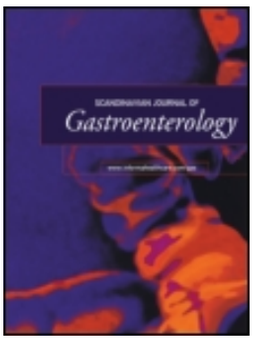

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Fasting Plasma Caffeine Concentration

A Guide to the Severity of Chronic Liver Disease

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Fasting plasma caffeine concentrations (FPCC) were measured in 86 outpatients being examined for suspected or known liver disease. Seven patients (8%) who avoided caffeine consumption had nonmeasurable FPCC; they were dropped from further consideration. The remaining 79 subjects were divided into 4 diagnostic groups: surgical shunt ($n = 11$); alcoholic, posthepatitic, or primary biliary cirrhosis ($n = 29$); miscellaneous liver disease ($n = 23$); and normal liver ($n = 16$). FPCC was highest (mean, 17.8 μmol/l) in the shunt group, followed by the cirrhosis (12.3), miscellaneous liver diseases (4.6), and normal liver (2.1) groups. FPCC seemed to reflect severity of functional impairment, further supported by highly significant correlations with quantitative liver function tests, such as aminopyrine breath test ($R_s = -0.89$; $n = 66$), indocyanine green disappearance ($R_s = -0.85$; $n = 65$), and galactose elimination capacity ($R_s = -0.70$; $n = 75$). A careful dietary history showed no significant difference in caffeine consumption among the groups. It is suggested that in regular coffee drinkers FPCC might serve as a simple and convenient guide to the severity of functional impairment in chronic liver disease.

Key words: Aminopyrine breath test; caffeine; indocyanine green disappearance; liver function tests

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It is generally acknowledged that the conventional liver tests, while useful for screening patients with suspected or for monitoring patients with known liver disease, are not good indicators of the different liver functions. This is because their pathophysiological basis is not fully understood. In addition, the rate of synthesis, volume of distribution, or pathway and rate of disposition of the substances measured (such as enzymes) are not known. Finally, the concentrations of some markers (such as bilirubin) may be influenced by extrahepatic pathology.

Many attempts have been made to overcome these difficulties, usually by administering known amounts of an exogenous compound handled exclusively by the liver, whose fate is sufficiently well characterized. Examples include the elimination of galactose (1), sulfobromophthalein (2), indocyanine green (3), aminopyrine (4), and antipyrine (5). However, these tests are not without problems. They may be associated with hypersensitivity reactions, are relatively complicated to perform, tend to be costly, and are more or less inconvenient to the patient.

During the course of studies designed to validate a caffeine breath test (6), we were able to confirm the finding of Desmond et al. (7) that the plasma clearance of caffeine is markedly reduced in patients with liver disease. Furthermore, our results (6, 8) showed that over a wide range of clearance values there was a highly significant relationship to fasting plasma caffeine concentrations (FPCC). We therefore hypothesized that a simple measurement of the caffeine blood level—without prior dosing—might serve as an indicator of hepatic functional impairment. To

test this idea, FPCC was measured in 86 ambulatory subjects, and the results were compared with quantitative liver function test results.

MATERIALS AND METHODS

Subjects studied

Eighty-six consecutive patients, seen in our outpatient facility for examination of suspected or known liver disease were included in this study. Of these, seven patients had to be dropped from final analysis because of unmeasurable plasma caffeine concentrations as a result of caffeine avoidance. The other 79 patients were divided into 4 groups in accordance with the results of clinical, laboratory, histological and/or radiological findings (Table I).

Group A, designated shunt, consisted of 11 patients with end-to-side portacaval ($n = 7$) or distal splenorenal ($n = 4$) shunt because of biopsy-documented alcoholic cirrhosis ($n = 8$) or congenital hepatic fibrosis ($n = 3$). They had been operated on from 1 to 7 years (mean, 3 years) before this investigation. All patients were considered to be in a stable condition, although two had mild ascites.

