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Comparison of [^{14}C] Phenacetin and Amino[^{14}C]pyrine Breath Tests after Acute and Chronic Liver Injury in the Rat[1] (40843)

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Breath tests have become popular as a simple, noninvasive investigational technique in gastroenterology in recent years. They are used mainly as tests of small bowel function (1) and as measures of hepatic drug metabolizing capacity (2). A variety of substrates have been used in assessing hepatic dysfunction with the aminopyrine breath test being the most widely used. The aminopyrine breath test was first described by Lauterburg and Bircher (3) using various animal models of hepatic dysfunction and subsequently Hepner and Vesell (4) have demonstrated it to be abnormal in patients with cirrhosis. Both groups have shown the breath test to correlate with the disappearance of aminopyrine from plasma. Recently Breen *et al.* (5) reported that [^{14}C] phenacetin can also be used as substrate in a breath test and showed that its elimination is also abnormal in cirrhosis. The metabolism of phenacetin is mediated via cytochrome *P*-448 (6) while aminopyrine is metabolized via cytochrome *P*-450 (7). Therefore, another potential for breath tests is to use various substrates to explore the effect of liver disease on different routes of drug metabolism.

Despite the increasing use of breath tests it remains unknown how well a change in breath test reflects the extent of hepatic damage. This study was designed, therefore, to examine the [^{14}C]phenacetin and amino[^{14}C]pyrine breath tests in a model system in which the degree of hepatic damage was varied. In addition, we have compared the influence of acute and chronic liver injury in the rat on each route of metabolism.

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Methods. A. Experimental models of liver injury. Two groups of male Sprague–Dawley rats and their appropriate controls were studied. Animals were fed Purina rat chow and water *ad lib.*

1. Acute liver injury. Rats (175–225 g) were used in all experiments. Graded liver injury was induced by intraperitoneal injection of 0.2, 0.4, 0.8, 1.0, and 1.5 ml/kg carbon tetrachloride (CCl_4) as a 25% solution in corn oil. Control rats received 0.8 ml of corn oil intraperitoneally. Breath tests were performed after a 12-hr fast, 24 hr after the administration of CCl_4 or corn oil. Blood was taken from the aorta at the end of the experiment for aspartate aminotransferase (AST; SGOT) determinations by the malate dehydrogenase/NADH SMA 11 automated procedure. Livers were removed and fixed in formalin prior to section and stained with hematoxylin and eosin and examined microscopically. Sections were coded and read blindly by two observers (P.D. and S.S.) for the extent of hepatic necrosis. The extent of necrosis was graded 0–5 based on the amount of cell damage extending from the central vein to the adjacent portal tracts. Thus damage covering about 20% of the area around the central vein was graded as 1, 40% as 2, 60% as 3, etc.

2. Chronic liver injury. Experimental cirrhosis was produced in the rat as previously reported (8). Briefly cirrhosis was produced by simultaneous administration of CCl_4 and phenobarbital. Phenobarbital was added to the drinking water at a concentration of 0.5 g/liter and CCl_4 was administered twice weekly by exposure to CCl_4 in air (inhalation). Animals received at least 20 inhalations of 10 min each and studies were performed 8 days after ceasing phenobarbital and CCl_4. Histological examination of the liver was performed in all animals and only

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those which showed evidence of cirrhosis (presence of nodular regeneration and increased fibrosis) were included in this group. Control animals received only phenobarbital.

B. Breath tests. [^{14}C]phenacetin[2] (0.25 ml, 1 μCi/0.2 mg/ml, labeled on ethyl group) or 1 ml of amino[^{14}C]pyrine (29 μg/1.5 μCi/1.5 ml, labeled on both methyl groups) (Amersham Searle Corp.), was injected into the peritoneal cavity of unanesthetized rats. Rats were housed in individual airtight restraining cages; exhaled $^{14}CO_2$ was drawn through concentrated sulfuric acid to remove water and then through a scintillation vial containing 10 ml of a 2:1 (v/v) methanol–ethanolamine mixture to trap all expired CO_2. Previous experiments with two vials placed in series demonstrated that all expired $^{14}CO_2$ was trapped in the first vial. All CO_2 expired was collected during eight consecutive 15-min periods starting 60 min after the test drug was administered. Trapped radioactivity was determined after adding 10 ml A.C.S. (Amersham, Arlington Heights, Ill.) and counting in a liquid scintillation counter, using the automatic external standardization procedure to correct for quenching. The rate of exhaled $^{14}CO_2$, as measured by the total disintergrations per minute per collection period, decreased exponentially during the collection period and the elimination rate constant (K_{el}) was calculated by a least square regression analysis of the logarithm of the amount of $^{14}CO_2$ produced with respect to time. The elimination half-life was calculated by dividing 0.693 by the elimination rate constant.

