Gut, 1978, **19**, 40-45

Assessment of the (^{14}C) aminopyrine breath test in liver disease

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SUMMARY Different methods of performing the (^{14}C) aminopyrine breath test have been assessed. A tracer dose of 2 μCi without a loading dose and with a single breath collection at two hours was the method selected, since it gave the best discrimination between patients with hepatocellular diseases and normal subjects (5·2 ± 0·2%, mean ± SEM). Reduced values occurred in patients with chronic active hepatitis (with and without cirrhosis) (1·5 ± 0·2%), alcoholic cirrhosis (1·7 ± 0·4%) and hepatitis (2·5 ± 0·3%), and late primary biliary cirrhosis suggesting defective microsomal function with respect to demethylation. Normal results were common in early primary biliary cirrhosis. Two weeks of prednisolone therapy caused some improvement in the breath test in nine of 10 patients with chronic active hepatitis. It is concluded that the (^{14}C) aminopyrine breath test is a simple test for detecting hepatocellular dysfunction, but has no obvious diagnostic advantage over the determination of serum aspartate transaminase and two hour post-prandial bile-acids.

Microsomal enzymes play an important role in drug metabolism. Evidence that their activity may be reduced in liver disease (Schoene *et al.*, 1972) is indirect and is limited to studies with selected drugs or chemicals, involving different enzymes for their metabolism. Lauterburg and Bircher (1976) showed in animal studies that the $^{14}CO_2$ exhaled after intravenous administration of aminopyrine specifically labelled at the two 4-N-methyl groups (Fig. 1) gives quantitative information on the hepatic demethylation of the drug. Subsequently a breath test, after oral administration of (^{14}C) aminopyrine, has been developed which provides a simple procedure for the quantitative assessment of microsomal function in man (Hepner and Vesell, 1974; Bircher *et al.*, 1976a).

Several different procedures for the test have been proposed (Hepner and Vesell, 1975; Bircher *et al.*, 1976a, b) and these have been evaluated in this study. The value of the test in patients with primary biliary cirrhosis (PBC) and alcoholic liver disease has also been examined. In addition, the effect of prednisolone therapy in chronic active hepatitis on the results obtained in the test has been investigated.

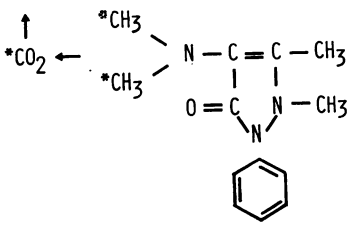

Fig. 1 *Formation of $^{14}CO_2$ from (^{14}C) aminopyrine resulting from microsomal demethylation. *Radioactive carbon atom.*

Methods

SUBJECTS

The patients were grouped according to the diagnosis, which was made on clinical, biochemical, and histological grounds. Twenty-seven (14 men and 13 women, aged 25-81 years) suffered from chronic active hepatitis (CAH) with and without cirrhosis and 21 (one man, 20 women, age range 30-84 years) from symptomatic primary biliary cirrhosis (PBC). Twenty-six suffered from alcoholic liver disease,

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Received for publication 5 October 1977

11 with cirrhosis (four men, seven women, age range 35-70 years), seven with hepatitis without cirrhosis (four men, three women, age range 36-61 years) and eight patients with fatty change (six men, two women, age range 29-57 years). In addition patients with acute hepatitis (three), hepatic malignancy (four), neoplastic large bile duct obstruction (three), and extrahepatic portal vein obstruction (three) have been investigated.

The 15 normal controls (eight men, seven women, age range 17-82 years) comprised four healthy laboratory staff and 11 patients with normal liver function. None of the patients or controls had taken drugs known to induce hepatic microsomal function for at least 15 days before the test (Levi *et al.*, 1968). Permission for the study was given by the Hospital Ethics' Committee and informed consent was obtained from each patient and control.

LIVER FUNCTION TESTS

Serum aspartate transaminase, bilirubin, and albumin determinations were performed. Some patients had their prothrombin times and two hour post-prandial bile acids estimated (Barnes *et al.*, 1975).

