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Caffeine: A Model Compound for Measuring Liver Function

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The effects of liver disease on caffeine plasma clearance (Cl) and on exhalation of $^{14}CO_2$ following i.v. injection of 2 μCi of [3-methyl-^{14}C]caffeine together with 125 mg of the unlabeled compound were measured in 15 patients with cirrhosis, 11 subjects with miscellaneous liver disease, and 10 normal volunteers. Compared to mean values for Cl (2.02 ± S.D. 0.68 ml per min per kg) and t½ (3.8 ± 0.9 hr) in normal volunteers, cirrhotics were characterized by highly significant reductions in Cl (to 0.76 ± 0.40) and prolongation in t½ (to 13.7 ± 13.0), whereas the volume of distribution (VD) remained relatively unchanged (0.57 ± 0.16 vs. 0.64 ± 0.13 liter per kg in normals). Cumulative $^{14}CO_2$ production and specific activity of $^{14}CO_2$ in breath decreased in parallel (r = 0.83) with Cl. Patients with miscellaneous liver disease exhibited only small changes in Cl and t½; however, $^{14}CO_2$ parameters in breath appeared more sensitive in indicating the slight functional derangement. In view of the correlation (R_s = 0.83) of cumulative $^{14}CO_2$ excretion with the initial disappearance constant for bromosulfophthalein, the caffeine breath test may be considered as a quantitative measure of hepatic microsomal activity; based on a surprisingly close, hyperbolic relationship between Cl and fasting caffeine plasma concentrations, the latter might serve as a simple guide to severity of liver disease.

In recent years, there have been increasing efforts to develop liver function tests based on a more precise definition of the fate in the body of the substrate used. Two such tests "nominated but not yet elected" (1), namely the aminopyrine breath test (ABT) and the serum bile acid level (SBAL), have been the subject of particular attention. Although for the ABT, a chemically pure exogenous compound with reasonably well-defined pharmacokinetic behavior is used and an endproduct of hepatic microsomal metabolism is measured (2–5), the procedure requires administration of radioactivity, the equipment to count ^{14}C, and has the drawback of using a substance with potentially adverse effects. Measurement of SBAL offers numerous advantages. With radioimmunoassay techniques available (6), determination of SBAL is simple; the drawing of a single fasting blood sample represents the only risk. Bile acids are endogenous compounds; however, their fate depends on many variables including absorption, hepatic uptake, conjugation, biliary excretion, gallbladder emptying, and the influence of the gut flora (7–9). The relative importance of these determinants of SBAL is only partially known. Furthermore, a rational interpretation of SBAL (as, e.g., of some drug levels) is not possible in view of the lack of simple pharmacokinetic modeling.

Caffeine, an exogenous substance which is extensively consumed in beverages and food stuffs, undergoes practically complete absorption (10), is metabolized almost exclusively in the liver (11) and, in single doses (equivalent to 1 to 2 cups of coffee), may be considered innocuous. Its pharmacokinetics in normal man are now well elucidated (10, 12) and, as expected, its plasma disappearance in subjects with cirrhosis is delayed (13). Since demethylation apparently represents an important metabolic pathway, labeling of methyl groups—comparable to aminopyrine—offers the possibility of a breath test.

Based on studies in animals, the feasibility of a caffeine breath test (CBT) in normal man was demonstrated (14). We now report the use of this approach in patients with liver disease. The results support the contention that measurement of $^{14}CO_2$ in breath following administration of ^{14}C-labeled caffeine closely reflects the plasma clearance of the compound. Presumably, therefore, the CBT yields quantitative information concerning hepatic functional derangement. Since, in addition, plasma clearance appears to be the main determinant of fasting plasma levels, the use of caffeine as agent for testing liver function may combine the advantages of both ABT and SBAL.

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MATERIALS AND METHODS

MATERIALS

Caffeine as a sterile caffeine sodium benzoate solution in water for injection use containing 125 mg of caffeine and 125 mg of sodium benzoate per 1 ml ampoule was obtained from Dr. H. Gattiker, Pharmazeutische Praeparate, Zurich, Switzerland.

[3-methyl-^{14}C]Caffeine was synthesized and purified as described elsewhere (14). In the final preparation, less than 0.1% radiochemical impurity could be detected. Ampoules were prepared containing 2 μCi of the labeled caffeine in 2 ml of 0.9% NaCl. They were sterilized in the hospital pharmacy of the Inselspital, Berne and tested for pyrogenecity in the Swiss Red Cross Control Laboratory, Berne. All solutions for i.v. injection were stored at 4°C until use.

SUBJECTS STUDIED

Table 1 summarizes the clinical and laboratory data of the three groups of *outpatients* studied.

Group A consisted of 8 patients, aged 34 to 66 years ($\bar{x}$ = 55), with clinically established and (with the exception of patients P.A. and R.S.) biopsy-proven cirrhosis of the liver due to alcoholic liver disease or due to chronic active hepatitis. Three cirrhotics (R. S., R. B., and P. R.) had clinically detectable ascites at the time of study.

Seven females, aged 42 to 73 years ($\bar{x}$ = 56), with primary biliary cirrhosis (PBC, Stage 4) belonged to Group B. All had positive antimitochondrial antibodies (titer > 1:100), elevated IgM concentrations (>380 mg per dl) in plasma, and a compatible histology.

