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STUDIES OF THE METABOLISM OF *N*-METHYL CONTAINING ANTI-TUMOUR AGENTS

$^{14}CO_2$ BREATH ANALYSIS AFTER ADMINISTRATION OF ^{14}C-LABELLED *N*-METHYL DRUGS, FORMALDEHYDE AND FORMATE IN MICE

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Abstract—The $^{14}CO_2$ content of the breath was analysed after administration of the following *N*-$^{14}CH_3$ labelled drugs to mice: aminopyrine, hexamethylmelamine (HMM), pentamethylmelamine (PMM), procarbazine and caffeine. Except for aminopyrine, the $^{14}CO_2$ exhalation rate plots declined monophasically with half lives of 91 min ([^{14}C]-HMM), 97 min ([^{14}C]-PMM), 68 min ([^{14}C]procarbazine) and 92 min ([^{14}C]caffeine). The $^{14}CO_2$ exhalation rate peaked rapidly after aminopyrine administration and declined bi-phasically with an initial $t_{\frac{1}{2}}$ of 15 min and a terminal $t_{\frac{1}{2}}$ of 126 min. The $^{14}CO_2$ plots after both [^{14}C]-HMM and [^{14}C]aminopyrine were influenced by pre-treatment of mice with proadifen. Pretreatment with phenobarbitone shortened the $t_{\frac{1}{2}}$ of the $^{14}CO_2$ appearance rate after [^{14}C]HMM by 24% but did not change the $^{14}CO_2$ curve after administration of [^{14}C]aminopyrine. The $^{14}CO_2$ exhalation rate plots after administration of H^{14}CHO and H^{14}COOH were virtually identical with that obtained after [^{14}C]aminopyrine and not influenced by either proadifen or phenobarbitone pretreatment. The $^{14}CO_2$ exhalation rate profile obtained on metabolism of [^{14}C]aminopyrine in mice thus appears to be determined by the rate of the oxidation of formaldehyde or formate to CO_2. Only 24% of the label injected with the *N*-methyl moieties of [^{14}C]HMM and 21% of the label in [^{14}C]procarbazine were exhaled as $^{14}CO_2$, whereas 49% of the *N*-$^{14}CH_3$ in [^{14}C]aminopyrine were metabolized to $^{14}CO_2$. It remains to be determined whether this difference and the difference in the shapes of the $^{14}CO_2$ exhalation profiles obtained with the cytotoxic *N*-$^{14}CH_3$ drugs as compared to [^{14}C]aminopyrine, are related to the biochemical processes mediating their antineoplastic activity.

The major biotransformation pathway for most drugs containing *N*-methyl moieties is the hydroxylation of the carbon atom with subsequent cleavage of the N–C bond:

$$\text{>N-CH}_3 \rightarrow \text{>N-CH}_2\text{OH} \rightarrow \text{>N-H} + \text{HCHO}$$

The exact fate of the drug derived C_1 fragment in the mammalian organism is not very well understood. Part of it is metabolised to formaldehyde, thence to formate and finally CO_2 which is exhaled with the breath. Aminopyrine (Fig. 1), an obsolete analgesic, undergoes extensive *N*-demethylation, and the quantitative analysis of $^{14}CO_2$ appearing in the breath after administration of ^{14}C-labelled aminopyrine is used as clinical test for evaluating hepatic functions [1] and as a noninvasive method for estimating the rate of oxidative xenobiotic metabolism *in vivo* in animals [2].

Two pharmacological aspects of the fate of drug derived C_1 fragments have received little attention: Firstly, it is feasible to conceive that the metabolic *N*-demethylation leads to an accumulation of the potentially toxic species formaldehyde and formate. Formaldehyde has been shown to be cytotoxic [3] and the sequestration of formate has been associated with ocular toxicity [4]. Secondly, the antineoplastic *N*-methyl containing drug molecules hexamethylmelamine (HMM), procarbazine (Fig. 1) and the aryldimethyltriazenes (e.g. dacarbazine) are dependent on the presence of *N*-methyl moieties for cytotoxic activity [5]. Furthermore, these agents require metabolic activation. These two phenomena may indicate that the fate of drug derived C_1 metabolites is associated with biochemical events leading to antitumour activity. As part of our efforts to elucidate the chemical and biochemical features which confer antineoplastic activity on a molecule containing an *N*-methyl group, we investigated the disposition of drug derived $^{14}CO_2$ after administration of *N*-^{14}C-methyl labelled HMM, pentamethylmelamine (PMM) and procarbazine, and compared it with the $^{14}CO_2$ disposition after dosing mice with two *N*-^{14}C-methyl labelled xenobiotics devoid of antitumour activity, caffeine and aminopyrine. Although the [^{14}C]aminopyrine breath test has been the subject of several investigations [1, 2, 6–8], this is to our knowledge the first report of its application in mice. The appearance in the breath of CO_2 formed by the metabolism of an *N*-methyl group is the last step in a series of biophysical and biochemical processes, e.g. transfers across membranes and oxidative reactions of the C_1 unit. To test the hypothesis that the

