The Evaluation of the Drug-metabolizing Capacity in Patients with Diabetes Mellitus

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SUMMARY

Hepatic drug-metabolizing capacity was investigated in 56 diabetics. The antipyrine test was selected as an in vivo index, since its kinetics indirectly reflect the metabolically active liver mass. Hepatic cytochrome P-450 (P-450), determined from the biopsy samples, was used as an in vitro parameter, since it is a direct measure of microsomal drug-metabolizing enzyme activity. There was a wide interindividual variation in the indexes of drug metabolism in the diabetics: 40 fold in P-450 content and eightfold in antipyrine metabolism. P-450 levels were higher and antipyrine metabolism faster in the subjects with normal liver than in those with fatty liver, parenchymal inflammatory changes, or cirrhosis. Thus the in vivo and in vitro parameters of drug metabolism were related to the alterations in liver histology. On the other hand, the diabetes per se did not seem to alter the drug-metabolizing capacity of the liver. Also, drug metabolism in diabetics classified by treatment regimen did not differ significantly.

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Experimental studies have revealed alterations in drug metabolism in diabetic animals,[1–9] and an association has been shown between impaired metabolism of hexobarbital, chlorpromazine, codeine, acetophenetidin, and aminopyrine and enhanced metabolism of aniline and prolonged, hexobarbital, sleeping time.[1–6] Changes in the ultrastructure of the hepatocyte (especially an increase in the smooth endoplasmic reticulum[1,2]) and the presence of a cytoplasmic inhibitor of drug metabolism correlating with an elevated hepatic cyclic AMP concentration have been suggested as possible mechanisms of altered drug metabolism.[5,10,11] Reports of changes in liver microsomal cytochrome P-450 content in diabetic animals are conflicting,[5–7] and the influence of experimental diabetes on drug metabolism is thus controversial. Insulin seems to affect drug metabolism through the correction of an insulin-deficient state.[5–7]

Only a few studies have been carried out on drug metabolism in human diabetics. Daintith et al.[12] observed faster metabolism of antipyrine in insulin-treated diabetics than in controls, while Dajani et al.[13] found slower acetophenetidin metabolism in diabetics than in controls, this being correctable with insulin. Ueda et al.[14] have suggested that the disappearance rate of tolbutamide from the blood is not affected by the metabolic disorders of diabetes mellitus, but it is prolonged by severe hepatic or renal impairment. Major interindividual variations in the steady state concentrations of tolbutamide and chlorpropamide in diabetics have been reported by Melander et al.,[15] and it has recently been suggested that monogenic control of tolbutamide metabolism exists in man.[16] Tokola et al.[17] have observed greater in vitro activities of drug-metabolizing enzyme systems in liver samples obtained during abdominal surgery from diabetics treated with tolbutamide than in those from other patients, although Redman et al.[18] failed to demonstrate any induction of liver microsomal enzyme activity by tolbutamide in diabetics. These results do not provide any clear picture of the possible alterations in drug metabolism in patients with diabetes mellitus. The need for systematic studies is further emphasized by the results of the University Group Diabetes Program study,[19,20] in which oral treatment was carried out with a standard dose with no appreciation of individual drug handling.

The present study was undertaken to assess hepatic drug metabolism in patients with diabetes mellitus. Diabetics from whom a liver biopsy had been obtained were investigated by comparing parameters of drug metabolism, antipyrine kinetics, and cytochrome P-450 content in biopsy samples in relation to liver histology and treatment regimens.

MATERIALS AND METHODS

Subjects. Fifty-six diabetics, 33 women and 23 men, were investigated; 54 of these had maturity-onset-type diabetes

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and two juvenile-onset-type diabetes. These patients were all referred to the Clinical Research Unit because of poor control of diabetes or disturbances in liver function tests. The mean age of the patients was 56.8 yr (SD, 8.9 yr). The mean duration of diabetes was 5.5 yr, ranging from 1 to 20 yr. Their per cent ideal bodyweight was 136%, ranging from 88 to 190% (the estimations were based on Home Economics Research Report Nr 10,ARS,USDA). Cardiac failure, if present, was compensated in every case, and renal function, as judged by creatinine clearance, was normal throughout. Percutaneous liver biopsy was performed for diagnostic purposes. Thirteen nondiabetics, six women and seven men, whose mean age was 43.6 yr (SD, 14.2 yr), were taken as controls. All these patients were admitted because of abnormal results in liver tests noted earlier, but they had a normal liver histology at the time of biopsy. Informed consent was obtained from each patient before the antipyrine test. Neither the diabetics nor the controls were being treated with any compounds known to have a major capacity for inducing drug metabolism.