EXPERIMENTAL TECHNIQUES

(^{14}C) *aminopyrine breath test*

(Dimethylamine-^{14}C) aminopyrine (12·2 mCi/mmol), specifically labelled at the two N-methyl groups, was obtained from the Radiochemical Centre, Amersham. It was diluted with 154 mM NaCl to give a solution of 1 μCi/ml, which was kept at 4°C. After an overnight fast the patients were given an oral dose of 2 μCi (164 nmol) dissolved in approximately 50 ml water. They remained recumbent for the duration of the test and blew through a glass tube, with an anhydrous calcium chloride filter into a counting vial containing 2 ml hyamine/ethanol (1:1, v/v) with 0·6% (w/v) thymolphthalein as indicator. Ten millilitres scintillant containing 2,5-diphenyl oxazole (4 g) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (50 mg) in 1 litre toluene, were then added and the radioactivity of the sample determined in a Phillips liquid scintillation spectrometer. For each test 1 ml of the absorbent solution was titrated with 0·1 M HCl until the blue colour of the indicator disappeared; the number of mmol CO_2 absorbed is therefore 10/titration (ml). The results were calculated as dpm/mmol CO_2 and expressed as a percentage of the administered dose assuming that the endogenous products of carbon dioxide were 9 mmol kg^{-1} (Winchell *et al.*, 1970). An accurate assessment of the dose administered was obtained by diluting an aliquot of the stock ^{14}C aminopyrine with 10% (v/v) aqueous ethanol to give a solution with a concentration of 1 μCi per litre and then adding 1 ml to 10 ml scintillant. All estimations were made in duplicate.

Determination of decay constant of specific activity of $^{14}CO_2$ in breath (K_B)

Samples of $^{14}CO_2$ were collected before the test and then half, one, two, three, five, and eight hours after the administration of the aminopyrine. The specific activities were plotted on semi-logarithmic paper and the value for the half life in hours ($T_{\frac{1}{2}}$) calculated by least square analysis of the results obtained after two hours, when the specific activities declined exponentially. The decay constant K_B was calculated from the formula $K_B = \frac{0{\cdot}693}{T_{\frac{1}{2}}} \times 100$ and the results expressed as percentage per hour.

Fractional disappearance of aminopyrine from plasma (K_p)

Twenty subjects received a dose of non-labelled aminopyrine (9 mg/kg) simultaneously with the isotope. Plasma samples were obtained at zero time and two, four, six, and eight hours later. The aminopyrine content was assayed spectrophotometrically at 260 mμ in acid after extraction into ethylene dichloride; allowance was made for the formation of the metabolites, N-acetyl 4-aminoantipyrine and 4-amino-antipyrine by the method of Brodie and Axelrod (1950).

Two hour breath test (SA_{2h})

Expired $^{14}CO_2$ was estimated two hours after the administration of the ^{14}C aminopyrine. It was assumed that the CO_2 production rate did not change between $1\frac{1}{2}$ and $2\frac{1}{2}$ hours. Results were expressed as a percentage of the dose administered as previously indicated. The values obtained were less than those of Hepner and Vesell (1975), who determined the cumulative excretion over two hours. They also differ from the two hour values of Bircher *et al.* (1976a b,), who did not allow for endogenous CO_2 in their calculations. Results for the groups were compared using Student's *t* test with a confidence limit of 95%.

Results

COMPARISON OF METHODS

Examples of the specific activity curves for CO_2 following the administration of (^{14}C) aminopyrine are given in Fig. 2. The peak of radioactivity excretion was reached within two hours after the drug administration in 45 of the 47 patients in whom K_B