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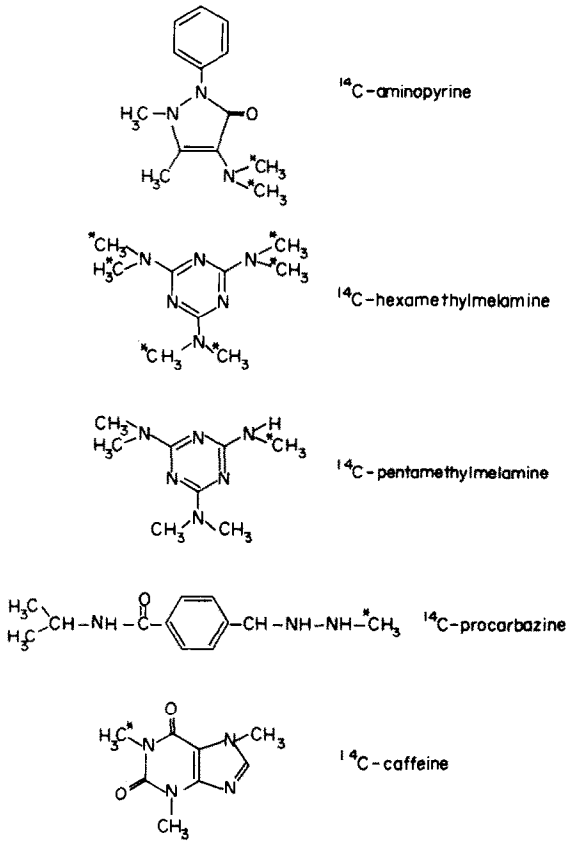

Fig. 1. Chemical structures of drugs used in this study. Asterisks indicate positions of ^{14}C label.

metabolic transformation of formaldehyde and formate, both metabolic intermediates of oxidative *N*-demethylations, to CO_2 does not determine the overall rate of formation of drug derived CO_2 [9], we compared the $^{14}CO_2$ formation rate plots after administration of $H^{14}CHO$ and $H^{14}COOH$ with those obtained from drugs containing *N*-^{14}C-methyl groups.

MATERIALS AND METHODS

Compounds. Procarbazine hydrochloride was the gift of Roche Pharmaceuticals, Welwyn Garden City, U.K. Caffeine and aminopyrine were commercially available, and HMM and PMM were synthesised in our laboratories. The following labelled drugs were generously provided by Dr. R. Engle, Chemical Resources Section, N.C.I., Bethesda, U.S.A.: 2,4,6-*N*($^{14}CH_3$)$_2$-hexamethylmelamine, 6-*N* $^{14}CH_3$-pentamethylmelamine, *N* $^{14}CH_3$-procarbazine. 1-$^{14}CH_3$-Caffeine, *N*($^{14}CH_3$)$_2$-aminopyrine, [^{14}C]formaldehyde and [^{14}C]sodium formate were purchased from New England Nuclear, Southampton, U.K., and The Radiochemical Centre, Amersham, U.K.

Animals and drug administration. Male CBA/Lac mice (25–30 g) were injected i.p. with compounds at the following doses: aminopyrine 38 mg/kg, HMM 40 mg/kg, PMM 40 mg/kg, procarbazine 100 mg/kg, caffeine 40 mg/kg, formaldehyde 2 mg/kg, sodium formate 2 mg/kg. 2 mg Formaldehyde constitute 20% and 2 mg formate 14% of the total portion of the dose of [^{14}C]aminopyrine which can be metabolized to labelled formaldehyde or formate. Amounts of radioactivity injected together with unlabelled drugs were 0.6 μCi in the case of aminopyrine and procarbazine, 1 μCi in the case of HMM, formaldehyde, formate and caffeine and 2 μCi in the case of PMM. Procarbazine (100 mg/kg) was also injected into a tail vein. Drugs were dissolved in saline (procarbazine, caffeine, aminopyrine, formaldehyde, formate) or in arachis oil (HMM, PMM). Volumes injected were 0.1 or 0.2 ml.