Group B, designated cirrhosis, was composed of 29 patients. In 15, the cirrhosis was considered to be of alcoholic origin (prolonged intake of >150 g alcohol/day), 10 were diagnosed as having posthepatitic cirrhosis (PHC) (with accompanying chronic active hepatitis in 8) and 4 as having primary biliary cirrhosis (PBC) (stage 4, positive AMA > 1:100, IgM levels > 3.8 g/l). Histologic confirmation was available in all except five with alcoholic cirrhosis (AC), in whom needle biopsy was considered too risky because of prolonged prothrombin time and/or thrombocytopenia.

Group C, designated miscellaneous liver disease, included 23 patients, of whom 10 had biopsy-documented chronic active hepatitis (CAH) without cirrhosis, 5 had chronic persistent hepatitis (CPH), and 5 had noncirrhotic alcoholic liver disease (ALD). In one each, the diagnosis of remitting hepatitis A, porphyria cutanea tarda, and *Echinococcus multilocularis* infection was made on typical clinical, biochemical, and serological grounds.

Sixteen subjects, designated controls, formed group D. After referral to our department for study of various abdominal complaints or for assessment of liver function before disulfiram treatment, liver disease was ruled out by physical examination, routine laboratory tests, and ultrasound of the abdomen, which all gave normal results. Six subjects had as sole abnormality a slight increase in the γ-glutamyltranspeptidase, which in four was thought to be due to excessive alcohol consumption, in one to antiepileptic drugs, and in one to either one or both of these factors. In one additional subject, the aspartate aminotransferase level was slightly abnormal (28 IU/l).

Further characterization of patients in terms of sex, age, and smoking habits, the mean values and ranges of routine liver test results, and details concerning concomitant medication are summarized in Table I.

Experimental procedure

After a 12-h overnight fast, routine study (starting at 0800 h) included a complete history and physical examination, preceded by the drawing of blood for routine laboratory tests and for measurement of the FPCC. A careful evaluation of the caffeine intake was made during the day before the study. Estimation of this intake was based on the assumption that a cup of coffee contains on the average 100 mg, a cup of tea 50 mg, and a serving of cola beverage 40 mg of caffeine (9). Subsequently, where indicated, at least two (and, when feasible, three) quantitative liver function tests were performed in the recumbent subject. Indocyanine green (ICG; Hynson, Westcott and Dunning, Baltimore, Md., USA) was injected intravenously (0.5 mg/kg body weight), followed by collection of blood samples from the opposite arm at regular intervals between 3 and 21 min. Thereafter, galactose (E. Merck, Darmstadt, FRG) was administered intravenously (0.5 g/kg body weight) and blood samples obtained between 20 and 45 min. Finally, the aminopyrine breath test (ABT) was performed with collection of breath samples during 60 min after intravenous injection of a tracer dose (1.5 μCi) of (^{14}C-dimethylamino)antipyrine

Table I. Clinical and laboratory data (x ± SD; range in parentheses)

Group	*n*	Women, no.	Age, years	Smoker, *n*	Fasting conjugated bile acids, μmol/l	Total serum bilirubin, μmol/l	Aspartate aminotransferase, IU/l	Alkaline phosphatase, IU/l	Prothrombin time, %	Serum albumin, g/l	Concomitant drug treatment*, no. of patients in parentheses	
Shunt, A	11	3	45 ± 13 (22–60)	9	51 ± 37 (14–135)	37 ± 41 (15–163)	57 ± 54 (15–205)	70 ± 30 (31–120)	69 ± 19 (45–100)	37 ± 4 (30–46)	1 (4) 2 (1) 3 (1) 4 (1)	5 (1) 6 (1)
Cirrhosis, B	29	10	52 ± 12 (21–76)	7	24 ± 20 (2–88)	32 ± 56 (7–320)	38 ± 23 (11–126)	75 ± 61 (24–276)	77 ± 16 (46–100)	41 ± 7 (28–53)	1 (12) 2 (3) 3 (1) 4 (1)	5 (3) 6 (2) 7 (10) 8 (2)
Miscel. liver disease, C	23	12	42 ± 17 (17–74)	8	10 ± 14 (1–50)	19 ± 29 (5–148)	64 ± 122 (6–610)	57 ± 54 (20–276)	82 ± 19 (37–100)	44 ± 5 (36–52)	1 (2) 3 (2) 5 (1) 6 (4)	7 (4) 8 (1)
Controls, D	16	4	37 ± 12 (25–68)	9	3 ± 2 (1–6)	12 ± 7 (5–24)	15 ± 7 (2–28)	28 ± 6 (18–40)	91 ± 9 (76–100)	47 ± 4 (42–53)	6 (3) 9 (2)	
Normal range					0–6	0–26	0–20	14–47	70–100	3.75–6.0		