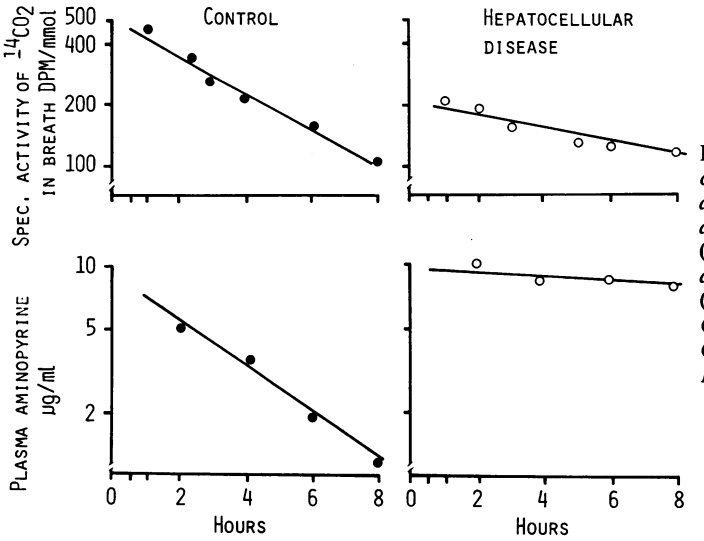

Fig. 2 *A comparison of disappearance curves of specific activity of $^{14}CO_2$ in breath and aminopyrine in plasma after administration of ^{14}C-aminopyrine (2 µCi; 9 mg/kg) in a control subject (●) and a patient with hepatocellular disease (chronic active hepatitis with cirrhosis) (○). Control: K_B = 20·9%/h, K_p = 25·5%/h, CAH + cirrhosis: K_B = 7·0%/h, K_p = 2·0%/h.*

was determined (30 at 30 minutes, eight at one hour and seven at two hours) and an exponential rate of decay was then observed. Very much flatter curves were obtained in patients with hepatocellular disease than in the control subjects. Similar observations were obtained for the plasma disappearance curves (Fig. 2). The values for the decay constants of $^{14}CO_2$ specific activity in breath (K_B) and plasma aminopyrine clearance (K_p) correlated reasonably well in seven subjects with normal liver function tests (mean K_B = 18·7%/h, mean K_p = 21·0%/h) and 11 patients with liver disease (r = 0·70, P < 0·01). A better correlation was obtained when only the six patients with hepatocellular disease were considered (r = 0·89, P < 0·05). The values for CO_2 specific activity at 2h(SA_{2h}) also correlated well with K_p (r = 0·88, n = 20, P < 0·001).

When the results for SA_{2h} were compared with those for K_B in 47 patients a good correlation was also obtained (r = 0·72, P < 0·001). In a comparison between six controls and nine cirrhotic patients, using the results for SA_{2h}, only one of the cirrhotic patients had a value within the range of 95% of the controls, whereas with the results for K_B four patients had normal results. This suggested that the determination of SA_{2h} provided the best test of discrimination between cirrhotics and normal subjects. No improvement in discrimination was obtained using values for peak $^{14}CO_2$ specific activity, or cumulative radioactivity in breath for the first two hours of the test. All subsequent studies were therefore performed using a single determination of $^{14}CO_2$ specific activity at two hours. Studies in four controls and seven patients with liver disease showed that the values for SA_{2h} tended to be slightly lower when a loading dose was given, but the difference between the mean values was not statistically significant.

REPRODUCIBILITY OF TWO HOUR BREATH TEST

Six patients with liver disease and two normal subjects had the two hour breath test repeated after five days. The coefficient of variation for the two sets of results, which had mean values of 3·48% and 3·53% respectively, was 6·1%.

PATIENT STUDIES WITH TWO HOUR BREATH TEST (Figs. 3 and 4)

The results for the 15 control subjects ranged from 4·1 to 6·5% (5·2 ± 0·2%, mean ± SEM). In contrast, the values for the 27 patients with CAH, with and without cirrhosis, ranged from 0·2 and 3·8%; the mean value of 1·5 ± 0·2% was significantly different from that for the controls (P < 0·001).

The mean value for 21 patients with symptomatic primary biliary cirrhosis was 4·1 ± 0·3%. This was significantly lower than that for the controls (P < 0·001) and higher than that for the CAH patients (P < 0·001). Fifteen of the 21 PBC patients, however, had values within the normal range; all those who had subnormal results had histological and biochemical evidence of late PBC.

