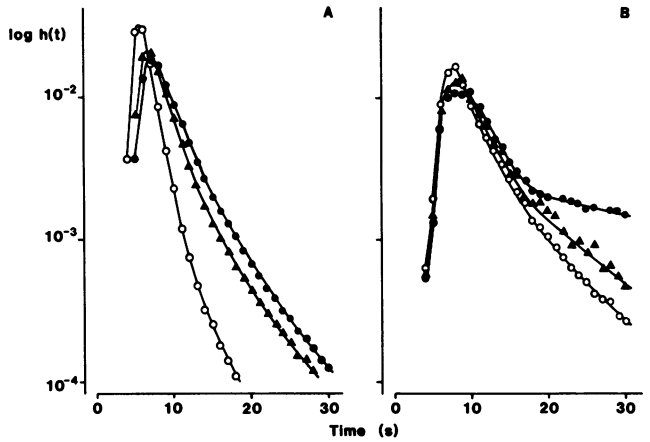

Figure 1. Multiple indicator dilution curves of ^{51}Cr-erythrocytes (○), ^{99m}Tc-albumin (▲) and [^{14}C]sucrose (●) in a control (*A*) and cirrhotic (*B*) rat liver. The decadic logarithm of the frequency function h(t) is given. In normal liver (*A*), the indicators appear in the flow-limited fashion typical for liver: the erythrocytes appear earlier and peak higher than albumin and sucrose due to the distribution of the latter into the extravascular space of Disse. The sucrose is slightly more delayed than the albumin due to its smaller molecular weight. No evidence of diffusional hindrance is seen. In the cirrhotic liver, by contrast, (*B*) the albumin curve is almost superimposed upon the erythrocyte curve. This is due to sinusoidal capillarization that does not permit the albumin to leave the sinusoid; the sucrose shows a break from the smooth decay at ~ 20 s due to diffusional exchange at the newly formed capillary wall.

the resulting blood flow was not significantly different from controls. Portal resistance was markedly increased in the cirrhotic animals. Intrahepatic shunting, measured by the passage of 15-μm microspheres, was negligible in the control group and highly variable in the cirrhotic animals, ranging from normal to up to 10% (Table II).

Results of a set of indicator dilution curves in a control and a cirrhotic rat liver are shown in Fig. 1. The control animal shows the classical pattern seen in normal liver, i.e., the extravascular indicators, albumin and sucrose, are delayed compared to the erythrocyte curve due to their larger distribution into the space of Disse. In the cirrhotic liver, by contrast, the albumin curve is almost superimposed upon the erythrocyte curve since the exchange of albumin is hindered while the sucrose curve shows evidence of diffusional exchange. This pattern has been shown to be due to sinusoidal capillarization (5, 18, 30). All but three cirrhotic animals showed evidence of marked sinusoidal capillarization by this technique. The spaces calculated from these curves are shown in Table III. While there was no difference in erythrocyte space between the two groups, the extravascular albumin space was markedly reduced and the extravascular sucrose space increased in the cirrhotic group (Table III).

The single pass extraction of all three substances tested, taurocholate, propranolol, and aminopyrine, was significantly reduced in the perfused liver of cirrhotic rats (Table III). The decrease compared to controls averaged 32, 18, and 37%, respectively, with the decreases not differing statistically among each other as determined by analysis of variance ($P > 0.10$).