Protocol. The drug metabolism studies were carried out in the Clinical Research Unit. Blood samples for blood glucose determination were taken four times a day—after an overnight fast, at noon, in midafternoon, and in the evening—except in the cirrhotic group, where they were taken only after fasting. Blood samples for the liver function tests were taken after an overnight fast. Three to four hours later, a percutaneous liver biopsy was performed with a ThruCut needle.[21] All biopsy samples were used for histologic study and part of each specimen was utilized for the determination of cytochrome P-450 content (P-450). The next morning, after an overnight fast, antipyrine (20 mg/kg dissolved in 100 ml of fruit juice) was administered to each patient. Venous blood samples were drawn at zero time and 1, 3, 6, 9, 12, 24, and 48 h after antipyrine ingestion.

Serum biochemistry. Serum concentrations of albumin and total bilirubin and the activities of alanine aminotransferase (Alat) and alkaline phosphatase (AP) were measured using standard AutoAnalyzer techniques (Technicon). Blood glucose concentration was determined by a hexokinase method (Glucoquant, Boehringer Mannheim GmbH, Germany).

Liver histology. The studies on liver histology were carried out by the methods of Ahlqvist.[22] The patients were classified on the basis of their histologic findings as follows: (1) Normal liver (NL); (2) Fatty liver (FL), more than 10% triglyceride by quantitative analysis;[23] (3) Fatty liver with nonspecific inflammatory changes with or without fibrosis (FL+I), patients with viral hepatitis and chronic active hepatitis being excluded; and (4) Cirrhosis (Ci).

Drug metabolism studies. Plasma antipyrine concentrations were determined by the gas-liquid chromatography method of Prescott et al.[24] with phenacetin as an internal standard, as used earlier.[21] Cytochrome P-450 content was determined in the total homogenate of the biopsy material according to the method of Greim et al.,[25] as reported previously.[21]

Calculations. The plasma antipyrine half-life (T/2) was read from the time concentration curve on a semilogarithmic graph. Plasma clearance (CL) was obtained by dividing the dose by the area under the plasma concentration time curve, as calculated by the trapezoidal rule. The apparent volume of distribution (aVd) was determined from the relationship: aVd = CL/k, where k equals the elimination rate constant.

The mean value of all glucose determinations was calculated for each patient to serve as an estimate of diabetic control.

Statistical analysis of the data was performed by the Student's t test and regression analyses.

RESULTS

Drug metabolism in patients with diabetes mellitus. The results are given in Tables 1 and 2 and in Figure 1. There were great interindividual variations in the parameters of drug metabolism in the patients with diabetes mellitus: P-

TABLE 1
Indices of drug metabolism in patients with diabetes mellitus classified by liver histology

Subjects	Antipyrine			Cytochrome P-450
	T/2 (h)	CL (ml/min)	aVd (L/kg)	nmol/g
Diabetics				
Normal liver (N,4)				
Mean	6.0*	80.6†	0.58*	15.0†
SD	2.0	33.5	0.06	4.5
Fatty liver (N,13)				
Mean	11.4‡	41.0§	0.41§	7.6§
SD	5.1	13.7	0.09	3.3
Fatty liver with inflammatory changes (N,33)				
Mean	12.2§	33.0§	0.42§	4.8§
SD	4.0	13.3	0.13	2.4
Cirrhosis (N,6)				
Mean	16.7§	26.1§	0.42§	6.4§
SD	3.3	9.8	0.07	1.0
Controls with normal liver (N,13)				
Mean	8.4	43.0	0.47	10.2
SD	2.0	13.5	0.07	1.9