* Concomitant drug treatment: 1 = diuretics: spironolactone, furosemide, chlorthalidone, amiloride; 2 = beta-blockers: propranolol, atenolol; 3 = digitalis: digoxin; 4 = allopurinol; 5 = antidiabetics: metformin, insulin; 6 = psychotropics: nitrazepam, oxazepam, lorazepam, triazolam, pizotifen; 7 = prednisone; 8 = azathioprine; and 9 = antiepileptics: phenytoin, carbamazepin, phenobarbital.

(Amersham International, UK). Except for patients with clinically advanced liver disease, women of childbearing age were not submitted to the ABT.

During a follow-up study of 6 months, it was possible to obtain repeat measurements of the FPCC in 41 patients with clinically stable liver disease. In 22 of these subjects, the FPCC was determined within 4 weeks of the first measurement, and in the others within 6 months.

Analytical methods

All routine liver tests (Table I), with the exception of conjugated bile acids, were performed in the central hospital laboratory by standard methods. Conjugated serum bile acids were measured with a radioimmunoassay, using a commercial kit (Becton Dickinson, Orangeburg, N.Y., USA). Determinations of ICG and calculation of the elimination constant (K_1) (3), measurement of galactose and estimation of galactose elimination capacity (GEC) (1), and the ABT (4, 10) were carried out by established procedures.

For determination of caffeine concentrations, the recently described high-performance liquid chromatography method (11) was used. On each analysis run (24 samples), 2 control samples with known caffeine content were measured. The coefficient of intraday variation ($n = 8$) was 2.9%; the coefficient of interday variations ($n = 18$) was 3.3%.

Calculations

For the calculations and the curve-fitting in Figs. 2 and 3 a Hewlett Packard HP 85 computer and modified standard programs (Hewlett Packard, Palo Alto, Calif., USA, 1980) were used. Linear transformation for the best fit was performed assuming a hyperbolic relationship between the FPCC and the ICG K_1 or aminopyrine breath test, respectively. This procedure also yielded the smallest values of variance for all correlations tested. The equation used was

$$(y + A)^{1/2} = B \cdot 1/(x - C) + D,$$

where x denotes the ICG K_1 or aminopyrine breath test, and y the FPCC. B is the slope of the transformed relationship, and C and D^2-A represent the asymptotes on x or y axes, respectively. For calculation of 90% confidence limits in the figures, standard procedures were applied (12).

RESULTS

The three groups of patients (groups A to C) differed clearly in the severity of the liver disease and the extent of spontaneous or surgically induced portosystemic shunting. These differences were evident already in some of the routine laboratory tests (Table I). As indicated by the ranges, however, there was considerable overlap between groups; in addition, normal values were found for each of the tests—except bile acids—even in groups A and B.

In contrast, the extent of functional derangement was reflected by all the three quantitative tests in a more comparable fashion (Table II). Whereas in the shunt group ICG K was on an average reduced to approximately 25%, in the cirrhotic group it approached 50%, and in the miscellaneous liver disease group 85% of controls. Although the results of the ABT paralleled those of ICG K in the patients with shunt, impairment of demethylation was relatively more pronounced in the cirrhotic group. When the GEC is used as a standard of comparison, it must be kept in mind that approximately 2.5 mg/kg/min of galactose are removed by extrahepatic mechanisms (14). Nevertheless, GEC was somewhat better maintained, being on the average 40% of controls in group A, 50% in group B, and 70% in group C.