The enrichment and recovery of microsomal and cytosolic marker enzymes did not differ between control and cirrhotic rats (data not shown). Total hepatic cytochrome P-450 content averaged 383±50 and 230±124 nmol in control and cirrhotic rats, respectively ($P < 0.002$). The activities of the rate-limiting enzymes of aminopyrine and galactose metabolism are shown in Table IV. In accordance with the in vivo results, activity of aminopyrine *N*-demethylase, but not of galactokinase, was decreased in cirrhotic as compared to control rats. The reduction

Table II. Hemodynamic Parameters in the Perfused Rat Liver of Control (n = 10) and Cirrhotic (n = 16) Rats. Means±1 SD Are Given

	Controls	Cirrhotics
Portal pressure ($cm\ H_2O$)	8.9±0.9	14.2±2.8*
Portal flow ($ml \cdot min^{-1} g^{-1}$)	1.33±0.22	1.57±0.49
Resistance ($dyn \cdot s \cdot cm^{-5} \cdot 10^{-3}$)	19.3±3.1	34.3±17.8‡
Shunting (%)	0.7±0.8	2.2±2.6

Differences between the two groups were evaluated by Student's *t* test. Portal pressure was measured in vivo. The livers were then perfused at that pressure and flow was determined volumetrically. Shunting is defined as the passage of 15 μm ^{57}Co-microspheres. * $P < 0.001$; ‡ $P < 0.02$.

Table III. Hepatic Spaces (ml/g) and Extraction (%) of Endo- and Xenobiotics in the In Situ Perfused Liver of Control (n = 10) and Cirrhotic (n = 16) Rats

	Controls	Cirrhotics
Space		
Erythrocyte	0.189±0.015	0.177±0.021
Extravascular albumin	0.050±0.008	0.028±0.017*
Extravascular sucrose	0.068±0.012	0.126±0.047‡
Extraction		
Taurocholate	97.4±1.2	66.2±30.3*
Propranolol	99.2±0.3	81.0±18.3*
Aminopyrine	65.1±9.9	41.3±18.2‡

Means±1 SD are given. Differences were evaluated by Student's *t* test. * $P < 0.005$; ‡ $P < 0.001$.

in ABT activity was due solely to a decrease in V_{max}, K_m being unaffected (Table IV).

Microscopic examination of control livers showed a normal lobular architecture with cell plates one cell thick. Hepatocellular structure was preserved. Treated animals, by contrast, showed micronodular cirrhosis in all 16 animals with diffuse fibrous septae envelopping nodules of hepatocytes. The hepatocyte structure was well preserved, necrosis or fatty metamorphosis involving < 1% of the cells. Central veins, when discernible, were displaced towards the periphery of the nodules. A moderately pronounced ductular proliferation was seen in the portal tracts of treated animals.

The quantitative data on histology are reported in Table V. The most striking finding was a significant reduction in both, relative and absolute hepatocellular volume which was compensated for by a seven- and fivefold increase in connective tissue and bile ducts, respectively. Hepatocellular volume fraction showed a bimodal distribution (Fig. 2) with 5/16 animals having a normal V_v. The same held true for absolute hepatocellular volume but 8/16 cirrhotic animals having a maintained hepatocellular volume (Fig. 2).

The determinants of the different liver function tests were then evaluated by stepwise logistic regression analysis. The different determinants of serum bile acid levels are shown in Fig. 3. The conventional liver tests (ALT), the extravascular albumin and sucrose space, taurocholate extraction, bile acid clearance, shunt fraction, (PF), portal pressure, and hepatocellular volume were analyzed. Although different parameters showed a statistically significant correlation with serum bile acid levels (see Fig. 3), the model selected intrahepatic shunting as the sole determinant (Fig. 3 *A*).

The determinants of the aminopyrine breath test in vivo are shown in Fig. 4. Instead of taurocholate extraction and bile acid clearance, cytochrome P-450 content, intrinsic clearance and aminopyrine extraction were analyzed. All aspects of microsomal function showed a statistically significant correlation with the aminopyrine breath test as did aminopyrine extraction. Logistic regression analysis selected the EVA and PF as sole determinants, however.

In the analysis of the galactose breath test the microsomal parameters were replaced by galactokinase activity, the other parameters being the same as above. Again, the activity of the rate-limiting enzyme correlated with the galactose breath test as did hepatocellular volume (Fig. 5). Logistic regression analysis picked the same parameters as for the aminopyrine breath test; thus, both tests showed a significant direct relationship with extravascular albumin space and an inverse relationship with portal flow.