Group C was composed of 11 patients, aged 24 to 71 years ($\bar{x}$ = 52), suffering from miscellaneous liver disease including noncirrhotic alcoholic liver disease, echinococcosis, chronic persistent hepatitis, noncirrhotic chronic active hepatitis, hemochromatosis treated by regular phlebotomy, and Gilbert's syndrome. The diagnosis was based on typical clinical and laboratory data, and except in patients P. B., P. A., and E. S., confirmed by histology.

All patients had normal serum creatinine values, and none but four had relevant concomittant illness: Patient E. R. had aortic valve replacement 16 years previously for aortic stenosis; Patient A. M. had insulin-dependent diabetes mellitus; Patient P. R. had insulin-dependent diabetes mellitus and porto-caval end-to-side anastomosis 14 years previously, and Patient E. G. had lactose intolerance. The various drugs taken by the patients are given in Table 1.

In addition to routine tests performed in the central hospital laboratory according to standard methods, work-up of these patients included, whenever possible, measurement of the initial bromosulfopthalein (BSP) plasma disappearance rate (k_i) following i.v. injection of 5 mg per kg of body weight (15) and determination of the galactose elimination capacity (GEC) after i.v. administration of 0.5 gm per kg of body weight (16). In addition, fasting conjugated bile acids were measured using a commercial radioimmunoassay kit (Becton Dickinson, Orangeburg, NY). The caffeine study was performed within 1 to 3 weeks of this work-up.

As a *control group*, 10 healthy male volunteers aged 27 to 56 years ($\bar{x}$ = 38) were studied. All had normal clinical findings, and routine laboratory tests, including transaminases, γ-glutamyl transpeptidase, alkaline phosphatase, bilirubin, prothrombin time, and serum creatinine yielded normal values. Individual age and body weight are shown in Table 2. None of this group was taking any drugs the day before or during the study.

EXPERIMENTAL DESIGN

Informed consent was obtained from all participants. They had to abstain from caffeine-containing beverages and food stuffs at least during the 12 hr prior to investigation. All experiments were performed in subjects who were quietly resting in bed, starting at 8 a.m., following an overnight fast. Samples of venous blood and breath were taken before the start of each experiment. Following 30 min of bed rest, during which endogenous CO_2 production was expected to become basal, 2 μCi [3-methyl-^{14}C]caffeine together with 125 mg unlabeled caffeine were injected i.v. in a cubital vein. Venous blood from a vein of the opposite arm and breath samples were collected at regular intervals during 3 hr. After the 3-hr sampling, participants were allowed to leave for a caffeine-free lunch of their own choice. They returned and were again immobilized in bed 6.5 hr after injection of caffeine for collection of the last two plasma and breath samples at Hours 7 and 8.

METHODS

BLOOD SAMPLES

Venous blood samples were drawn into heparinized plastic tubes. The blood was immediately centrifuged (10 min, 3,000 rpm) and the plasma stored at −20°C until use.

Following alkaline extraction of 0.5 ml of plasma with chloroform (analytical grade, Merck, Darmstadt, Germany) caffeine was determined as described by Cohen et al. (17) using GLC (Perkin-Elmer 3920) with a glass column 180 x 0.2 cm i.d. packed with OV 17 3% on 80- to 100-mesh chromosorb WHP (Applied Science Laboratories, State College, Pa.), phosphorous-nitrogen sensitive detection at 600°C (Perkin-Elmer PN-Detector), and mepivacaine (Scandicaine® 2%, AB Astra-Bofors, Södertälje, Sweden) as internal standard.

BREATH SAMPLES

Collection of exhaled air was accomplished by blowing through a straw equipped with a one-way valve directly into a counting vial containing 4 ml of hyamine, 2 mmoles in methanol/ethanol (1:1, v/v), and phenolphthalein as indicator (2).

^{14}C-Radioactivity in breath samples was counted in a Packard liquid scintillation counter (TRI-CARB 2660) after addition of 5 ml of scintillation cocktail containing toluene (800 ml), Triton X-100 (200 ml), 2,5-diphenyloxazole (5 g) (PPO, Merck, Darmstadt, Germany), and 1,4-bis-(5-phenyloxazole-2-yl)benzene 100 mg (POPOP, Merck, Darmstadt, Germany).

TABLE 1. CLINICAL AND LABORATORY DATA OF THE PATIENTS STUDIED[a]