The 27 patients with alcoholic liver disease were grouped according to histological diagnosis (Fig. 4). Eleven had alcoholic cirrhosis with a mean value of 1·7 ± 0·4%; this was significantly different from that of the controls (P < 0·001) but not from the CAH

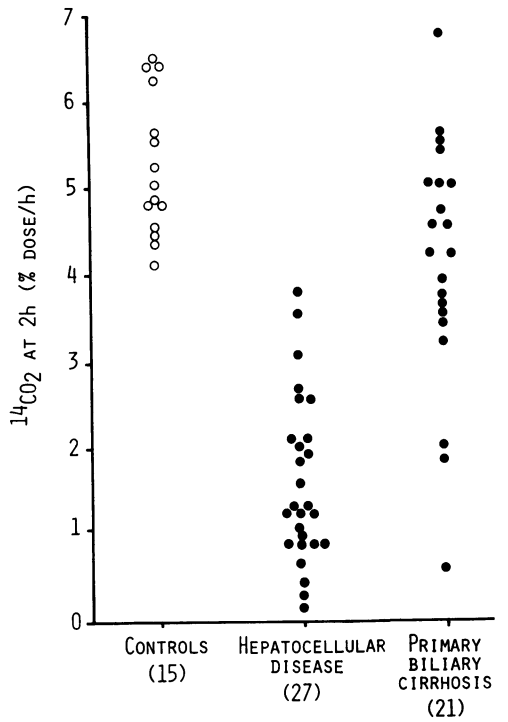

Fig. 3 *Results for two hour breath test (SA_{2h}) on patients with hepatocellular disease (CAH cirrhosis, 27) and primary biliary cirrhosis (21) compared with controls (15) (expressed as % dose/h).*

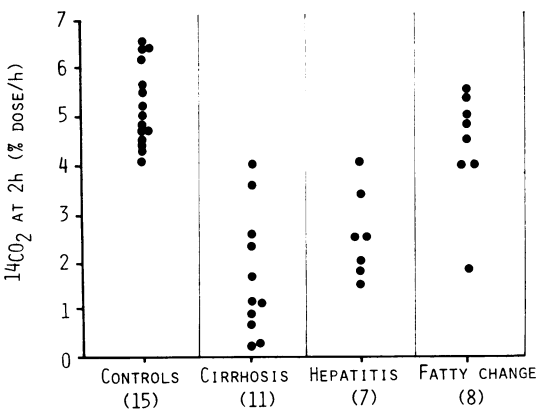

Fig. 4 *Results for two hour breath test (SA_{2h}) in alcoholic patients separated into three subgroups according to histological diagnosis: cirrhosis (11), hepatitis (seven), fatty change (eight).*

group. Two of the alcoholic cirrhotics had values within the normal range (3·6 and 4·0%). The seven patients with alcoholic hepatitis without cirrhosis had a mean value of 2·5 ± 0·3%; this differed significantly from the controls ($P < 0·001$) but not from the alcoholic cirrhotics ($P < 0·10$). The eight patients who had only fatty change on histological examination had a mean value of 4·4 ± 0·4%; this is significantly higher than the two previous subgroups ($P < 0·001$) and lower than the controls ($P < 0·05$).

A miscellaneous group of patients was also studied. Three patients with acute hepatitis (two viral and one isoniazid-related) had reduced values (mean 1·8%, ranging from 0·2-3·8%), which is in agreement with the observations of Hepner and Vesell (1975). The mean value for four patients with hepatic malignancy was 3·1% (2·4-4·0%). Three patients with neoplastic large bile duct obstruction had a mean value of 2·6% (1·2-3·6%). Three patients with extrahepatic portal vein obstruction had a mean value of 4·3% (3·4-5·8%).

EFFECT OF PREDNISOLONE AND AZATHIOPRINE IN CAH

Eight patients with histologically confirmed HB_sAg negative CAH and five patients with HB_sAg positive CAH were studied using the two hour test before starting prednisolone (20-30 mg daily); the test was repeated after one week in 12 patients and after two weeks in 10 patients. After one week the two hour breath test had improved in 10 of the 12 patients (mean improvement 0·73 ± 0·87%) and after two weeks had improved in nine of 10 patients (mean improvement 0·81 ± 0·94%). Six of these increased values shown in Fig. 5 were higher than could be expected from day to day variation. The overall improvement in the results for the 13 patients, however, just failed to reach conventional significance ($P < 0·10$).