Table IV. In Vitro Activity of the Rate Limiting Enzymes of ABT and Galactose Elimination in Control (n = 10) and Cirrhotic (n = 16) Rat Liver

	Controls	Cirrhotics
ABT		
V_{max} (*μmol/h*)	143±25	85±53*
K_m (*mmol/liter*)	0.9±0.2	1.1±0.3
Cl_{int} (*ml/h*)	167±54	96±66‡
Galactokinase (*μmol/min*)	12.5±3.7	11.4±5.0

Enzyme activities are calculated for the whole liver. Cl_{int} was calculated as the ratio V_{max}/K_m. Means±1 SD are given.
* $P < 0.005$; ‡ $P < 0.01$.

Table V. Morphometric Analysis of Control (n = 10) and Cirrhotic (n = 16) Rat Liver

	Controls	Cirrhotics
Volume fraction (V_v)		
Hepatocytes	0.784±0.028	0.652±0.077*
Nonhepatocytes	0.159±0.021	0.121±0.025‡
Bile ducts	0.008±0.004	0.039±0.020‡
Vessels	0.029±0.007	0.045±0.017*
Connective tissue	0.021±0.008	0.153±0.062*
Absolute volumes (*ml*)		
Hepatocytes	16.3±1.4	12.0±3.6*
Nonhepatocytes	3.3±0.4	2.3±0.8*
Bile ducts	0.2±0.1	0.7±0.3*
Vessels	0.6±0.2	0.8±0.3
Connective tissue	0.4±0.2	2.7±1.0*

Means±1 SD are given; differences were evaluated by Student's t test.
* $P < 0.002$; ‡ $P < 0.001$.

Relationships between hepatocellular volume and different aspects of function are shown in Fig. 6. The predictors of hepatocellular volume were analyzed in two ways. In the first analysis, all measured parameters were offered for logistic regression analysis. In this case, cytochrome P-450 (P450) and galactokinase activity (GK) were selected. The equation of this regression was $y = 5.00 + 0.02P450 + 0.18GK$ ($F = 44.42$, $P < 0.00001$). Since we were interested in the functional rather than the biochemical aspects, the aspects of function in subcellular fractions were then removed, offering only ALT, serum bile acids, aminopyrine and galactose breath test, pressure, flow and intrahepatic shunting for further analysis. In this case only PF and IHS were picked as predictors according to the following regression equation: $y = 20.13 - 3.36PF - 1.28IHS$ ($F = 25.74$, $P < 0.0001$). Thus, both parameters predict an inverse relationship between hepatocellular volume and flow or shunt.

Discussion

We have analyzed multiple aspects of hepatic clearance function and correlated them with different hemodynamic, micro-

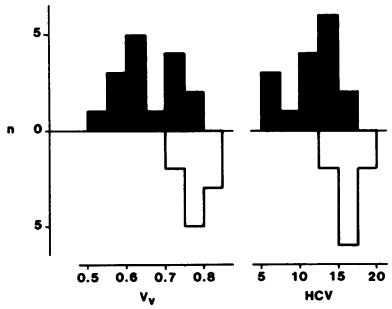

Figure 2. Histogram of the distribution of the volume fraction (V_v) and HCV of hepatocytes in cirrhotic (■) and normal (□) livers. While the control livers show a relatively narrow, normal distribution, the distribution of both, V_v and HCV in cirrhotic livers appears to be bimodal.