Patient	Sex	Age (yr)	Body-weight (kg)	GEC (mg/min/kg)	BSP[b] k_i (%/min)	Fasting conjugated bile acids (μmoles/liter)	Total serum bilirubin (mg/100 m)	Albumin (gm/100 ml)	SGOT (μmoles/min/liter)	Alkaline phosphatase (μmoles/min/liter)	Diagnosis[c]	Concomitant[d] drug treatment
Normal values:				6.0–9.1	9.5–16.0	0–6	0.2–1.5	3.8–5.4	0–20	14–47		
						A. Cirrhotic Liver Disease (n = 8)						
R. S.	M	66	70	3.9	3.2	20	2.9	3.6	46	92	AC	3, 4
P. A.[e]	M	63	100	4.4	6.0	22	1.3	3.7	55	47	CAH	1
R. B.[e]	M	53	57	3.8	3.3	210	1.5	3.2	17	56	AC	3, 7
C. W.[e]	M	64	55	5.3	4.8	14	1.0	3.3	36	38	CAH	5
H. G.	F	51	41	5.5	—	6	1.2	5.2	55	47	CAH	1
J. S.	F	53	78	4.7	6.0	10	1.4	4.5	32	28	CAH	1, 3, 4, 8
P. R.	M	34	64	4.6	—	84	0.2	2.3	122	121	CAH	3, 4, 7, 9
A. C.	M	52	60	5.3	6.0	22	0.8	3.5	38	45	CAH	5, 4
$\bar{x}$		55	66	4.7	4.9	49	1.3	3.7	50	59		
S.D.		10	18	0.6	1.3	70	0.8	0.9	32	31		
						B. PBC (n = 7)						
Z. Z.	F	42	68	3.8	—	65	5.5	3.8	89	153	PBC	2
G. R.	F	61	65	4.1	3.3	18	2.9	3.6	71	109	PBC	4, 6
H. M.	F	42	65	5.8	6.0	95	2.3	4.0	68	255	PBC	2, 5, 6
H. L.	F	44	49	5.5	7.9	70	7.0	4.1	173	142	PBC	2, 6
B. L.	F	73	55	5.0	6.0	48	3.1	3.0	38	119	PBC	5, 13
E. G.	F	56	50	4.0	—	39	1.9	4.3	77	197	PBC	1, 11, 12, 14
E. R.	F	71	49	6.1	—	48	1.2	3.3	48	170	PBC	1, 3, 8, 10
$\bar{x}$		56	57	4.9	4.0	55	3.4	3.7	81	164		
S.D.		13	8	0.9	1.9	25	2.1	0.5	44	50		
						C. Miscellaneous Liver Disease (n = 11)						
K. K.	F	71	55	7.0	10.6	6	0.4	3.4	11	66	ECH	16
P. A.	M	52	118	6.9	—	4	0.3	3.8	18	32	ALD	1
H. Lu.	M	59	85	5.4	7.7	2	0.7	4.6	17	37	CPH	—
I. L.	F	53	61	5.7	8.7	38	0.4	4.6	13	27	ECH	1
P. B.	M	49	79	—	—	1	1.6	4.0	14	21	GIL	—
A. M.	M	50	70	5.4	6.6	18	1.0	3.7	18	32	ALD	9
E. S.	M	56	96	6.1	—	1	0.9	3.8	23	31	ALD	15
A. L.	M	61	80	5.9	7.7	5	0.7	4.3	19	30	CAH	1, 5
R. F.[e]	M	24	102	7.1	8.9	4	0.6	4.2	20	40	CPH	—
F. R.	M	53	65	8.7	—	<1	0.6	4.1	16	33	HEMO	1
H. I.[e]	M	47	75	6.2	7.3	3	0.7	4.3	20	40	ALD	—
$\bar{x}$		52	81	6.4	8.2	8	0.7	4.1	17	35		
S.D.		12	19	1.0	1.3	11	0.4	0.4	3	12		

[a] The patients in this table have been arranged according to the values of caffeine clearance (see Table 3) for each group.

[b] Initial bromosulfophthalein disappearance constant.

[c] Abbreviations denote the following: AC, alcoholic cirrhosis; CAH, chronic active hepatitis; ECH, echinococcosis; GIL, Gilbert's syndrome; CPH, chronic persistent hepatitis; ALD, alcoholic liver disease, HEMO; hemochromatosis.

[d] Numbers denote the following: 1, benzodiazepines; 2, penicillamine; 3, furosemide; 4, spironolactone; 5, prednisone/prednisolone; 6, colestyramine; 7, lactulose; 8, digoxine; 9, insuline; 10, sulfinpyrazone; 11, reserpine; 12, butizide; 13, azathioprine; 14, clopamide; 15, allopurinol; 16, mebendazole.

[e] Smokers.

CALCULATIONS

The *specific activity* of $^{14}CO_2$ *in breath* was corrected for dose and body weight and expressed as specific activity = % dose × kg body weight per mmoles CO_2. The area under the $^{14}CO_2$-exhalation-time-curve was calculated using the trapezoidal rule.

Caffeine plasma disappearance was considered to be a first order process, and the apparent elimination rate constant (k) was calculated by log-linear regression anal-

TABLE 2. DATA OF CAFFEINE METABOLISM IN NORMAL VOLUNTEERS

Volunteer (n = 10)	Sex	Age (yr)	Body weight (kg)	Cl[a] (ml/min/kg)	VD[b] (liters/kg)	$t_{1/2}$[c] (hr)	B_0[d] (μg/ml)	Cumulative $^{14}CO_2$ excretion in breath		Specific activity in breath	
								0–60 min	0–120 min	at 60 min	at 120 min
								$\left(\frac{\%\text{ dose} \times \text{kg} \times \text{min}}{\text{mmoles } CO_2}\right)$		$\left(\frac{\%\text{ dose} \times \text{kg}}{\text{mmoles } CO_2}\right)$	
E. R.[e]	M	27	80	1.30	0.53	4.7	0.3	14.4	34.9	0.32	0.34
M. T.	M	33	82	1.32	0.54	4.7	0.5	19.8	45.0	0.40	0.44
U. M.	M	29	67	1.52	0.60	4.6	0.4	16.2	38.4	0.32	0.40
H. P.[e]	M	27	75	1.73	0.62	4.1	0.3	19.8	47.8	0.43	0.48
E. C.[e]	M	54	83	1.84	0.48	3.0	0.1	29.3	65.4	0.57	0.63
P. H.	M	34	70	1.85	0.66	4.1	NM[f]	16.7	37.9	0.35	0.36
F. W.[e]	M	40	80	2.27	0.62	3.2	0.2	34.8	75.8	0.63	0.63
H. S.[e]	M	56	86	2.28	0.69	3.5	NM[f]	31.8	69.0	0.59	0.61
C. B.[e]	M	48	58	2.55	0.97	4.4	0.3	29.7	68.5	0.62	0.66
B. D.	M	28	58	3.56	0.66	2.1	0.1	37.7	81.9	0.63	0.73
$\bar{x}$		38	74	2.02	0.64	3.8	0.3	25.0	56.5	0.50	0.53
S.D.		11	10	0.68	0.13	0.9	0.1	8.5	17.5	0.15	0.14