Proadifen (SKF 525 A, 2-diethylaminoethyl 2.2-diphenylvalerate) dissolved in saline was injected i.p. at a dose of 60 mg/kg 30 min prior to the administration of aminopyrine or HMM. Phenobarbitone dissolved in saline was administered i.p. at a dose of 50 mg/kg on four consecutive days before breath analysis.

Breath analysis. After drug administration the mice were placed in a plexiglass desiccator which served as breath collection chamber. The exhaled CO_2 was trapped continually in 15 ml of a methanol/ethanolamine (4:1 v/v) mixture. The mixture was renewed at the end of 10 min intervals for 2 hr after drug administration, then in 20 min intervals for the following 100 min, at 30 min intervals for a further 60 min period and at 60 min intervals for a final period of 3 hr. CO_2 in the breath was trapped for 7 hr essentially as described by Lauterburg and Bircher [2]. Air was drawn through the breath collection chamber at a rate of 200–400 ml/min. Samples (2 or 3 ml) of the trapping fluid were made up to 5 ml with methanol to which 15 ml scintillant (2,5 bis(5-tert.-butylbenzoxazol-2yl)thiophen 0.4% w/v in toluene) were added and the radioactivity was counted in either a Beckman LS 230 or a Packard TriCarb 2660 Scintillation Counter. To assess whether the *N*-methyl drugs had a marked effect on the rate of exhalation of endogenous CO_2, mouse breath was trapped with 0.3 mmoles of NaOH. Neutralisation of the NaOH was indicated by the colour change of phenolphthalein. The average rate of CO_2 production within 6 hr after i.p. injection of arachis oil was 0.73 ± 0.12 mmoles/hr (obtained from 11 collections using 2 mice); after i.p. saline 0.89 ± 0.1 mmoles of CO_2 were exhaled per hour (10 collections). After administration of HMM the CO_2 production was only insignificantly reduced compared with the oil treated mice. After aminopyrine the CO_2 production was unchanged compared with the saline treated mice.

Preparation of plasma samples. 2,3,4,5 and 6 hr after the administration of [^{14}C]HMM, mice were anaesthetized with halothane and blood was obtained by cardiac puncture. The blood was centrifuged immediately and the radioactivity in 0.2 ml plasma samples was counted after addition of 10 ml of NE 260 (New England Nuclear) scintillant.

Sleeping time determinations. Duration of pentobarbital anaesthesia (sleeping time) was measured as the interval between the loss and the voluntary recovery of the righting reflex. Pentobarbital sodium was injected i.p. at a dose of 60 mg/kg and mice were placed in dorsal recumbancy during the period

they were anaesthetised. The mean duration of anaesthesia in 5 untreated mice was 201 ± 19 min. Pretreatment with phenobarbitone (50 mg/kg i.p. for four days) decreased the sleeping time by 84%.

Expression of data. In Figures 2 to 6, the percentage of administered radioactivity exhaled per min as $^{14}CO_2$ is plotted semilogarithmically against time after drug administration. The experimental points in these figures are the mean ± S.D. of observations made in at least four mice. To obtain rates, cumulative radioactivity per collection period was divided by the collection period. In Table 1 the peak *N*-demethylation rate was calculated on the basis of the number of all labelled CH_3 moieties within the drug molecule which could be biotransformed to $^{14}CO_2$.

The linear phases of the $^{14}CO_2$ exhalation rate curves were evaluated by linear regression analysis and the slopes thus obtained were used to calculate half lives. In case of aminopyrine, formaldehyde and formate, the method of residuals [10] was employed to resolve the descending part of the curve into two exponential components.