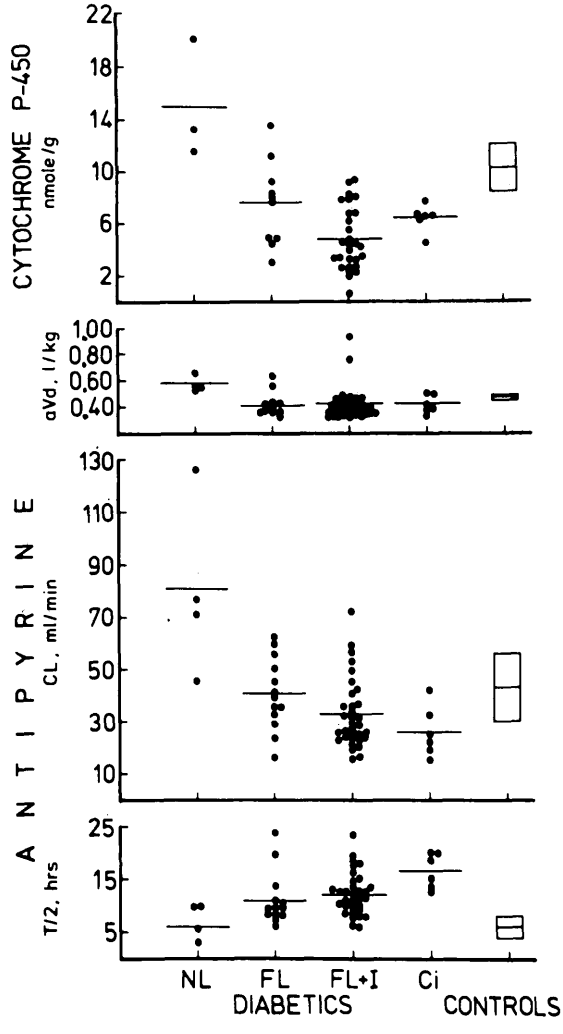
P values for differences between the means: (1) Controls compared with diabetics with normal liver * $P < 0.05$, † $P < 0.005$, and (2) diabetics with liver alterations compared with normal liver ‡ $P < 0.05$, § $P < 0.005$.

TABLE 2
Per cent ideal body weight, blood glucose, liver function tests, and antipyrine clearance in patients with diabetes mellitus classified by liver histology

Histology	Body weight % IBW*	Blood glucose mmol/L	Liver function tests: Bil µmol/L	Alat U/L	AP U/L	Alb g/L	Antipyrine clearance ml/min
Normal liver (N,4)							
Mean	103	11.8	10	27	233	41	80.6
SD	16	4.1	3	14	78	2	33 5
Fatty liver (N,13)							
Mean	149†	10.6	11	44	203	42	41.0‡
SD	23	3.1	6	46	115	3	13.7
Fatty liver with inflammatory changes (N,33)							
Mean	139†	10.5	10	68§	223	44	33.0‡‖
SD	18	2.8	4	49	71	2	13 3
Cirrhosis (N,6)							
Mean	139†	9.3	19	70†	277	42	26.1‡‖
SD	17	2.9	12	28	169	5	9.8
Normal levels in our laboratory		(3.3-5.5)	(2-17)	(40)	(60-250)	(40-54)	(30-56)

* IBW = ideal body weight.
P values for differences between the means: (1) Diabetics with normal liver compared with those with altered liver: † $P < 0.05$, ‡ $P < 0.005$, and (2) diabetics with normal or fatty liver compared with the others: § $P < 0.05$, ‖ $P < 0.005$.

FIGURE 1. Individual values for antipyrine half-life (T/2), plasma antipyrine clearance (CL), apparent volume of distribution (aVd), and cytochrome P-450 content in diabetics, classified by liver histology (NL = normal liver, FL = fatty liver, FL + I = fatty liver with nonspecific inflammatory changes with or without fibrosis, and Cl = cirrhosis), and in normal liver controls.



450 from 0.5 to 20.0 nmol/g (mean, 6.2 ± 3.6 nmol/g); antipyrine T/2 from 3.2 to 24.0 h (mean, 12.2 ± 4.6 h); antipyrine CL from 15.0 to 126.5 ml/min (mean, 37.5 ± 19.4 ml/min); and aVd from 0.32 to 0.94 L/kg (mean, 0.43 ± 0.12 L/kg). This variability in drug metabolism was related to alterations in liver parenchyma. The subjects with intact liver had higher P-450 values and they metabolized antipyrine at a faster rate than those with parenchymal alteration in the liver.