[a] Total plasma clearance.
[b] Apparent volume of distribution.
[c] Plasma half-life.
[d] Fasting caffeine plasma concentration.
[e] Smoker.
[f] Not measurable.

ysis of caffeine plasma concentrations from 15 to 480 min. The apparent volume of distribution (VD) of caffeine was calculated VD = dose i.v./(C_{0EX} - C_{0M}), where the measured plasma caffeine concentration of the fasting sample drawn just before caffeine injection (C_{0M}) was subtracted from the extrapolated caffeine concentration at time 0 (C_{0EX}). The total caffeine plasma clearance (Cl) and the caffeine plasma half-life ($t_{1/2}$) were calculated as Cl = VD x k and $t_{1/2}$ = ln2/k, respectively.

All results are given as mean ± S.D. Group comparisons are based upon the Mann-Whitney test; $p < 0.01$ was regarded as statistically significant.

RESULTS

CAFFEINE CLEARANCE AND BREATH TEST IN VOLUNTEERS

A representative example depicting the time course of plasma concentrations of unlabeled caffeine, and of the specific activity of $^{14}CO_2$ in breath in a normal volunteer—open circles (compared with a cirrhotic—open triangles) is shown in Figure 1. It is evident that disappearance of caffeine from plasma in the normal may be interpreted to follow first order kinetics. Simultaneously, specific activity of $^{14}CO_2$ in breath appears within 5 min after i.v. injection of the labeled compound, achieving a peak between 2 and 3 hr, followed by a relatively rapid decline thereafter. Since the general appearance of these concentration-time curves was similar in all 10 volunteers studied, standard pharmakokinetic analysis was considered feasible. The results are summarized in Table 2.

Although total plasma clearance ranging from 1.30 to 3.56 ml per min per kg, tended to be higher in smokers consuming 5 to 40 cigarettes per day, there was considerable overlap with nonsmokers. With one exception, the values for volume of distribution clustered closely around the mean of 0.64 liter per kg of body weight. In spite of undoubted differences in caffeine intake prior to the study, the results suggested an inverse relationship between fasting caffeine plasma concentrations (B_0) and plasma clearance. Plasma half-lives ($t_{1/2}$) exhibited considerable variation, extending from 2.1 to 4.7 hr.

The pertinent results of the breath test have been given either as cumulative $^{14}CO_2$ excretion (AUC in breath) during 1 and 2 hr, or as instantaneous specific activities at 1 and 2 hr, respectively.

Specific activities averaging 0.50 and 0.53% dose x kg per mmole CO_2 were comparable, attesting to the plateau character of the $^{14}CO_2$ expiration curve during this time period. As expected, during the second hour of breath collection, average cumulative $^{14}CO_2$ excretion was more than doubled. Evidently, all breath parameters changed in parallel with caffeine plasma clearance.

CAFFEINE CLEARANCE AND BREATH TEST IN PATIENTS WITH LIVER DISEASE

As depicted in a representative study (Figure 1, open triangles), the plasma concentration-time curves in patients with liver disease maintain their monoexponential character, while exhibiting differences in disappearance rate. The breath curves assumed alterations in configuration and magnitude. Depending upon severity of the liver disease, specific activity of $^{14}CO_2$ was markedly reduced, showing little rise during the 8 hr of observation.

In Table 3, the results of pharmacokinetic evaluation of plasma and breath curves are summarized. Although the patients are again listed according to nosologic entities, the similarity of results in Groups A (cirrhotic liver disease) and B (primary biliary cirrhosis) is at once apparent. Thus, compared to normal volunteers, plasma clearance in both groups was on the average reduced to