RESULTS

The rate of appearance of $^{14}CO_2$ in the breath of mice after i.p. injection of [^{14}C]aminopyrine peaked rapidly and declined in a biphasic fashion (Fig. 2), a finding compatible with information on the [^{14}C]aminopyrine breath test in rats [6]. Resolution of the post absorptive phase of the $^{14}CO_2$ exhalation rate plot into two exponential components revealed two half lives, an initial $t_{\frac{1}{2}}$ of 15 min and a terminal $t_{\frac{1}{2}}$ of 126 min (Table 1). In rats a comparable dose of [^{14}C]aminopyrine was reported to lead to a $^{14}CO_2$ formation rate plot with $t_{\frac{1}{2}}$ α of 40 min and $t_{\frac{1}{2}}$ β of 116 min [6]; guinea pigs on administration of 75 mg/kg aminopyrine were shown to exhibit a CO_2 curve with a peak CO_2 formation rate lower than those described for rats and determined here for mice and a terminal phase with a $t_{\frac{1}{2}}$ of 105 min [7].

The $^{14}CO_2$ formation rate plots after administration of [^{14}C]-HMM, [^{14}C]-PMM (Fig. 3), [^{14}C] procarbazine (Fig. 4) and [^{14}C]caffeine (Fig. 5) all markedly deviate from the plot obtained after [^{14}C]aminopyrine. They peak after 75 min (HMM), 130 min (PMM), 45 min (procarbazine) and 100 min (caffeine) and exhibit peak $^{14}CO_2$ formation rates which are considerably smaller than the equivalent value for aminopyrine (Table 1). They decline monophasically with half lives of 91 min for HMM, 97 min for PMM, 68 min for procarbazine and 92 min for caffeine (Table 1). Whereas 49 per cent of the *N*-^{14}C-methyl moieties injected with the aminopyrine were exhaled as $^{14}CO_2$ within 7 hr post injection, only 24 per cent of the six labelled *N*-methyl groups in the HMM molecule were expired with the breath. In case of PMM labelled only on the secondary *N*-methyl group merely 3 per cent were expired. The percentages of the dose exhaled after administration of procarbazine and caffeine labelled on one *N*-methyl moiety each are 21 per cent and 12 per cent respectively.

For HMM the $t_{\frac{1}{2}}$ of the $^{14}CO_2$ formation rate plot is appreciably longer than the reported plasma half

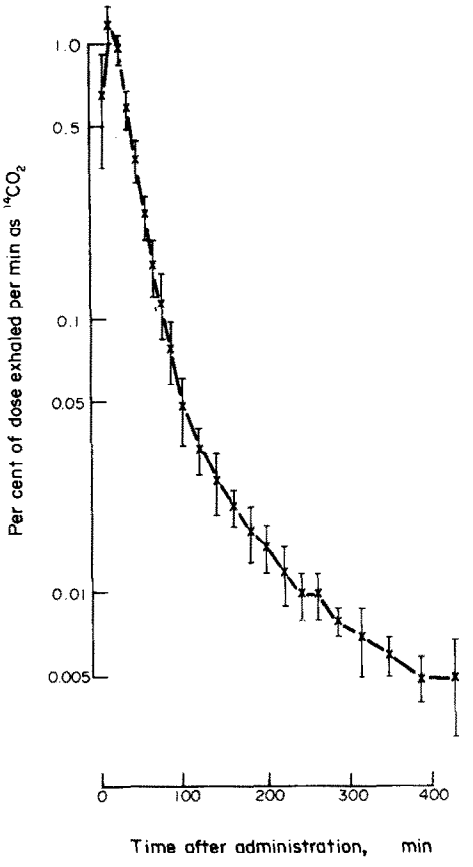

Fig. 2. $^{14}CO_2$ formation rate plot after administration of [^{14}C]aminopyrine. Details of the breath collection and data representation under Methods.

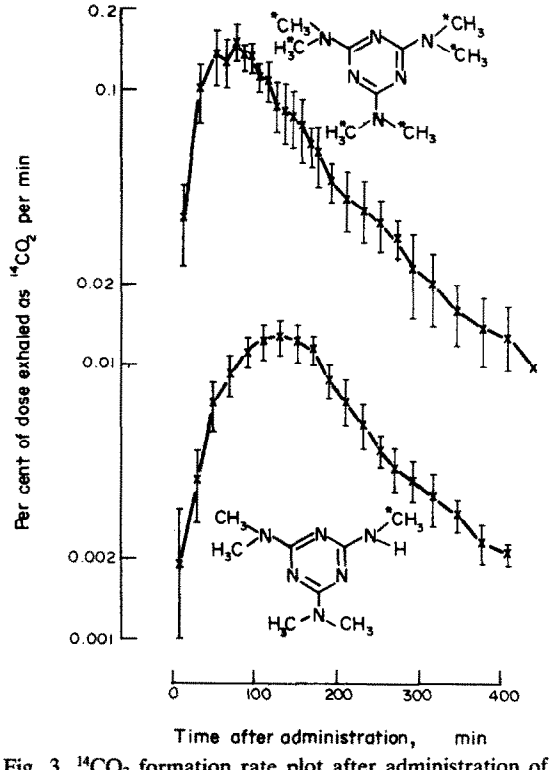

Fig. 3. $^{14}CO_2$ formation rate plot after administration of [^{14}C]-HMM and [^{14}C]-PMM.