Diabetics with normal liver were less overweight, had a longer duration of the disease, and were younger than those with altered liver structure, but the differences were not statistically significant. Blood glucose values varied within the same range in all the liver histologic classes among the diabetics (Table 2).

Relationship between in vivo and in vitro parameters of drug metabolism. Since it is not always possible to determine P-450 levels in patients with diabetes mellitus, we set out here to compare plasma antipyrine clearance with the P-450 content in the biopsy specimens. As shown in Figure 2, a significant correlation emerged between these two measures.

Drug metabolism and diabetes therapy. Figure 3 shows the parameters of drug metabolism in diabetics classified by treatment regimens. If comparison were made between subjects with moderate liver alterations (excluding those with normal liver or cirrhosis) in order to obtain more homogenous groups, no significant differences were found between these groups (Table 3).

Liver histology and liver function tests in diabetics. The various histologic groups among the diabetics overlapped in their conventional liver test values (Table 2). It was nevertheless possible to distinguish diabetic groups with normal and fatty liver from the others by reference to plasma antipyrine clearance.

Relationship between liver function and the parameters of drug metabolism. Possible correlations between liver function and drug metabolism were also investigated. Only in the diabetics with liver alterations did any relation

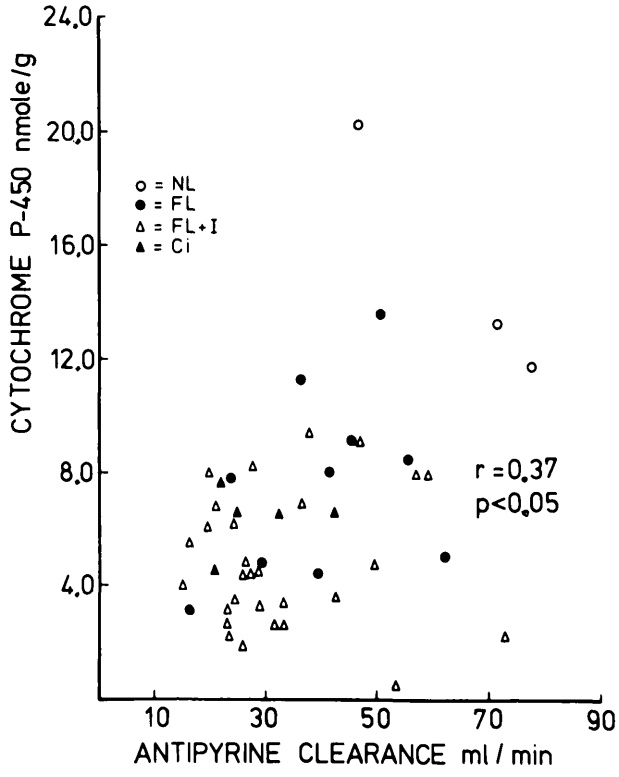


FIGURE 2. Relationship between hepatic cytochrome P-450 content and plasma antipyrine clearance in the whole series of 48 diabetics.

emerge between the plasma antipyrine half-life and serum albumin concentration ($r = 0.30$, $P < 0.01$); plasma antipyrine clearance was similarly related to serum albumin ($r = 0.23$, $P < 0.05$). In the whole series of diabetics, there were no significant correlations between the parameters of drug metabolism and conventional liver tests.

Relationship between the diabetic state and drug metabolism. To evaluate the role of the diabetic state on drug metabolism, we compared the present data with earlier results from this center in alcoholics[21] and in epileptics[26–28] (Table 4). Mean P-450 levels in the diabetics with normal