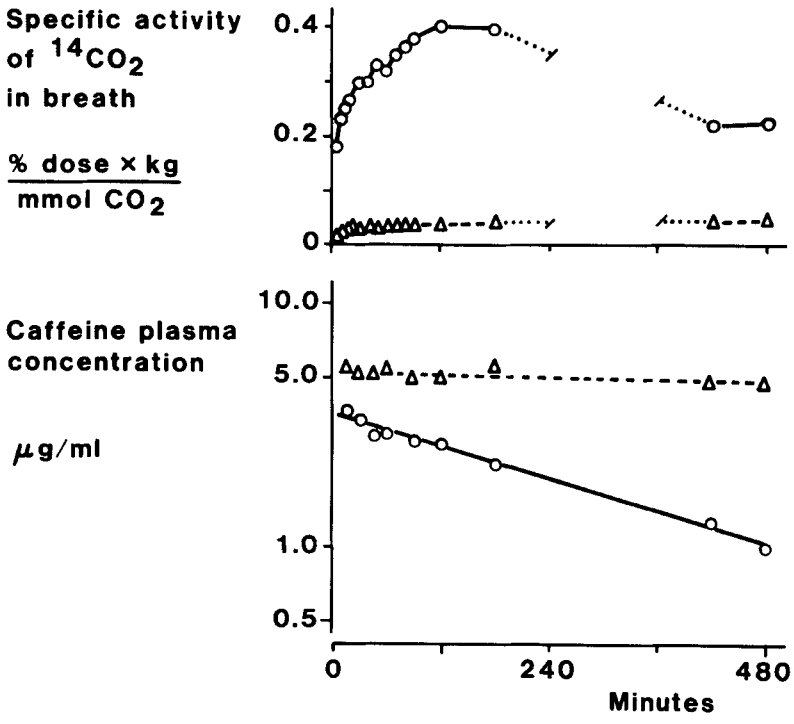

FIG. 1. Representative curves of $^{14}CO_2$ in breath (*upper panel*) and caffeine disappearance in plasma (*lower panel*) in a normal volunteer (O) and a patient with cirrhosis (Δ). The decreased rate of caffeine metabolism in the cirrhotic is reflected by the slowed plasma disappearance and the diminished specific activity of $^{14}CO_2$ in breath.

less than one-half (0.67 and 0.87, compared to controls of 2.02 ml per min per kg, $p < 0.001$ for both comparisons). Surprisingly, volume of distribution remained relatively unchanged (0.57 and 0.57, compared to 0.64 liter per kg). Excepting R. S. and R. B. with excessively prolonged half-lives, t½ in both groups of cirrhotics was on the average 2.5 times that in normal controls. As a reflection of the impaired caffeine disposition, B_0 averaged 4- to 8-fold that in volunteers.

The changes in cumulative $^{14}CO_2$ excretion and specific activities in breath are also comparable in the two groups. Thus, with the exception of H. L., there is no overlap with results in normal volunteers, all values being reduced by some 60 to 75%. Once again, the changes in breath parameters closely reflect changes of caffeine clearance.

Since Group C (miscellaneous liver disease) was, in general, characterized by mild functional derangement, it was not surprising to find plasma clearance and t½ closer to normal. In 7 of the 11 subjects, plasma clearance values were within the range of those for normal volunteers. Abnormalities of breath parameters were present in up to one-half of the patients, thus accounting for the observed differences in mean values for cumulative $^{14}CO_2$ excretion (16.5 at 60 min and 38.6 at 120 min vs. the normal of 25.0 and 56.5, respectively, $p < 0.025$) and for specific activity in breath (0.34 at 60 min and 0.39 at 120 min vs. 0.50 and 0.53, $p < 0.025$, respectively).

CHANGES IN CAFFEINE METABOLISM RELATED TO HEPATIC FUNCTIONAL IMPAIRMENT

Comparison of the results of the CBT with indices of hepatic functional impairment required prior validation of a relationship between plasma clearance and the $^{14}CO_2$ parameters in breath.

Thus, as shown in Figure 2, over a wide range of plasma clearance values there exists a close and highly significant ($p < 0.001$) linear relationship between plasma clearance and both cumulative $^{14}CO_2$ excretion ($r = 0.83$) and specific activity ($r = 0.83$) in breath. Although the data depicted in this figure are restricted to the 2-hr values, virtually identical results were obtained using breath parameters at 1 hr ($r = 0.82$ and 0.83, respectively) and at 3 hr ($r = 0.84$ and 0.83).

The initial disappearance constant for bromsulfhthalein (BSP-K_i) and the GEC may be viewed as quantitative measures of functional derangement. Thus, their relationship to the CBT (Figure 3) was of particular interest. A much closer correlation is observed between the cumulative $^{14}CO_2$ excretion and BSP-K_i ($R_s = 0.83$, $p < 0.001$) than GEC ($R_s = 0.39$, $p < 0.025$). A positive intercept of the regression line at the GEC axis is consistent with extrahepatic galactose metabolism.

DISCUSSION

The present study represents the first dual approach to the investigation of caffeine metabolism in patients with liver disease, simultaneously assessing the plasma kinetics of the parent compound and evaluating the appearance in breath of a metabolic end product of demethylation. The justification for this approach is based on the following facts:

(i) Caffeine disposition in man occurs almost exclusively by hepatic metabolism, less than 3% of metabolites in urine are trimethyl xanthines (11).

(ii) Demethylation represents the most important metabolic step. During the first 2 hr after caffeine administration, approximately 80% of labeled CO_2 is derived from position 3, i.e., the paraxanthine pathway (18).