Mean FPCC appeared to indicate the severity of liver disease almost as well as the quantitative tests. With 6 µmol/l ($\bar{x} + 2$ SD) representing the upper limit of 'normal', there was, with the exception of a patient with splenorenal shunt due to congenital fibrosis, no overlap of values between shunts and controls (Fig. 1, upper panel). Only 5 of the 29 cirrhotic patients fell within the range observed in controls. As evidenced by the results of the dietary history, these differences in mean FPCC were not simply explained by differences

Table II. Fasting plasma caffeine levels and quantitative liver function tests ($x \pm SD$; range in parentheses)

	Total no. of patients, *n*	Indocyanine green elimination constant (K)		Aminopyrine breath test		Galactose elimination capacity		Fasting plasma caffeine concentration, μmol/l
		n	min^{-1}	*n*	% dose × kg/ mmol CO_2	*n*	mg/min/kg	
Normal range			0.14–0.28		0.68–1.08		6–9	1–6
Shunt, A	11	9	0.05 ± 0.02 (0.03–0.08)	11	0.21 ± 0.16 (0.05–0.58)	10	4.2 ± 0.8 (3.3–5.3)	17.8 ± 9.4 (0.6–32)
Cirrhosis, B	29	24	0.10 ± 0.04 (0.02–0.17)	27	0.31 ± 0.21 (0.03–0.79)	29	4.7 ± 1.0 (2.7–7.3)	12.3 ± 10.5 (0.7–50.6)
Miscel. liver disease, C	23	20	0.17 ± 0.05 (0.08–0.28)	17	0.61 ± 0.25 (0.18–1.01)	21	5.7 ± 1.2 (4.0–7.7)	4.6 ± 4.1 (0.6–17.3)
Controls, D	16	12	0.19 ± 0.03 (0.15–0.25)	11	0.84 ± 0.12 (0.70–1.12)	15	7.0 ± 0.8 (6.0–8.4)	2.1 ± 1.6 (0.1–4.9)

in caffeine consumption, since the latter was on the average comparable in all groups (Fig. 1, lower panel). In addition, despite the inducing effect of smoking on caffeine metabolism (15, 16), there was considerable overlap in FPCC between smokers and nonsmokers.

As shown in Figs. 2 and 3, both the elimination of ICG and the metabolism of aminopyrine had an apparently hyperbolic, highly significant relationship to caffeine concentration; the R_s values for untransformed data were -0.85 ($n = 65$) and -0.89 ($n = 66$), respectively. Considerably more scatter was evident in comparing FPCC with GEC, particularly with GEC values <5 mg/min/kg; nevertheless, R_s was -0.70 ($n = 75$).

Table III summarizes the R_s values obtained when comparing conventional liver tests with ICG fractional clearance and the ABT in our study group. Hepatic functional impairment, as measured by these two indices, is rather poorly reflected in aspartate aminotransferase, prothrombin, or serum albumin levels. On the other hand, fasting conjugated bile acid concentrations suggest a close relationship to flow-limited function (13).

Comparison of FPCC with the ABT also gave some indications concerning the sensitivity of plasma caffeine levels in attesting to the presence or absence of liver disease. In eight subjects with miscellaneous liver disease (one CPH, one hepatitis A, two CAH, four ALD) and two with cirrhosis (one PBC, one AC) the ABT was >0.68% dose · kg/mmol CO_2, thus being falsely negative. Although three of these reached 6 μmol/l, the upper limit of normal, they all were falsely negative in terms of FPCC as well. Conversely, of all 45 subjects with an abnormal ABT, only 7 (or 14%) had an FPCC within 'normal' range.

Intrapatient comparison of FPCC during follow-up study was possible in 41 subjects. In 22 of these, FPCC was, with an average of 10.2 μmol/l (obtained within 4 weeks), comparable to the initial level of 10.7 μmol/l (coefficient of variation, 18.6%); the other 19 had levels of 7.2 μmol/l (obtained within 6 months), compared with 6.6 μmol/l at the outset (coefficient of variation, 14.5%).