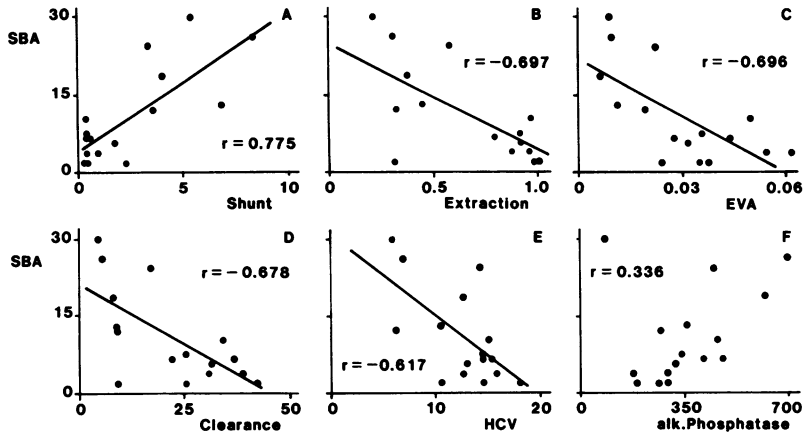

Figure 3. Determinants of SBA (in micromoles per liter) in cirrhotic livers. The relationship between intrahepatic shunt (percent), taurocholate extraction efficiency, EVA (in milliliters per gram), bile acid clearance (in milliliters per minute), hepatocellular volume (in milliliters) and serum alkaline phosphatase activity (international units per liter) are shown. The correlation coefficients are given in the figure. Multiple stepwise regression analysis selected intrahepatic shunting as the sole determinant, the regression equation being SBA = 3.98 + 2.84 × shunt (F = 20.99, P < 0.001).

circulatory, biochemical, and morphometric measurements in a rat model of liver cirrhosis. Our results suggest that sinusoidal capillarization, as determined by the extravascular space accessible to albumin, and portal perfusion are the main determinants of a microsomal (ABT) and cytosolic (galactose phosphorylation) function. Serum bile acids, by contrast, reflect mainly intrahepatic shunting. Conversely, when the main predictors of hepatocellular volume are searched for, one finds excellent correlation with different functional aspects but logistic regression analysis selects only portal perfusion and intrahepatic shunting.

Sinusoidal capillarization has been identified as a main determinant of hepatic function in both, cirrhotic man (5) and rats (18, 30); our investigation is the first to clearly show that sinusoidal capillarization is a factor more important than intrinsic clearance or hepatocellular volume for both a microsomal and a cytosolic liver function test. We have arbitrarily selected the extravascular albumin space as a dynamic measure of sinusoidal capillarization; this seems justified in view of the correlation shown in cirrhotic man between the reduction in extracellular albumin space and sinusoidal capillarization (5). Neither aminopyrine nor galactose are extensively albumin bound; therefore, we do not mean to imply causality from the correlation but rather that the extravascular albumin space measures an important aspect of the distortion of microvascular exchange in cirrhotic liver. The dependence of both functions on flow is more difficult to understand. Neither substance is thought to be cleared from the liver by a flow-limited regimen (1, 15, 16). Aminopyrine has recently been shown to have an extraction much higher than initially believed (31), a result confirmed in this study; nevertheless, in cirrhotic animals extraction was < 50% (Table III) and therefore one would not expect the clearance of the compound to depend on flow (1). In line with this is the fact that flow alone did not correlate with either aminopyrine (Fig. 4) or galactose (Fig. 5) metabolism. It is tempting to suggest, then, that volume of flow and extravascular albumin space are independent aspects of the reduction in "nutritional flow" to the liver and are the main determinants of these two metabolic functions.

The aminopyrine breath test has been shown to accurately reflect microsomal *N*-demethylation in the rat (15); in man it has been shown to be of utility in assessing severity of the disease (10) at least as well as the Child classification (32) and to have prognostic value in alcoholic liver disease and chronic hepatitis (33, 34). The same has been shown by our group in another model of liver cirrhosis in the rat (17). We have also demonstrated that it correlates well with morphometrically determined hepatocellular volume (8) a finding confirmed in the present study (Fig. 4). In agreement with earlier studies intrinsic clearance of aminopyrine, assessed by directly measuring activity of microsomal *N*-demethylase activity, was reduced mainly due to a decrease in V_{max} (8, 35) and correlated with in vivo aminopyrine metabolism (8). Different investigators have held a reduction in intrinsic clearance to be the main factor explaining decreased drug metabolism in cirrhosis both in man (12, 35) and rat (36). This is not in contradiction to our