TABLE 3. DATA OF CAFFEINE METABOLISM IN PATIENTS WITH LIVER DISEASE

Patient	Cl[a] (ml/min/kg)	VD[b] (liters/kg)	t½[c] (hr)	B_0[d] (μg/ml)	Cumulative $^{14}CO_2$ excretion in breath		Specific activity in breath	
					0–60 min	0–120 min	at 60 min	at 120 min
					(% dose × kg × min / mmoles CO_2)		(% dose × kg / mmoles CO_2)	
A. Cirrhotic Liver Disease (n = 8)								
R. S.	0.14	0.60	50.2	2.3	1.6	3.6	0.03	0.04
P. A.[e]	0.20	0.37	19.9	2.9	2.9	7.2	0.06	0.07
R. B.[e]	0.25	0.72	34.0	5.4	2.2	5.8	0.05	0.06
C. W.[e]	0.82	0.72	10.1	2.1	4.7	10.5	0.09	0.10
H. G.	0.82	0.57	8.1	1.5	7.3	18.2	0.17	0.17
J. S.	0.82	0.39	5.5	NM[f]	10.8	25.6	0.23	0.28
P. R.	1.01	0.67	7.7	0.8	9.6	24.0	0.23	0.24
A. C.	1.29	0.50	4.5	NM[f]	10.1	23.3	0.21	0.26
x̄	0.67	0.57	17.5	2.5	6.1	14.8	0.13	0.15
S.D.	0.42	0.14	16.5	1.6	3.8	9.0	0.09	0.10
B. PBC (n = 7)								
Z. Z.	0.42	0.66	18.3	1.0	5.8	13.3	0.12	0.14
G. R.	0.58	0.71	14.3	0.5	5.9	14.2	0.14	0.14
H. M.	0.63	0.45	8.3	3.5	7.3	18.9	0.17	0.22
H. L.	0.77	0.86	12.8	0.7	18.0	44.8	0.41	0.49
B. L.	1.05	0.25	2.8	1.7	3.7	9.2	0.08	0.10
E. G.	1.32	0.45	3.9	0.2	7.1	18.7	0.17	0.22
E. R.	1.33	0.59	5.1	0.4	10.2	23.7	0.21	0.23
x̄	0.87	0.57	9.4	1.1	8.3	20.4	0.19	0.22
S.D.	0.37	0.20	5.9	1.2	4.7	11.7	0.11	0.13
C. Miscellaneous Liver Disease (n = 11)								
K. K.	0.61	0.55	10.4	0.7	6.2	15.6	0.14	0.17
P. A.	1.10	0.53	5.6	1.0	6.4	15.5	0.14	0.17
H. Lu.	1.22	0.42	4.0	1.1	19.2	43.7	0.39	0.43
I. L.	1.25	0.62	5.7	0.5	9.3	23.9	0.21	0.27
P. B.	1.31	0.61	5.4	0.5	12.3	31.2	0.28	0.36
A. M.	1.52	0.43	3.3	1.0	13.0	33.0	0.31	0.35
E. S.	1.72	0.57	3.8	NM[f]	26.1	57.7	0.52	0.54
A. L.	1.83	0.54	3.4	NM[f]	17.7	43.3	0.39	0.46
R. F.[e]	1.89	0.61	3.7	0.5	40.1	87.1	0.75	0.75
F. R.	1.90	0.58	3.5	0.4	19.4	41.4	0.37	0.39
H. I.[e]	2.34	0.60	3.0	0.3	12.2	31.9	0.27	0.35
x̄	1.52	0.55	4.7	0.7	16.5	38.6	0.34	0.39
S.D.	0.48	0.07	2.1	0.3	9.9	20.5	0.18	0.17

[a] Total plasma clearance.
[b] Apparent volume of distribution.
[c] Plasma half-life.
[d] Fasting caffeine plasma concentration.
[e] Smoker.
[f] Not measurable.

The demonstration of a close linear relationship between cumulative $^{14}CO_2$ exhalation (or specific $^{14}CO_2$ activity in breath) and plasma clearance of caffeine supports the idea that the CBT is a reliable indicator of caffeine disposition. Indeed, combining the results in volunteers and patients with liver disease, the correlation is maintained over a wide range (0.14 to 3.56 ml per min per kg) of clearance values (Figure 2). This is surprising when one considers the heterogeneity of hepatic diseases investigated, the potential influence of changes in caffeine plasma binding due to liver disease, and the possible effect of concomitant drug treatment (as outlined in Table 1).

Although the parameters of caffeine plasma kinetics in this investigation in normal volunteers and patients with cirrhosis are in good agreement with previously published studies (10, 12, 13), strict comparison is difficult owing to differences in experimental design. Thus, we elected to administer caffeine by i.v. injection to circumvent potential delays in absorption, particularly