Other tests of liver function were performed at the same time as the breath test. In the 10 patients who had two weeks treatment serum aspartate transaminase values fell significantly ($P < 0·01$) after steroid therapy but there was no significant improvement in the breath test ($P < 0·15$), serum total bilirubin ($P < 0·30$), or serum albumin ($P < 0·45$). The two hour post-prandial bile acid determinations made at the time of the breath test in six patients were not significantly altered by the prednisolone therapy.

Two patients, one with HB_sAg positive CAH and one with HB_sAg negative CAH, were studied before and after azathioprine (100 mg daily). The condition of the HB_sAg positive patient deteriorated and the results for the breath test decreased (1·9% before treatment; 1·4% after one week; 0·8% after two weeks), whereas the results for the HB_sAg negative patient did not change (1·6% before treatment; 1·5% after one week; 1·7% after two weeks).

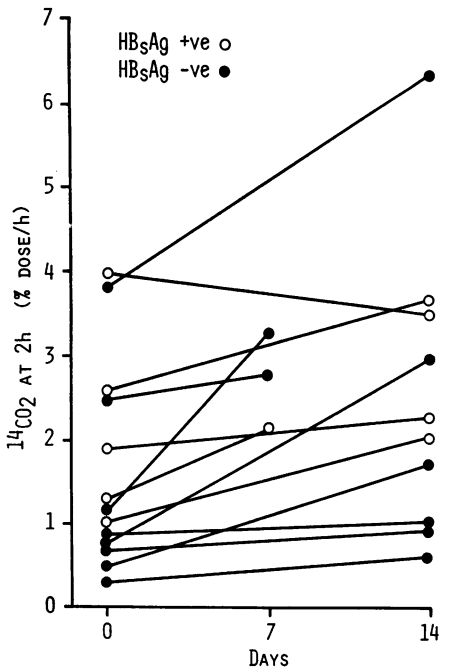

Fig. 5 *Effect of prednisolone therapy (20-30 mg/day) on two hour breath test in 13 patients with chronic active hepatitis (eight HB_sAg negative, five HB_sAg positive). The data at seven days is given only for the three patients who did not have a test after 14 days.*

CONVENTIONAL LIVER FUNCTION TESTS AND BREATH TEST IN HEPATOCELLULAR DISEASE

The results of conventional liver function tests and the breath test have been compared in 38 consecutive patients with hepatocellular disease (10 CAH without cirrhosis; 28 patients with either alcoholic cirrhosis or cirrhosis secondary to CAH). Of these 38 patients, three had normal results for the breath test and normal serum aspartate transaminase values, 10 had normal serum total bilirubin values, and 23 had a normal serum albumin concentration. Fourteen of 28 patients had a prothrombin time after vitamin K less than two seconds prolonged. Serum two hour post-prandial bile acids were performed in 24 of the 38 patients and only one patient had a normal result. The values for the two hour post-prandial bile acids correlated inversely with those of the aminopyrine breath test ($r = -0{\cdot}45$, $P < 0{\cdot}05$).

Discussion

It has been previously shown that aminopyrine is rapidly and completely absorbed from the small bowel and is then homogeneously distributed in the total body water. Microsomal demethylation, predominantly in the liver, accounts for at least 50-60% of its metabolism (Brodie and Axelrod, 1950), so that an estimate of the capacity of the liver for demethylation can be obtained by measuring the expired $^{14}CO_2$ after an oral dose of (^{14}C) aminopyrine. Determination of the decay constant K_B (Bircher *et al.*, 1976a) is certainly a more acceptable test for the patient than an enzyme assay on a liver biopsy specimen (Schoene *et al.*, 1972), but for outpatients it has the disadvantage of taking eight hours. The value of an empirical test, in which only a two hour measurement was made (Hepner and Vesell, 1975), was therefore investigated.