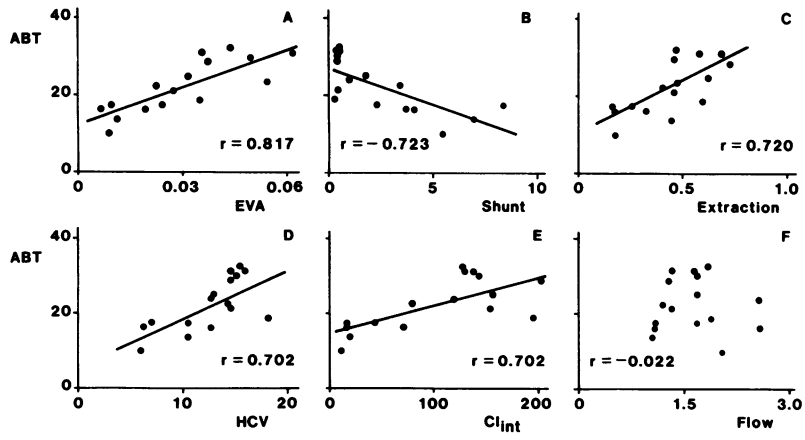

Figure 4. Determinants of the aminopyrine breath test in vivo (ABT_{AUC}, %dose). The relationship between EVA (milliliters per gram), intrahepatic shunt fraction (percent), aminopyrine extraction efficiency, HCV (milliliters) intrinsic aminopyrine clearance determined from microsomal *N*-demethylase (Cl_{int}, milliliters per hour) and PF (milliliters per minute per gram) are shown. The correlation coefficients are given in the figure. Multiple stepwise regression analysis selected extravascular albumin space and PF as the determinants of ABT_{AUC}, the regression equation being y = 18.63 + 403.07 × EVA − 5.42 × PF (F = 23.98, P < 0.0001).

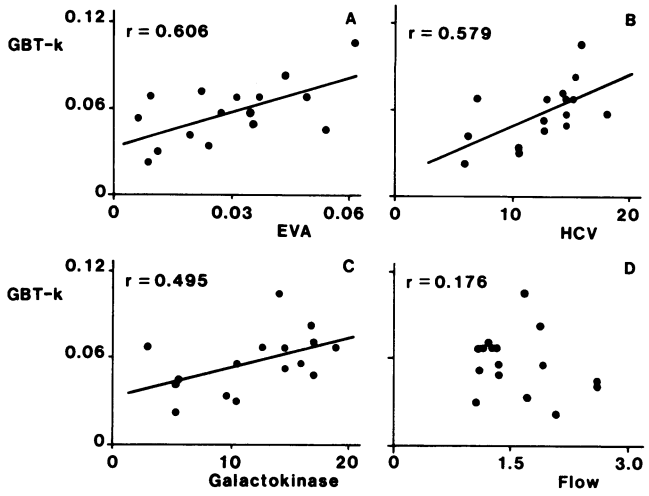

Figure 5. Determinants of the galactose elimination capacity in vivo (elimination rate constant GBT-k, $\% \cdot h^{-2}$). The relationship between EVA (milliliters per gram), HCV (milliliter), cytosolic galactokinase activity (micromoles per minute) and portal flow (milliliters per minute per gram) are shown. The correlation coefficients are given in the figure. Multiple stepwise regression analysis selected both EVA and PF as determinants of GBT-k, the regression equation being GBT − k = 0.06 + 1.01 × EVA − 0.02 × PF ($F = 7.96$, $P < 0.02$).

result, since in those studies neither sinusoidal capillarization had been assessed nor multifactorial regression analysis been performed.