C. Statistics. Statistical evaluations were performed with the two-tailed unpaired Student's t test, and linear regression analysis was performed to compare dose, elimination rate constant and SGOT values. In both cases $p < 0.05$ was taken to be the minimal level of statistical significance.

Results. Increasing doses of carbon tetrachloride given acutely caused a dose-related degree of hepatocellular damage as judged by both histology and SGOT levels (Table I). There was a significant association between the dose of CCl_4 and histological damage ($r = 0.80$, $P < 0.001$) and the dose of CCl_4 and SGOT ($r = 0.76$, $P < 0.001$) (Fig. 1). Acute CCl_4 induced dose-dependent changes in both phenacetin and aminopyrine elimination (Table II). The elimination of phenacetin was not different after the lowest dose of carbon tetrachloride as compared to controls. At a dose of 0.4 ml/kg carbon tetrachloride the elimination of phenacetin became abnormal and at a dose of 1 ml/kg the rate of excretion of $^{14}CO_2$ was reduced to such an extent that it could not be accurately estimated over a 2-hr time collection, indicating a reduction in the rate of elimination of at least 80%. Similarly, CCl_4 produced a dose-dependent reduction in the rate of aminopyrine elimination although it was not until a dose of 0.8 ml/kg carbon tetrachloride that the test became significantly different from control values. At higher doses of carbon tet-

TABLE I. EFFECT OF INCREASING DOSE OF CARBON TETRACHLORIDE ON EXTENT OF HISTOLOGICAL DAMAGE AND ASPARTATE AMINOTRANSFERASE LEVELS

Dose of carbon tetrachloride	Histological damage (graded 0–5)[a]	Aspartate aminotransferase (U/liter)
Control (n = 8)	0	65 ± 15[b]
0.2 ml/kg (n = 5)	2.4 ± 0.7	515 ± 175
0.4 ml/kg (n = 5)	2.5 ± 0.5	666 ± 325
0.8 ml/kg (n = 5)	3.9 ± 0.6	1114 ± 555
1.0 ml/kg (n = 5)	4.1 ± 0.6	1210 ± 352
1.5 ml/kg (n = 5)	3.9 ± 0.6	1955 ± 1117

[a] Extent of damage between central vein and portal tract (see Methods).
[b] Mean ± SD.

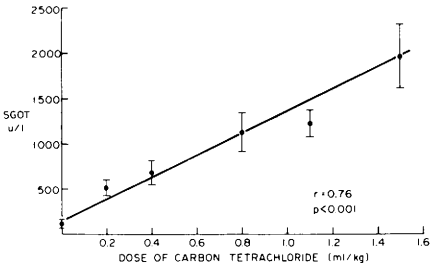

FIG. 1. Correlation between dose of carbon tetrachloride and SGOT levels.

[2] Provided by Dr. Ian Calder, University of Melbourne, Victoria, Australia.

TABLE II. EFFECT OF INCREASING DOSE OF CARBON TETRACHLORIDE ON PHENACETIN AND AMINOPYRINE BREATH TESTS (MEAN ± SD)

Dose of carbon tetrachloride (ml/kg)	Phenacetin breath test		Aminopyrine breath test	
	Half-life (min)	Elimination rate constant (min^{-1})	Half-life (min)	Elimination rate constant (min^{-1})
Control	32 ± 2	0.022 ± 0.001	52 ± 18	0.013 ± 0.005
0.2	33 ± 2	0.021 ± 0.001	62 ± 15	0.011 ± 0.003
0.4	40 ± 3*	0.017 ± 0.001*	68 ± 16	0.010 ± 0.002
0.8	127 ± 56*	0.005 ± 0.002*	108 ± 40	0.006 ± 0.002*
1.0	>200*	<0.003*	127 ± 36*	0.005 ± 0.002*
1.5	>200*	<0.003*	172 ± 68*	0.004 ± 0.002*

* $P < 0.05$ as compared to appropriate control.

rachloride the aminopyrine elimination was influenced to a lesser extent than the phenacetin breath test. There was a linear dose–response relationship when log·dose was compared to the change in elimination rate constant (K_{el}) of phenacetin ($r = 0.92$, $P < 0.001$) and of aminopyrine ($r = 0.76$, $P < 0.001$) (Fig. 2). The slope of the phenacetin dose response curve was significantly steeper than that of aminopyrine. By contrast (Fig. 2) the change in half-life for both drugs with the dose of CCl_4 was curvilinear.