Table 1. Breath analyses of $^{14}CO_2$ produced from N-$^{14}CH_3$ labelled drugs

Drug		$t_{\frac{1}{2}}$ (min)	% of dose excreted as $^{14}CO_2$	Peak N-demethylating activity (nmoles $^{14}CO_2$/min)
Aminopyrine 38 mg/kg	(6)	α = 14.8 ± 1.6 β = 125.8 ± 8.2	49.2 ± 3.3	105.4 ± 10.0
Aminopyrine after proadifen	(3)	84.5 ± 13.0	36.5 ± 2.5	24.0 ± 0.5*
Aminopyrine after phenobarbitone	(4)	α = 14.5 ± 1.2 β = 110.5 ± 21.6	49.5 ± 2.5	90.3 ± 4.7
Hexamethylmelamine 40 mg/kg	(5)	90.5 ± 5.6	23.5 ± 3.4	44.3 ± 5.6
Hexamethylmelamine after proadifen	(5)	215.0 ± 72.6	24.3 ± 4.1	24.9 ± 5.1*
Hexamethylmelamine after phenobarbitone	(4)	68.6 ± 9.5*	20.8 ± 1.5	52.3 ± 4.6
Pentamethylmelamine 40 mg/kg	(4)	96.5 ± 3.0	2.6 ± 0.6	0.06 ± 0.01
Procarbazine 100 mg/kg	(4)	68.0 ± 4.0	21.0 ± 2.3	17.5 ± 2.5
Procarbazine, i.v. injection, 100 mg/kg	(3)	63.6 ± 12.3	20.7 ± 3.1	17.9 ± 1.3
Caffeine 40 mg/kg	(4)	91.9 ± 2.1	12.0 ± 2.0	3.4 ± 0.7

Numbers in brackets give numbers of mice used. Asterisks indicate that the values are significantly different from the relevant control animals ($P < 0.05$).

life of 45 min in mice measured by g.l.c. [11]. On the other hand, the plasma concentration of total radioactivity after [^{14}C]-HMM decreased with a $t_{\frac{1}{2}}$ of 152 ± 17 min (n = 3).

The metabolic formation of $^{14}CO_2$ from [^{14}C]aminopyrine on the one side and [^{14}C]HMM on the other was further characterised by comparing the $^{14}CO_2$ profiles obtained from animals under the influence of inhibitors and inducers of mixed function oxygenases, the enzymes which catalyse xenobiotic N-demethylations. Pretreatment of mice with proadifen, an inhibitor of these enzymes, led to a decrease in the peak $^{14}CO_2$ formation rate for both drugs, altered the biphasic nature of the aminopyrine $^{14}CO_2$ plot which declined as one phase with a $t_{\frac{1}{2}}$ of 85 min, and the $t_{\frac{1}{2}}$ of the HMM $^{14}CO_2$ plot was prolonged by 136 per cent to 215 min (Table 1). Surprisingly, pretreatment of the animals with phenobarbitone, an enzyme inducer, at a dose which decreased the pentobarbital induced sleeping time by 84 per cent

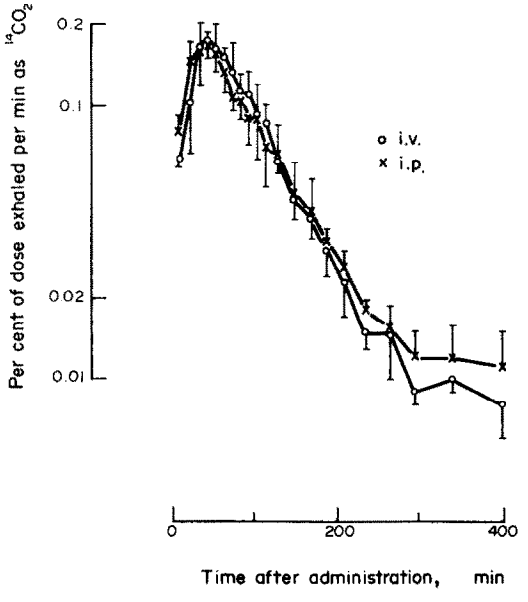

Fig. 4. $^{14}CO_2$ formation rate plot after administration of [^{14}C]procarbazine.