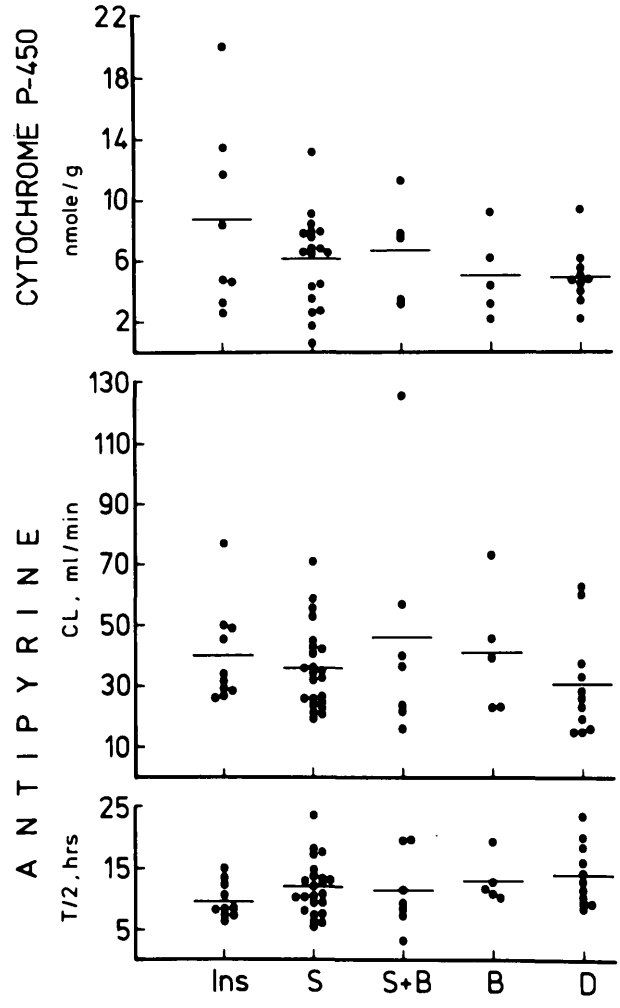


FIGURE 3. Individual values for antipyrine half-life (T/2), plasma antipyrine clearance (CL), and cytochrome P-450 content in patients with diabetes mellitus grouped according to the treatment regimen (Ins = insulin, S = sulfonylurea, S + B = sulfonylurea with biguanide, B = biguanide, and D = diet only).

TABLE 3
Liver function tests and parameters of drug metabolism in diabetics classified by treatment regimen (diabetics with fatty liver and with inflammatory changes included)

	Liver function tests				Antipyrine		Cytochrome P-450
Regimen of treatment	Bil μmol/L	Alat U/L	AP U/L	Alb g/L	T/2 h	CL ml/min	nmol/g
Insulin (N,8)							
Mean	7	46	230	44	10.4	34.8	6.2
SD	3	47	102	3	3.1	9.6	4.1
Sulfonylurea (N,19)							
Mean	12	49	218	43	12.1	34.8	5.2
SD	5	28	90	3	4.7	12.2	2.8
Sulfonylurea + biguanide (N,5)							
Mean	11	37	198	44	11.2	35.0	6.2
SD	5	30	100	3	5.0	15.5	3.8
Biguanide (N,5)							
Mean	10	106	214	44	13.1	41.2	5.0
SD	3	83	54	3	3.6	20.3	2.8
Diet (N,9)							
Mean	10	90	217	44	12.8	33.6	4.8
SD	5	52	81	2	4.7	17.8	2.0

TABLE 4
Indices of drug metabolism in diabetics, epileptics, and alcoholics classified by liver histology

Subjects/ Liver histology	Antipyrine			Cytochrome P-450 nmol/g	Reference
	T/2 (h)	CL (ml/min)	aVd (L/kg)		
Normal liver					
Epileptics (N,11)	3.5 ± 0.7	135.7 ± 35.5	0.55 ± 0.06	19.4 ± 3.5	26,27
Diabetics (N,4)	6.0 ± 2.0	80.6 ± 33.5	0.58 ± 0.06	15.0 ± 4.5	
Control patients (N,13)	8.4 ± 2.0	43.0 ± 13.5	0.47 ± 0.07	10.2 ± 1.9	
Alcoholics (N,6)	6.5 ± 2.3	78.9 ± 41.8	0.57 ± 0.18	12.6 ± 4.0	21
Fatty liver					
Diabetics (N,13)	11.4 ± 5.1	41.0 ± 13.7	0.41 ± 0.09	7.6 ± 3.3	
Alcoholics (N,8)	8.1 ± 2.5	58.2 ± 27.9	0.54 ± 0.14	7.9 ± 4.0	21
Inflammatory changes					
Diabetics (N,33)	12.2 ± 4.0	33.0 ± 13.3	0.42 ± 0.13	4.8 ± 2.4	
Alcoholics (N,7)	22.3 ± 15.1	28.6 ± 16.6	0.47 ± 0.12	2.5 ± 2.6	21
Cirrhosis					
Epileptics (N,4)	6.3 ± 1.1	63.1 ± 9.2	0.46 ± 0.04	13.9 ± 3.2	28
Diabetics (N,6)	16.7 ± 3.3	26.1 ± 9.8	0.42 ± 0.07	6.4 ± 1.0	
Alcoholics (N,6)	28.9 ± 8.7	15.2 ± 6.0	0.46 ± 0.18	5.9 ± 2.1	21