In the cirrhotic animals the phenacetin half-life was prolonged 17% as compared to their controls although this did not quite reach statistical significance. The aminopyrine elimination was prolonged by 33% in cirrhotics as compared to controls and these differences were significantly different (Table III).

Discussion. This study, employing breath tests, demonstrates that there is a relationship between the rate of elimination of both aminopyrine and of phenacetin and the extent of acute carbon tetrachloride-induced hepatic damage. Furthermore, there is a differential susceptibility of the metabolism of these compounds to acute and chronic liver injury (cirrhosis) induced by carbon tetrachloride in rats. Although there was a linear dose–response relationship with both aminopyrine and phenacetin after acute carbon tetrachloride administration, the slope of the phenacetin curve was much steeper than that of aminopyrine. This suggests that phenacetin metabolism is more sensitive and susceptible to this type of acute hepatic damage than aminopyrine. It is tempting to speculate that this is due to the different routes of metabolism of these compounds. Phenacetin is metabolized via deethylation and its rate of metabolism can be induced by type II inducers (3-methylcholanthrene) (6, 7) suggesting that its metabolism is cytochrome *P*-448 mediated. Aminopyrine is metabolized via two demethylation steps and its metabolism can be induced by phenobarbital (4, 7) suggesting that its metabolism is mediated via cytochrome *P*-450. One explanation of our findings may be that cytochrome *P*-448 is more susceptible to acute carbon tetrachloride-induced liver injury than cytochrome *P*-450 enzyme systems. An alternative explanation is that one of the subsequent steps in the production of $^{14}CO_2$ from these test substances becomes rate-limiting in these liver injuries and thus the $^{14}CO_2$ is produced via different pathways. Phenacetin is deethylated and the acetate group

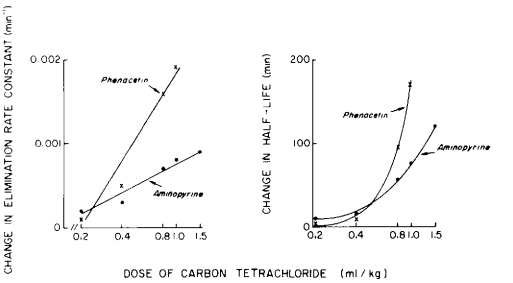

FIG. 2. Correlation between log·dose of carbon tetrachloride and change in elimination rate constant and in half-life of phenacetin and aminopyrine.

TABLE III. EFFECT OF EXPERIMENTAL CIRRHOSIS ON PHENACETIN AND AMINOPYRINE BREATH TESTS

	Control (n = 6)	Cirrhosis (n = 6)	Percentage difference
Phenacetin			
Half-life (min)	36 ± 5[a]	42 ± 9*	+16.7
K_{el} (min^{-1})	0.0193 ± 0.003	0.0165 ± 0.0037*	−14.5
Aminopyrine			
Half-life (min)	45 ± 8	60 ± 12*	+33.3
K_{el} (min^{-1})	0.0154 ± 0.0028	0.0116 ± 0.0024*	−25

[a] Mean ± SD.
* $P < 0.05$.

produced goes via the Krebs cycle to $^{14}CO_2$. In contrast, the methyl group from aminopyrine is converted to formaldehyde, formate, and then CO_2. It is therefore possible that carbon tetrachloride differentially affects these intermediate pathways of CO_2 production. In contrast to the acute liver injury, experimental cirrhosis impaired the elimination of aminopyrine to a greater extent than phenacetin.