The galactose elimination capacity, the oldest quantitative liver function test, is supposed to measure functional hepatocellular volume in man (16); in a morphometric study in another animal model of liver cirrhosis, we found excellent correlation between galactose elimination capacity and morphometrically determined hepatocellular volume (17), a finding confirmed in the present study (Fig. 5). This is in agreement with the correlation shown between galactose elimination and liver volume after different degrees of hepatic resection in man (37). Multivariate analysis selected again extravascular albumin space and hepatic perfusion rather than the rate-limiting enzyme or hepatocellular volume, however. The validity of this finding is supported by the excellent correlation between the two liver function tests.

Galactose elimination capacity is thought to reflect hepatocellular volume and under saturating conditions to be independent of hepatic perfusion (16); nevertheless, in normal liver galactose distributes into the space of Disse without evidence for diffusional hindrance (38). Keiding and Chiarantini have systematically investigated galactose elimination under conditions of varying flow and found the estimate of V_{max} to follow the sinusoidal distribution model (39). Therefore, our finding that galactose elimination capacity is determined by sinusoidal capillarization and flow probably reflects again alterations of nutrient flow, which is then the main determinant of hepatic function in cirrhosis.

Galactose elimination capacity was the only liver function test not separating cirrhotics from control animals. This is in agreement with clinical experience when unselected patients are studied (40, 41). This does not invalidate the use of galactose elimination capacity as a dynamic liver function test since it has been shown to be of prognostic value in patients with fulminant hepatic failure (42) and in patients undergoing portosystemic shunt surgery (43). Moreover, serial determinations of galactose elimination capacity may be of better value than single determinations in both patients (42, 44, 45) and animals (17) with liver cirrhosis.

In contrast to aminopyrine and galactose metabolism, shunt was selected as the sole determinant of serum bile acid levels (Fig. 3). An excellent agreement between a shunt index determined by an isotope technique and serum bile acid levels has been reported by Ohkubo et al. in man (14). Spillover into the systemic circulation due to intrahepatic shunting has been identified as the main determinant of serum bile acid levels in patients with chronic liver disease (46). In contrast, Poupon et al. found the intrinsic clearance to be the main determinant of bile acid levels in patients with liver cirrhosis and a portacaval shunt (47); they did not measure intrahepatic shunt, however, and speculated that it was presumably low in some of their patients. We have previously described defective uptake of bile acids in hepatocytes isolated from cirrhotic rats (48). Both, extraction and bile acid clearance, correlated with serum bile acid levels (Fig. 3) in line with the studies suggesting a correlation with intrinsic clearance (47, 48).

In man, determination of serum bile acid levels has been found to be a very sensitive indicator of chronic liver disease (49, 50) and to be of better prognostic value than the Child-Turcotte classification (51) or conventional liver tests (33). Our results suggest that serum bile acid level determination might convey additional information to that contained in the assessment of a microsomal or cytosolic function.

Morphometric analysis of the liver showed hepatocellular volume to be bimodally distributed (Fig. 2) in agreement with