liver were lower than in epileptics with normal liver, but higher than in our nondiabetic control patients or in alcoholics with normal liver. Respectively, antipyrine kinetics in the diabetics were slower than in epileptics, within the same range as in alcoholics, but higher than in our nondiabetic control patients. In the diabetics with fatty liver, P-450 was within the same range and antipyrine kinetics slightly slower than in alcoholics with fatty liver. Both in vivo and in vitro indexes were in the same range in the diabetics and alcoholics with liver inflammatory changes. In subjects with cirrhosis, both in vivo and in vitro indexes were higher in epileptics than in the diabetics or alcoholics.

DISCUSSION

Hepatic drug metabolism in man is subject to genetic control and is influenced by such various factors as age, sex, temperature, environmental chemicals, drugs, and diseases.[29–33] The hepatic handling of an orally administered compound depends on hepatic blood flow, protein binding in blood, uptake systems, and enzyme activity.[34–37] Since drug-metabolizing enzymes in the liver are located in the endoplasmic reticulum of the hepatocytes,[38] it is natural to assume that changes in the liver function or structure may be reflected in drug metabolism.[39] Conventional liver function tests do not provide reliable information about quantitative liver function,[40] and for this reason the present study was based on histologic changes in the liver.

The majority of our patients with diabetes mellitus had liver parenchymal alterations, primarily fatty liver or nonspecific inflammatory changes. Patients with viral or chronic active hepatitis were not included, but the contribution of ethanol in some subjects cannot be excluded. Thus it was assumed that the hepatic alterations were not associated with any unique etiologic factor. Most patients were well, except for the obese maturity-onset-type diabetics. Among them the reported frequency of fatty liver varies from 21 to 78%;[41] its pathogenesis is uncertain but has been related to hyperinsulinemia and insulin resistance.[41,42] The occurrence of cirrhosis in diabetics is reported to be twice as frequent as in a normal population,[41,43] although different opinions exist.[44] The reasons for the augmented prevalence of hepatocellular diseases in diabetics are not clear, but normal insulin levels or normal insulin/glucagon ratios are thought to have a role in the maintenance of hepatocyte integrity and in liver regeneration.[45–48] The fatty liver of diabetes only rarely leads to cirrhosis.[49,50] On the other hand, there is a high incidence of glucose intolerance in chronic liver diseases. Its mechanisms are not known but are often associated with abnormalities in the metabolism of insulin, glucagon, and growth hormone.[51]

Most drugs are metabolized by the hepatic microsomal enzyme system, the terminal component in this process being cytochrome P-450, a heterogenous group of hemoproteins.[52–54] According to present opinion, a drug combines to the binding sites of P-450 before being oxidized to a metabolite.[55,56] The existence of multiple forms of P-450 has been demonstrated in animals[57] and also proposed in man.[58–60] The activity of this enzyme system is increased by certain drugs[61] and reduced by such parenchymal liver diseases as hepatitis and cirrhosis.[21,61,62] The P-450 levels in our nondiabetic control patients agree well with previous data for human beings with intact liver, obtained by compatible methods.[63] Comparison between the present and earlier data from this center demonstrates that P-450 levels in diabetics with normal liver were lower than in epileptics with normal liver but higher than in our nondiabetic controls or in alcoholics with normal liver.[21,26–28] However, the number of diabetics with normal liver was small, so that one should be cautious about drawing any firm conclusions. Any parenchymal change, fatty accumulation, or increase in fibrotic bands and/or inflammatory cells had a lowering effect on P-450 levels in the diabetics. This could partly be explicable by fat or fibrotic tissue in the homogenates, since P-450 is determined per unit weight of tissue. Earlier reports,[21,64] however, have demonstrated that P-450 levels correlate with the amount of intact liver parenchyma. Thus the liver histology should be taken into account when evaluating P-450 values. No comparisons were made here between P-450 levels and DNA or protein content in the homogenates, and data from experimental studies suggest that there are no relations between these parameters.[65]