A constant of 9 mmol/kg/h for endogenous CO_2 production was included in the calculation so that the results could be expressed in terms of percentage of dose administered per hour at two hours; this constant is, however, influenced by such factors as physical exercise, which might be important when outpatients are studied. Physical activity was therefore restricted as far as possible in these patients and a second breath test was always performed under the same conditions as the first.

The results of the two hour breath test using a tracer dose were compared with those obtained after a loading dose of 9 mg/kg in 11 patients, since this dose had been used when the breath and plasma tests were performed simultaneously. The administration of a large dose of aminopyrine did not improve the discrimination capacity of the breath test, so a tracer dose only was given in subsequent studies.

In contrast to the breath test the rate of aminopyrine plasma disappearance rate reflects both demethylation and the different catabolic processes which the compound undergoes (Brodie and Axelrod, 1950). This could explain the small difference observed for the values of K_p and K_B. In our studies SA_{2h} correlated better with K_p than K_B, specially in normal subjects.

Our results are essentially similar to those of Bircher and coworkers (1976) and Hepner and Vesell (1975). Low values for the breath test at two hours are characteristic of hepatocellular disease. In contrast, normal values are common in PBC where hepatocellular failure is a late feature. Although more studies in those with more marked cholestatic jaundice are necessary, our data suggest that cholestasis *per se* is not as important as hepatocellular dysfunction in affecting demethylation activity. All the alcoholic patients studied had some degree of liver disease; reduced values were seen in the cirrhotic and hepatitic subgroups and, as would be expected, were related to the degree of hepatocellular failure. In the miscellaneous group low results were observed in patients with hepatocellular damage and metastatic liver disease.

An improvement in the breath test results has been shown in some of the HB_sAg positive and negative CAH patients after prednisolone. The improvement in the HB_sAg positive patients was of interest as steroids have not been shown to be very effective in treating this disease (Schalm *et al.*, 1976). This observation supports the hypothesis that corticosteroids may induce microsomal enzymes, possibly by antagonising the action of a post-transcriptional repressor (Gelehrter, 1973). It might, however, also reflect an improvement in the overall degree of hepatocellular function, rather than a specific inducing effect. In contrast, no effect was seen in two patients treated with azathioprine, which acts predominantly as an immunosuppressant. This is in agreement with previous studies with indocyanine green which showed no improvement in hepatic reserve (Rikkers and Sherlock, 1975).

The breath test appears to be more sensitive in detecting hepatocellular disease than liver function tests such as serum albumin, serum total bilirubin, and prothrombin time. Its efficiency as a screening test for hepatocellular dysfunction was, however, similar to that of serum aspartate transaminase and serum two hour post-prandial bile acids determinations. Others have shown that the results of the breath test compare well with such quantitative tests of hepatic function as bromosulphthalein excretion and galactose elimination capacity (Bircher *et al.*, 1976a, b). One limitation of the test is that it requires the administration of a small dose of radioactivity (2 μCi) but most of it is quickly eliminated in the breath. In centres where mass spectrometry is available (^{13}C) aminopyrine could be used as an alternative (Schneider *et al.*, 1975). Although a hypersensitivity reaction to aminopyrine has been described, this is rare (Palva and Mustala, 1970, 1972) and it has not been reported after a single dose; moreover, only a tracer dose (164 nmol) is given for this test.

The (^{14}C) aminopyrine breath test is easy to perform and involves a relatively non-invasive technique. It appears to be a useful screening test for hepatocellular disease, and complements other tests which measure different activities of the liver. Any real advantage over other standard laboratory investigations has, however, to be established.

Dr J. Galizzi was supported by Conselho Nacional de Desenvolvimento Cientifico E Tecnologico (Brasil). We are grateful to Dr S. Barnes for the serum bile-acid determinations, Professor P. J. Scheuer for liver biopsy interpretation, the Department of Medical Physics for advice, and the Department of Chemical Pathology for routine biochemical estimations. Dr J. Bircher and Professor J. Preisig kindly allowed us to read their paper before publication and gave us advice during the study.

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