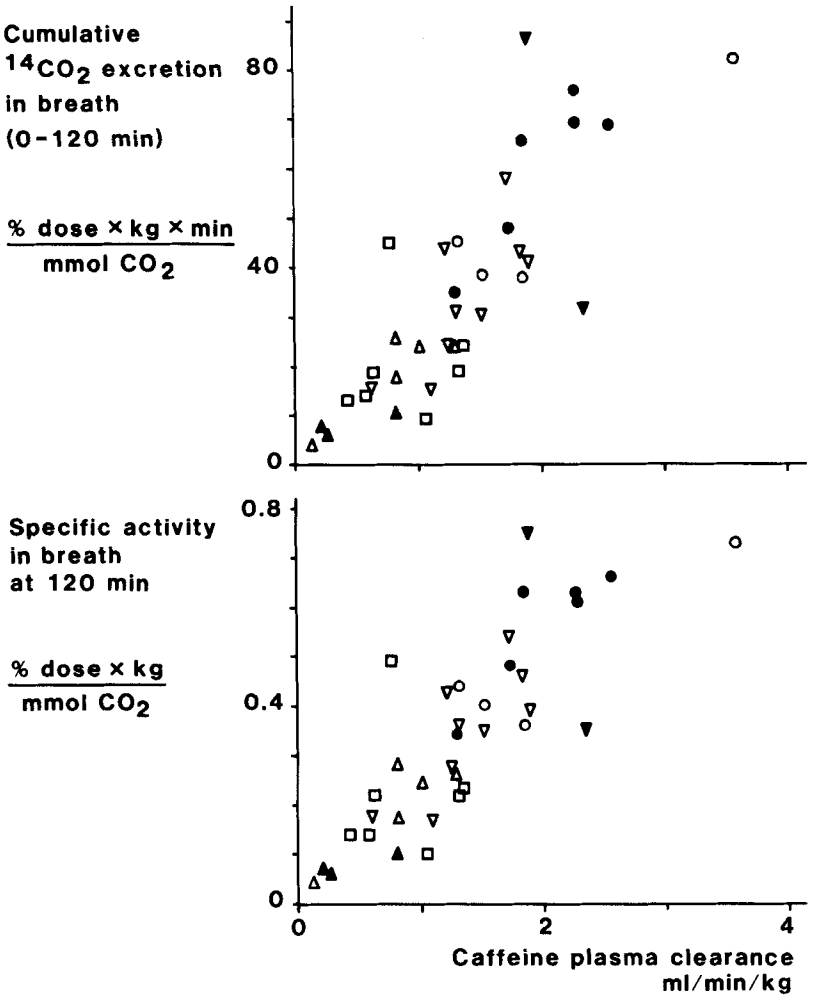

FIG. 2. Relationship of caffeine plasma clearance to cumulative $^{14}CO_2$ excretion (*upper panel*) and specific activity of $^{14}CO_2$ in breath (*lower panel*) in all 36 subjects studied; r = 0.83 and 0.83, respectively. The *filled symbols* represent smokers, the *open symbols* nonsmokers. ○ = volunteers; △, cirrhotic liver disease; □, PBC; ▽, miscellaneous liver disease. The regression equations are: y = 25.6x + 0.3 (*upper panel*) and y = 0.23x + 0.04 (*lower panel*).

in subjects with liver disease. As a consequence, we chose a small dose (125 mg) to avoid toxicity. (Neither in volunteers nor in patients did we ever observe any adverse effects.) Finally, since Desmond et al. (13) demonstrated that the monoexponential decline of plasma caffeine levels is maintained beyond 4 to 5 half-lives, we felt justified in limiting our collection period to 8 hr.

Despite these differences, the general effects of liver disease on caffeine plasma kinetics may be defined with some confidence. They consist of a prolongation in caffeine t½, a slight but statistically insignificant reduction in volume of distribution and a decrease of plasma clearance. This statement is based on the fact that the above changes are noted not only in those patients with clinically most advanced liver disease (Groups A and B), but also in the group with miscellaneous liver disease characterized by mild functional abnormalities. In addition, if the data on the relationship between albumin concentration and plasma caffeine binding are considered (13), there can be little doubt that the effects of hepatic disease in reducing clearance of unbound caffeine are further amplified. Furthermore, the differences in clearance values were confirmed when calculated according to Cl = dose/$AUC_{0-\infty}$, yielding 2.03 ± 0.66 ml per min per kg in normal volunteers (as compared to 2.02 ± 0.68) and 1.35 ± 0.73 in patients with liver disease (as compared to 1.34 ± 0.73).

The prolonged half-lifes (compared to the intervals of dosing) imply potential accumulation of caffeine in subjects with liver disease even following "normal" consumption of caffeine-containing beverages and food stuffs. The data in Figure 4 support this idea, since liver patients tend to have higher fasting caffeine plasma concentrations compared to controls. More important, perhaps, is the fact that in spite of differences in caffeine consumption, there is a relatively close—presumably hyperbolic—relationship to caffeine clearance. This correlation, which is reminiscent of the relationship between serum creatine concentrations and inulin clearance (19), might serve as a basis for using caffeine as a convenient and ubiquitous exogenous tracer for estimating hepatic functional impairment.

Recent concern with long-term toxicological implications of food additives has stimulated much interest in caffeine metabolism. Some 17 metabolic endproducts of caffeine have now been identified in man (11). Since it has been demonstrated that *N*-demethylation represents the first and quantitatively most important metabolic step, the idea of a CBT was immediately evident. On the basis of studies in the rat, we have showed that such an