Antipyrine is widely used to reflect hepatic drug metabo-

lism in man.[66,67] This is due to its unique characteristic of being absorbed completely from the gastrointestinal tract, bound to a negligible extent to plasma or tissue protein, distributed in total body water, almost totally metabolized by the liver through two hydroxylations and a N-demethylation, and having a low extraction ratio with a few side effects.[36,39,66,68] Its elimination from plasma is hence largely determined by the hepatic oxidizing enzyme activity, and therefore it may serve as a quantitative measure of liver function.[64,66,69] In this study the metabolism of antipyrine in the diabetics with normal or fatty liver varied within the levels of our control patients, whereas it was reduced in those diabetics with parenchymal inflammatory changes or cirrhosis. Antipyrine clearance was thus useful in distinguishing diabetic groups with normal or fatty liver from those with greater liver changes, even though the values for individual subjects overlapped. On the other hand, there was no correlation between the conventional liver tests and the liver histology or antipyrine metabolism.

Since multiple forms of P-450 exist, the value of the antipyrine test as a predictive model for the metabolism of other compounds has been questioned.[67,70,71] There are great interindividual variations in antipyrine kinetics in normal subjects, although the values for each subject are generally reproducible.[67] In parenchymal liver diseases, these variations are more restricted, and, moreover, the rate of antipyrine clearance from the plasma has been shown to be related to the metabolism of aminopyrine, diazepam, galactose, indocyanine green, lidocaine, paracetamol, and propranolol.[36,66,72–74] Although liver diseases may influence the metabolism of drugs with different parameters of disposition to a varied extent, antipyrine is considered as a suitable marker drug to reflect drug metabolism, even if it is not a model for all drugs.[36,37,39,67]

Since both diabetes and liver disease were present in most of our patients, it was not possible to firmly evaluate the role of the diabetic state per se. When we compared the present results with earlier data from this center,[21,26–28] we noticed that, in severe hepatocellular diseases, liver damage seemed to determine largely the drug-metabolizing capacity, even though this could be enhanced by inducing drugs.[39] In the diabetics with fatty liver, both the in vivo and in vitro indexes of drug metabolism were within the levels of our control patients and were not essentially different from those of alcoholics with fatty liver. Moreover, comparison between the diabetics and alcoholics with inflammatory changes did not yield noticeable differences, even though etiologies for the liver changes were thought to be different. Our results suggest, therefore, that, in the treated diabetics with liver abnormalities, drug metabolism does not seem to be related to diabetes to any great degree. Additional control studies, however, are needed to clarify the problem further.

Comparison between antipyrine clearance and the P-450 content of the homogenates revealed a significant correlation, although far from a perfect one. Thus the in vitro index, obviously, cannot be extrapolated without precautions in order to predict the in vivo metabolism of antipyrine. In vitro studies fail to take into account the total hepatic capacity for drug metabolism[36,75] and the role of transport processes, hormonal factors, and the availability of in vivo cofactors in the hepatocyte.[39]

It has been claimed that the rate of drug metabolism is higher in diabetics on insulin than in controls or diabetics treated by other regimens.[12] Experimental studies suggest that insulin does not affect drug metabolism in normal animals, whereas it does in diabetic animals, where it normalizes altered drug metabolism with no overshoot to a supranormal or infranormal rate of enzyme activity.[1–7] This suggests that the insulin effect on drug metabolism is not of primary importance and depends on correction of the state of insulin deficiency. Little is known about the influence of oral hypoglycemic agents on drug metabolism. The present findings do not confirm earlier results[12] but suggest that drug metabolism in diabetics does not seem to be influenced directly by different treatment regimens.

The results demonstrate the existence of wide interindividual variations in drug-metabolizing capacity in patients with diabetes mellitus. Both in vivo and in vitro parameters of drug metabolism were related to liver histologic changes. On the other hand, the diabetes per se or different treatment regimens did not seem to affect significantly drug metabolism. It follows that the variability in drug-metabolizing capacity in diabetics is an important point of consideration with selecting oral drugs for chronic use only in patients with liver abnormalities, or, when evaluating effectiveness and adverse reactions to drugs on a large scale.[19,20]

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