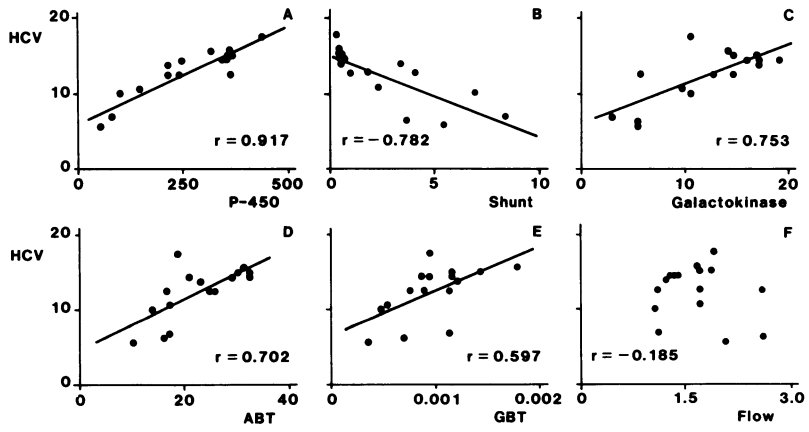

Figure 6. Determinants of morphometrically determined HCV (milliliter) in cirrhotic rat liver. The relationship between HCV and hepatic cytochrome P-450 content (P-450, nanomoles), intrahepatic shunt fraction (shunt, percent), cytosolic galactokinase activity (micromoles per minute), ABT_{AUC} (ABT, %Dose), GBT-k (GBT, $\% \cdot h^{-2}$) and PF (milliliters per minute per gram) are shown. The regression coefficients are given in the figure. Cytochrome P-450 and galactokinase were not further analyzed; among the remaining parameters (see text) stepwise multiple regression analysis selected IHS and PF as the sole determinants of HCV, the regression equation being HCV = 20.13 − 3.36 × PF − 1.28 × IHS ($F = 25.74$, $P < 0.0001$).

a previous publication from our laboratory (8). The functional equivalent of this heterogeneity is found in patients with liver cirrhosis (10, 11, 32, 40, 41, 44, 52) as well as in animal models (8, 17, 30, 35). The biological reasons for this heterogeneity are unknown at present. Some potential insight might be gained by our analysis of the determinants of hepatocellular volume (Fig. 6). By far the best correlation was seen with cytochrome P-450 content. This is not surprising in view of our previous observation that the cirrhosis model induced by chronic exposure to phenobarbital and carbon tetrachloride is compatible with the intact cell hypothesis (8). Determination of cytochrome P-450 in a representative sample of liver is not feasible in patients, however. Therefore, we did not include this factor into the multivariate analysis but restricted it to those factors that can be quantitated in vivo. Both quantitative liver function tests, ABT and galactose elimination, were statistically correlated with hepatocellular volume in agreement with earlier studies from our laboratories (8, 17); the best correlation was seen with the intrahepatic shunt fraction, however (Fig. 6). In the logistic regression model, shunt fraction and PF were selected as the best determinants of hepatocellular volume.

Shunt alone was an excellent predictor of hepatic volume showing an inverse relationship ($r = -0.783$, Fig. 6 *B*). This is not surprising in view of the fact that diversion of PF leads to hepatic atrophy (53–55). In this context, it is of interest to note that in a model of congenital hepatic portosystemic shunting, Bioulac-Sage described not only atrophy as seen with surgical shunts, but also the development of capillaries extending from the portal tracts in the hepatocellular parenchyma (56). The inverse relationship between hepatocellular volume and PF is more difficult to understand. Intact PF is necessary to maintain liver volume (57); therefore, one would predict a positive relationship between the two. It could be argued that PF and IHS are not independent parameters; there was no statistical correlation between the two, however ($r = 0.292$; data not shown). An alternative explanation could be that the animals with the highest PF were those with the most marked hyperdynamic circulation and, therefore, the most advanced disease (58, 59). Such a relationship could not be ascertained from our data. Cardiac output or noradrenaline levels should be determined to further follow up on this possibility.

In conclusion, our study correlating different aspects of hepatic function with hemodynamic and morphometrically determined structural parameters of rats with liver cirrhosis has identified sinusoidal capillarization and flow as the most important determinants of both, a microsomal and cytosolic function. By contrast, IHS is the main determinant of serum bile acid levels. IHS and PF are the main determinants of hepatocellular volume. Review of the literature shows that the findings reported here appear to be applicable to man. Further studies are needed to better correlate function with microcirculation in human liver. It also remains to be shown whether these relationships hold for liver disease of different etiologies. Finally, in future therapeutic investigations, the possibility to improve the microvascular exchange of substances and to maintain portal perfusion should be aggressively pursued.

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