Since breath tests were employed to assess drug elimination in these studies, their advantages and limitations warrant brief consideration. Breath tests are simple, noninvasive procedures which require little time for analysis but they are limited in that they measure the production of a metabolite ($^{14}CO_2$). There are a number of metabolic steps between the initial removal of the labeled carbon from the parent compound by demethylation or deethylation and the exhalation of $^{14}CO_2$. However, studies by both Lauterburg and Bircher (3) and Vesell and Hepner (4) suggest that the rate of $^{14}CO_2$ elimination does reflect accurately the rate of elimination of the parent compound, even in the presence of liver disease. Another limitation of the breath test is that it measures only a rate of elimination. This may be expressed as the rate constant of elimination (K_{el}) or more commonly as the half-life ($T½$). Whichever term is used, this parameter is a hybrid function dependent on the two physiological variables—the volume of distribution (V_d) and clearance (Cl), which is the measure of efficiency of drug elimination and for the purpose of this study is the optimal index of the capacity of the liver to metabolize drugs (9). For the present study, if we assume that there is no change in V_d and that the rate of elimination of $^{14}CO_2$ equals the rate of elimination of the parent compound in plasma, then clearance can be assessed by K_{el} or $T½$. Changes in clearance will be reflected by linear changes in K_{el} or reciprocal changes in half-life (Fig. 2). Thus, in situations where the effect of liver disease on drug kinetics is being examined and when only the elimination rate data are

TABLE IV. PERCENTAGE CHANGE IN HALF-LIFE, ELIMINATION RATE CONSTANT, AND PLASMA CLEARANCE IN CIRRHOTIC PATIENTS AS COMPARED TO CONTROLS

	Diazepam[a]	Chlordiazepoxide[b]	Meperidine[c]	Meperidine[d]	Pentazocine[c]	Clindamycin[e]
Half-life	+127	+163	+69	+119	+72	+30
Elimination rate constant	−56	−61	−40	−54	−42	−23
Plasma clearance	−48	−50	−36	−50	−46	−23

Note. Mean values from the literature in cirrhotics as compared to control subjects, expressed as a percentage of control values.

[a] Klotz, U., Avant, G. R., Hoyumpa, A., Schenker, S., and Wilkinson, G. R., J. Clin. Invest. 55, 347–359 (1975)
[b] Roberts, R. K., Wilkinson, G. R., Branch, R. A., and Schenker, S., Gastroenterology 75, 479–485 (1978)
[c] Neal, E. A., Meffin, P. J., Gregory, P. B., and Blaschke, T. F., Gastroenterology 77, 96–102 (1979)
[d] Klotz, U., McHorse, T. S., Wilkinson, G. R., and Schenker, S., Clin. Pharmacol. Ther. 16, 667–675 (1974)
[e] Avant, G. R., Schenker, S., and Alford, R. H., Amer J. Dig. Dis. 20, 223–230 (1975)

available, it is better to compare groups using K_{el} than half-life. This simple principle is illustrated in studies from the literature (Table IV) where the clearance, $T½$, and K_{el} (calculated) have been compared in control subjects and cirrhotic patients. K_{el} closely reflects changes in clearance while the percentage change in $T½$ overestimates the change in underlying function, this error increasing with increasing extent of change. In the present study, using the change in K_{el} as a measure of response, there was a linear dose–response relationship between the degree of acute hepatic damage and impairment of both phenacetin and aminopyrine elimination. A similar observation has also been observed by Sultatos *et al.* (10) for acetaminophen toxicity and the aminopyrine breath test. Using their data and plotting K_{el} and log·dose, a linear relationship is obtained. Carlisle *et al.* (11) likewise have shown that the aminopyrine breath test correlates well with the degree of hepatic necrosis in patients with alcohol-induced liver damage. This demonstrates well that the extent of impairment of the breath test reflects the extent of acute hepatic damage. However, it should be noted that at the lowest dose of carbon tetrachloride (0.2 ml/kg) the SGOT was elevated and histology showed some necrosis although both breath tests were normal. This confirms results reported by others in man (12) that SGOT will be a better marker of the presence or absence of acute necrosis than a breath test. The latter, however, should be a better indicator of the extent of functional impairment (i.e., metabolic capacity) in liver disease.

Summary. The elimination of both [^{14}C]phenacetin and amino[^{14}C]pyrine as judged by production of $^{14}CO_2$ in the breath showed a linear response relationship to the degree of hepatic necrosis. Further there was a differential sensitivity of the elimination of these test compounds to acute and chronic liver injury suggesting a variable susceptibility of the enzymes responsible for their metabolism. The study emphasizes the importance of using K_{el} rather than half-life when comparing the effect of liver injury on drug metabolism. Finally, the study demonstrates that breath tests will reflect the extent of hepatic damage and may also be used to examine the effect of liver disease on various routes of drug metabolism.

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