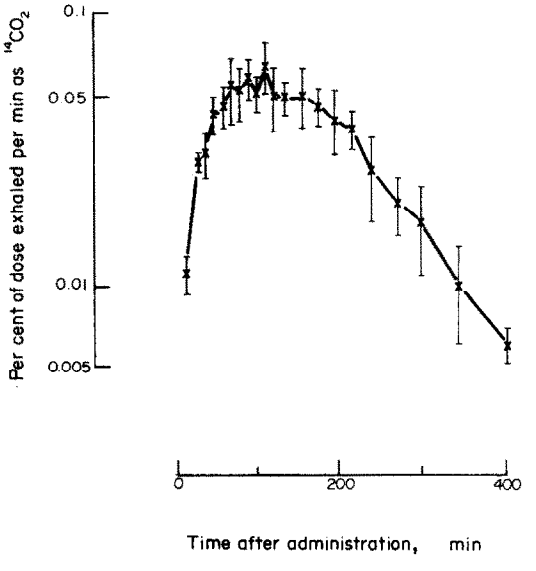

Fig. 5. $^{14}CO_2$ formation rate plot after administration of [^{14}C]caffeine.

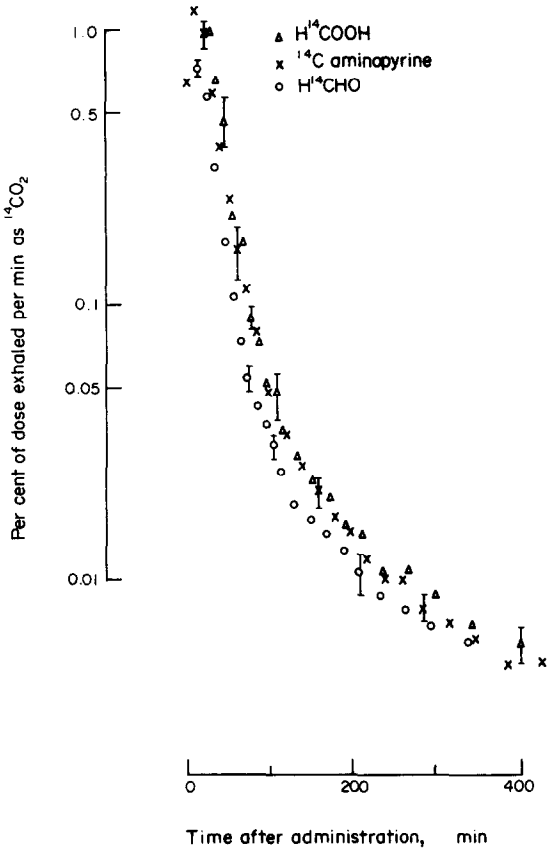

Fig. 6. $^{14}CO_2$ formation rate curves after administration of [^{14}C]aminopyrine, [^{14}C]formaldehyde and [^{14}C]sodium formate. For the sake of clarity of the representation standard error bars are only shown for some exhalation rates.

did not influence the shape of the $^{14}CO_2$ exhalation plot after aminopyrine. However, it caused a 24 per cent decrease in the $t_{\frac{1}{2}}$ of the HMM $^{14}CO_2$ plot to 69 min.

The route of administration for the drugs used in this study was intraperitoneal injection. The $^{14}CO_2$ plot after i.p. administration of [^{14}C]procarbazine 100 mg/kg was compared with that after i.v. injection and they appear to be identical (Fig. 4, Table 1).

When the animals were injected with ^{14}C-labelled formaldehyde and formate the resulting $^{14}CO_2$ exhalation rate plots were very similar to the $^{14}CO_2$ plot obtained after administration of aminopyrine (Fig. 6).

The $^{14}CO_2$ breath analysis after H^{14}CHO was not influenced by pretreatment of the mice with either proadifen or phenobarbitone (Table 2).