DISCUSSION

Caffeine may be considered an almost ideal compound for measuring liver function. After oral intake, it is rapidly and completely absorbed, shows little first-pass elimination, is distributed within body water, is only slightly bound by serum proteins (7, 17), and undergoes virtually complete hepatic metabolism (18). Since caffeine is con-

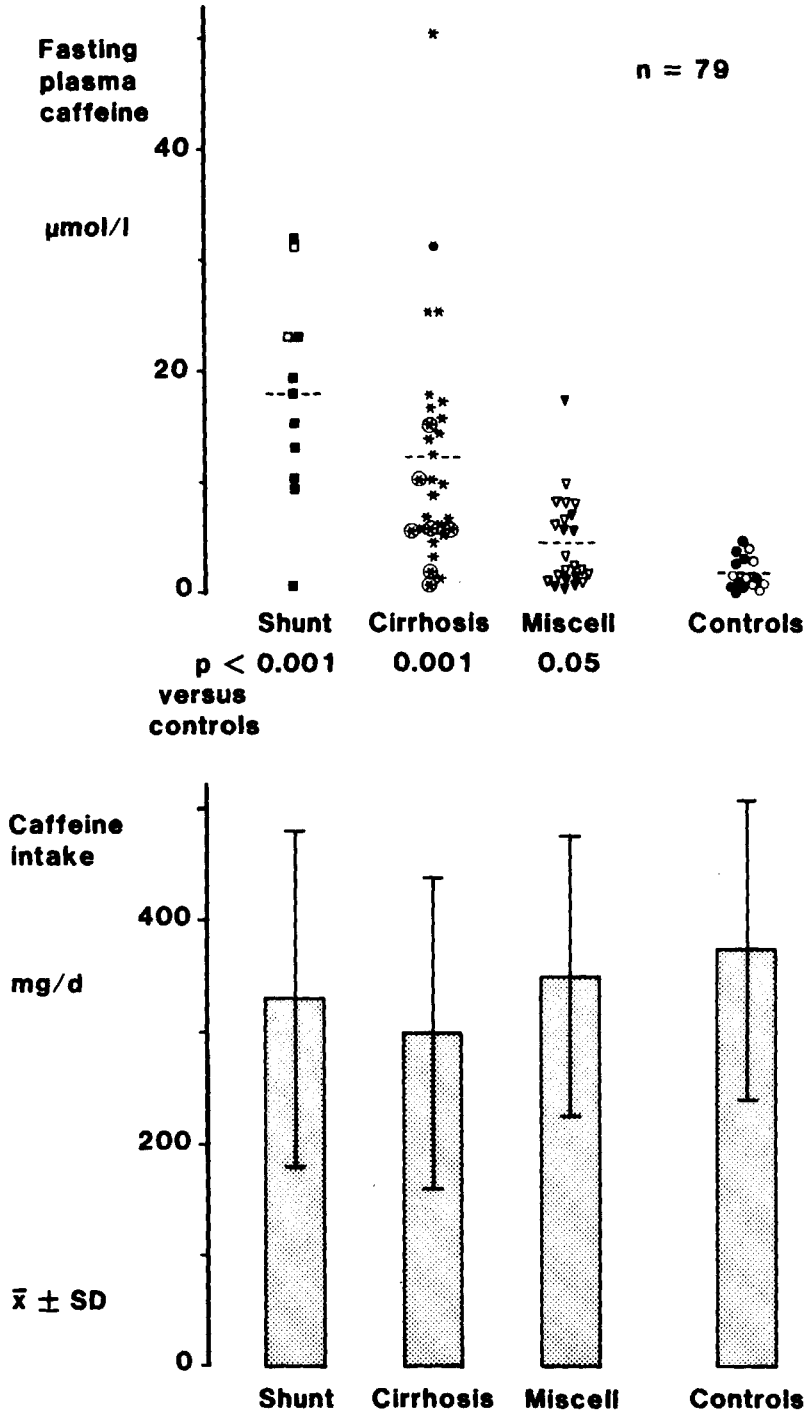

Fig. 1. In the upper panel the fasting plasma caffeine concentrations of all 79 subjects are arranged by diagnostic groups; the lower panel depicts the estimated caffeine intake during the day before study. Closed symbols (■, ▼, ●) and circled stars (⊛) denote smokers; open symbols (□, ▽, ○) and stars (★) nonsmokers.