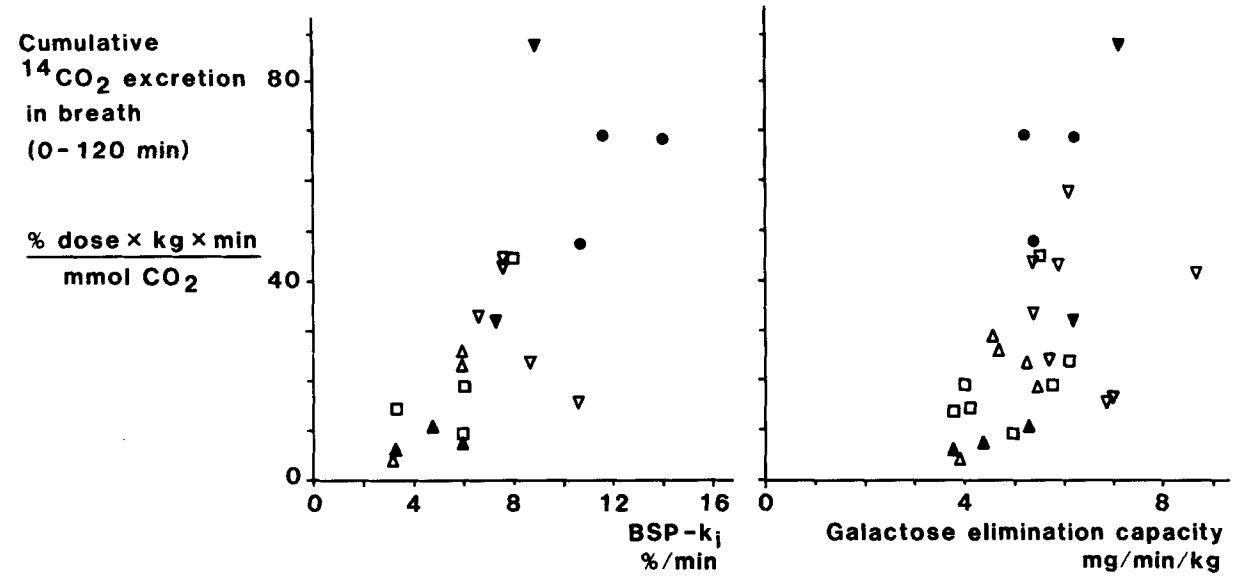

FIG. 3. Relationship between cumulative $^{14}CO_2$ excretion in breath and the initial bromosulfophthalein disappearance constant BSP-k_i (R_s = 0.83, n = 20, p < 0.001) and the GEC, respectively (R_s = 0.39, n = 28, p < 0.025). The *symbols* are identical to those used in Fig. 2.

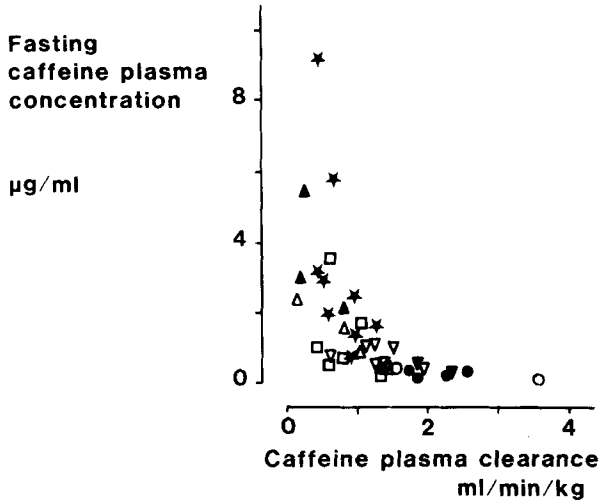

FIG. 4. Hyperbolic relationship between fasting caffeine plasma level and the subsequently measured caffeine plasma clearance (R_s = −0.81, n = 39, p < 0.001). The six subjects, in whom caffeine was not detectable in the fasting plasma, have been omitted from this graph. On the other hand, nine patients with biopsy-documented cirrhosis, who were given nonlabeled caffeine only, have been included; they are denoted with a ★. The other *symbols* are identical to those used in Fig. 2.

approach is feasible in man (14). Considering the importance of the paraxanthine pathway, we chose to use caffeine specifically ^{14}C-labeled in position 3, an approach which is supported by careful studies in normal volunteers (18). In contrast to other substrates hitherto used for breath tests, such as aminopyrine, *N*-demethylation of caffeine is apparently controlled primarily by the P-450 isoenzyme, P_1-450 arylhydrocarbon hydroxylase. It was not unreasonable, therefore, to anticipate that the enzyme induction produced by smoking (20–22) might—at least in part—counteract the effects of liver disease on the CBT. However, due to the small number of smokers (5 of 26) among the patients with liver disease, it is not possible to confirm or contradict such an assumption.

In agreement with the results obtained by Kotake et al. (18), analysis of the CBT data reveal that cumulative $^{14}CO_2$ exhalation exhibits the closest relationship with plasma clearance. This parameter was therefore used for a comparison with quantitative tests of liver function. As shown in Figure 3, changes in BSP-K_i are paralleled by corresponding alterations in $^{14}CO_2$ excretion, suggesting that the CBT—like the ABT—may be interpreted to reflect "functioning cell mass" (2). The data comparing microsomal (CBT) and cytosolic (GEC) functions exhibit much more scatter. This is surprising, since assessment of GEC is relatively blood flow-independent, and caffeine must be considered a low-extraction compound. Clearly, further work will be required for a better understanding of these discrepancies.

Using [^{14}C]aminopyrine, $^{14}CO_2$ yield in breath was higher in patients with chronic cholestatic liver disease (23, 24). Such differences between the subjects with PBC and other forms of cirrhosis were not evident in this study. This might be attributable to the fact that aminopyrine and caffeine are metabolized by different enzyme systems. Since all our PBC patients were in Stage 4 of their liver disease, the resultant reduction in metabolic capacity—also reflected in the comparable decrease of GEC values—may have obscured the potential "inducing" effects of cholestasis.

In summary, this study demonstrates that impaired elimination of caffeine in patients with liver disease due to changes in hepatic caffeine metabolism parallels alterations in "functioning cell mass" evident in diminished BSP-K_i. Since *N*-demethylation of caffeine, particularly in position 3, seems to be a prerequisite for further metabolism, the CBT yields quantitative information concerning the degree of hepatic functional derangement. Finally, in view of the surprisingly close relationship between fasting caffeine plasma concentration and caf-

feine plasma clearance, estimation of the caffeine level in a single plasma sample might serve as a semiquantitative guide to severity of liver disease.

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