DISCUSSION

Many *N*-methyl-containing drugs (e.g. chlorpromazine, diazepam, imipramine, morphine, aminopyrine) are sources of C_1 fragments, oxidised metab-

Table 2. Breath analysis of $^{14}CO_2$ produced from H^{14}CHO and H^{14}COOH (2 mg/kg)

Agent		$t_{\frac{1}{2}}$ (min)	% of dose excreted as $^{14}CO_2$	Peak $^{14}CO_2$ formation rate (nmoles $^{14}CO_2$/min)
H^{14}CHO	(5)	α = 14.2 ± 1.3 β = 125.6 ± 4.6	32.0 ± 1.6	12.1 ± 0.8
H^{14}CHO after proadifen	(3)	α = 15.3 ± 0.6 β = 107.3 ± 8.1	33.5 ± 4.0	11.2 ± 1.7
H^{14}CHO after phenobarbitone	(3)	α = 13.7 ± 1.5 β = 126.0 ± 8.2	32.0 ± 5.0	11.1 ± 3.2
H^{14}COOH	(3)	α = 13.7 ± 0.9 β = 135.7 ± 25.6	55.1 ± 3.3	12.5 ± 0.2

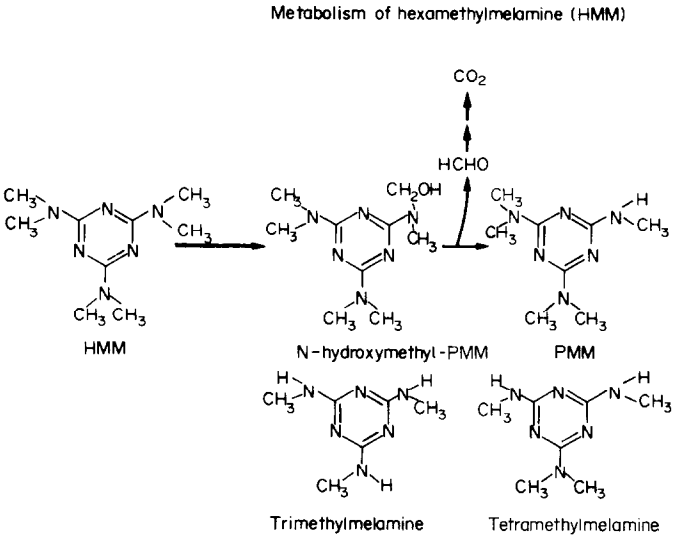

Fig. 7. Chemical structures of hexamethylmelamine metabolites.

olites of the *N*-methyl moieties, which potentially can be deposited in the mammalian organism, although toxic manifestations associated with formaldehyde or formate have not been implicated as side effects of these drugs. On the other hand, the cytotoxicity of *N*-methyl-containing antineoplastic drugs which require bioactivation has been putatively linked with the effect of formaldehyde or its *N*-hydroxymethyl-precursors on tumour growth [5, 12–14]. If indeed *N*-methyl metabolites play a role in the antitumour activity of these drugs one has to assume that there is a basic difference in the way in which different xenobiotic *N*-methyl moieties are metabolized depending on the chemical entity to which they are attached. This difference should explain why some *N*-methyl drugs are cytotoxic and others are not. To shed some light on this difference, we analysed the amount and time course of appearance of the ultimate *N*-methyl metabolite CO_2 in the breath of mice after administration of cytotoxic and non cytotoxic *N*-methyl xenobiotics. The $t_{\frac{1}{2}}$ of the $^{14}CO_2$ plot after [^{14}C]-HMM at 91 min is twice as long as that of the parent drug in plasma and appreciably shorter than that of total plasma radioactivity. The major plasma metabolites of HMM are tetra- and trimethylmelamine (Fig. 7). These metabolites undergo enzymatic *N*-demethylation to a much lesser extent than HMM [12]. Therefore, the half life of the $^{14}CO_2$ exhalation rate may well be influenced mainly by the disappearance of HMM from plasma and to a lesser degree by the disappearance of its metabolites. Consequently, the $t_{\frac{1}{2}}$ of 91 min may be a complex composite reflecting the disappearance of parent drug which undergoes rapid N–C cleavage and the disappearance of its metabolites which are *N*-demethylated more slowly. For aminopyrine the shape of the $^{14}CO_2$ curve has been suggested to reflect the disappearance of parent drug from plasma soon after administration and of predominantly monomethylaminoantipyrine, its major metabolite, during the terminal phase of the $^{14}CO_2$ plot [7]. Burg and Werner [15] reported a dose-dependent plasma half life of caffeine in mice of 102 min (at 25 mg/kg) which is not dissimilar to the value of 92 min derived from the $^{14}CO_2$ formation rate plot after ^{14}C-methyl caffeine reported here.