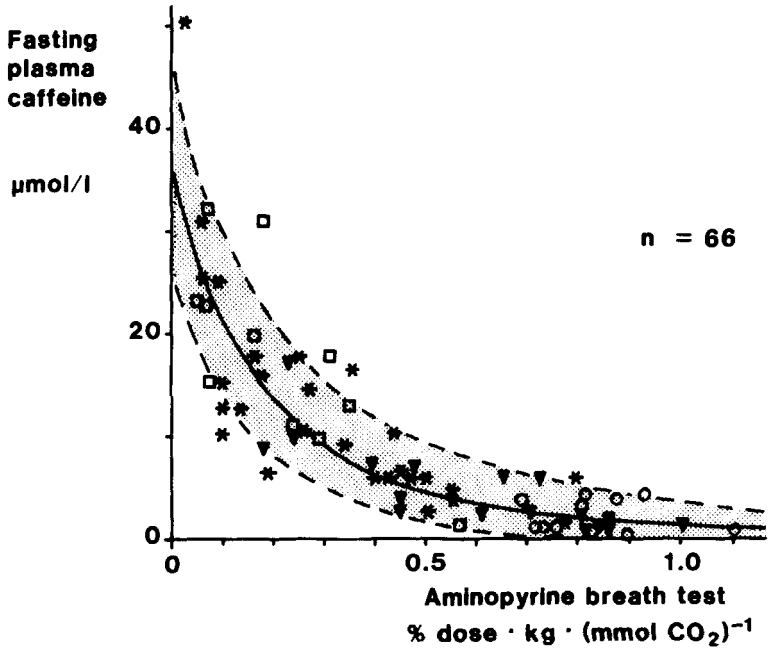

Fig. 2. Relationship between fasting plasma caffeine concentrations and results of the aminopyrine breath test. The broken lines denote the 90% confidence limits. The R_s value is -0.89. Best-fit linearization yielded $r = 0.88$. R_s values are -0.88 and -0.86, respectively, when nonsmokers ($n = 38$) and smokers ($n = 28$) are considered separately.

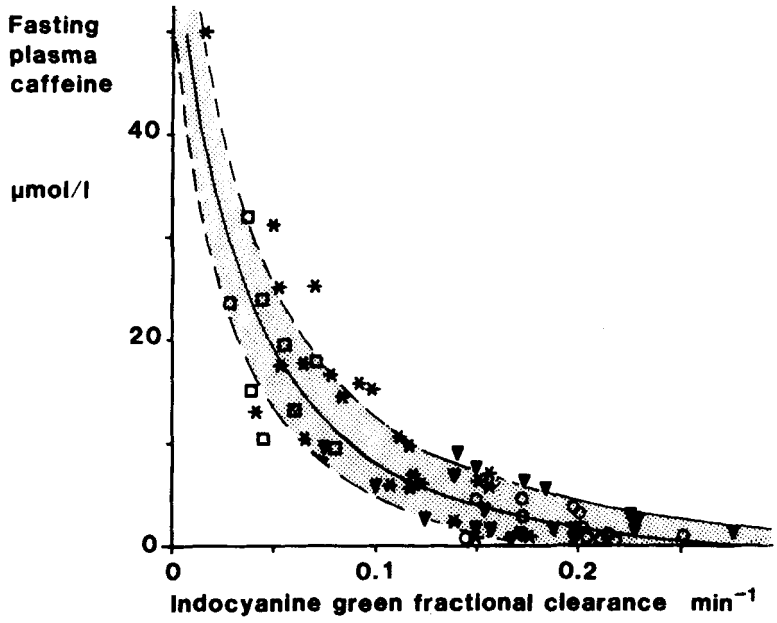

Fig. 3. Relationship between fasting plasma caffeine concentrations and the indocyanine green fractional clearance. The R_s value is -0.85. Best-fit linearization yielded $r = 0.89$. R_s values are -0.88 and -0.81, respectively, when nonsmokers ($n = 35$) and smokers ($n = 30$) are considered separately.

Table III. Spearman rank correlation coefficients (R_s)

	n	Aspartate amino-transferase	Prothrombin time	Serum albumin	Fasting conjugated bile acids	Fasting plasma caffeine concentration
Indocyanine green elimination constant (K)	65	−0.36	0.46	0.63	−0.78	−0.85
Aminopyrine breath test	66	−0.40	0.61	0.54	−0.67	−0.89

All values are significant at least at the 5% level.

sumed ubiquitously in various beverages and foodstuffs, measurable plasma levels are found in almost every subject. In view of its normal half-life of 3–5 h, concentrations are even detectable in coffee drinkers without liver disease after an overnight fast.

On the basis of previous results (6, 8) showing an inverse relationship between FPCC and caffeine plasma clearance we hypothesized that this tracer might serve as a guide to the hepatic handling of other low-extraction compounds. To test this idea, FPCC were obtained in three groups of patients differing in the severity of their liver disease and in a group of controls. Since most of these subjects were given an intravenous dose (1.5 μCi) of ^{14}C-aminopyrine for diagnostic purposes, this offered an opportunity to compare the handling by the liver of two low-extraction compounds. The fact that both compounds undergo oxidative demethylation, albeit mediated in part by different cytochromes (4, 19), seemed to make such a comparison even more relevant.

The results, attesting to a significant curvilinear relationship between FPCC and ABT, support the contention that a single fasting plasma level of caffeine may be representative of the hepatic capacity to dispose of low-extraction xenobiotics. The surprisingly close correlation probably reflects the fact that FPCC in most individuals represents a level that, in view of comparable dosing, may be considered a steady-state concentration primarily determined by disposition. Indeed, the hyperbolic shape of the curve suggests that the sensitivity of FPCC in indicating defective clearance increases with diminishing hepatic function.

Since a relationship between clearances of high- and low-extraction compounds was documented in previous investigations (20), the finding of a similar correlation of FPCC to ICG K was not unexpected. The fact, however, that both curves (Figs. 2 and 3) are virtually superimposable seems to support the idea of a common, major determinant for hepatic function. ICG—in contrast to aminopyrine—does not undergo significant hepatic metabolism (21); the common denominator must therefore be hepatic clearance. Since clearance may be expressed as $F \cdot E$ (where F = hepatic plasma flow, and E = extraction ratio), and since clearance of high- and low-extraction substances is dominated by F and E, respectively, our results seem to support the contention that liver disease results in proportional changes of both parameters. In an attempt to explain this phenomenon an 'intact hepatocyte hypothesis' has been advanced (22), suggesting that liver disease may be viewed as resulting in a diminished, normally perfused and normally functioning cell mass. Attractive as this idea may be, reduction to such a simple denominator is difficult to visualize, considering the complex changes of chronic liver disease. Furthermore, experimental support for the hypothesis is as yet limited (23). An alternative explanation could be based on the well-established phenomenon that in liver disease extraction of high-extraction compounds is reduced to the level of low-extraction substances (24). Presumably, their hepatic removal is then largely determined by the remaining mass of abnormally functioning cells (25).

Albeit differing in the underlying mechanisms, the similarity of the relationships between serum creatinine concentration and inulin clearance (26)

and between FPCC and ABT or ICG K is striking indeed. In view of this, and taking into account the extensive clinical experience with serum creatinine as an estimate of glomerular filtration in renal failure, FPCC might be considered a similar index of hepatic functional impairment. The recent introduction of simple immunoassays for caffeine will bring FPCC measurements within reach of routine hospital laboratories (27); thus, determinations of FPCC might become clinical routine, provided its relevance as an indicator of hepatic disposition can be expanded to clinically important drugs.

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