Even though procarbazine has been used in the treatment of malignancies for more than a decade, information on its distribution and plasma clearance has not been published. This is presumably due to the problems associated with the analytical determination of this highly unstable compound. The *in vivo* *N*-demethylation of [^{14}C]procarbazine in mice and rats has been investigated before [16, 17] and at 10.3 nmoles $^{14}CO_2$/min/mouse the $^{14}CO_2$ exhalation rate determined by Baggiolini *et al.* [16] resembles the rate of 17.5 reported in this study. The kinetic evaluation of the breath $^{14}CO_2$ content however has not been attempted before and the $^{14}CO_2$ plot in Fig. 4 gives some clue as to the persistence in the body of drug derived species capable of undergoing *N*-demethylation. On the assumption that the $^{14}CO_2$ formation is (a) related to the concentration of the drug in the hepatic circulation and (b) dependent on the rate of *N*-methyl hydroxylation, the fact that the $^{14}CO_2$ exhalation curves after i.p. and i.v. administration are identical (Fig. 4) indicates that in the mouse this drug does not undergo extensive *N*-demethylation on its first pass through the liver.

The HMM used in this study was labelled on all six methyl moieties. Labelled PMM was available with the ^{14}C-methyl label only on the secondary amine function. The rate of oxidative N–C cleavage of the dimethyl moieties *in vivo* is likely to be faster than that of the oxidation of the monomethyl groups in HMM metabolites as HMM is metabolized more rapidly *in vitro* than trimethylmelamine [12]. Therefore, it is not surprising that only 3 per cent of the label in PMM is transformed to $^{14}CO_2$ compared to 20 per cent of an equivalent dose of HMM with six labelled methyl groups. The shapes of both $^{14}CO_2$ exhalation rate curves are however very similar with half lives of 91 and 97 min (Fig. 3).

The output of CO_2 is the last step in a series of processes starting with the absorption of the parent drug and ending with the expiration of CO_2. If all of these processes were first order reactions that occurred in series, the disappearance rate of $^{14}CO_2$ would be determined by the rate limiting step in this sequence. It is difficult, however, to state *a priori* which of the various transfer rates or disposition rates of the drugs and their metabolites are reflected by the breath analysis. The similar shapes of the $^{14}CO_2$ exhalation rate plots after differently labelled HMM and PMM indicate that the steps which determine the rate of appearance of $^{14}CO_2$ with the breath are the same for both drugs irrespective of the actual *N*-demethylation rates at the hepatic enzyme sites. This is interesting in view of the fact that the plasma half life of PMM in mice is only 8 min as compared to 45 min for HMM [11]. The rate determining step in the disposition of HMM and PMM derived C_1 is unlikely to be the oxidative metabolism of formaldehyde or formate as the shape of their $^{14}CO_2$ exhalation rate plots is very different from the plots for HMM and PMM. Most of the volatile radioactivity after $H^{14}CHO$ and $H^{14}COOH$ was eliminated via the breath at a very fast rate and a small portion was expired with a $t_{\frac{1}{4}}$ of 126 min (HCHO) and 136 min (HCOOH) (Table 2). However, the striking similarity of the $^{14}CO_2$ formation rate plots after $H^{14}CHO$ and $H^{14}COOH$ with that after [^{14}C]aminopyrine (Fig. 6) suggests that in the mouse, the rate of metabolism of the aminopyrine *N*-methyl to CO_2 is determined by the rate of oxidation of the metabolic intermediates HCHO or HCOOH. Proadifen and phenobarbitone pretreatment did not alter the rate at which $H^{14}CHO$ was transformed to $^{14}CO_2$ (Table 2). It is pertinent to consider that some aldehyde dehydrogenases in rodents are inducible by phenobarbitone [18], yet in this study, there was no change in the rate of the metabolism of HCHO to CO_2 in mice after phenobarbitone. Thus it is feasible to hypothesize that it is the oxidation of formate which determines the rate of transformation of HCHO and also of the *N*-methyl moiety in aminopyrine to CO_2. This interpretation offers an explanation of the peculiar insensitivity of the [^{14}C]aminopyrine breath analysis in the mouse to pretreatment with phenobarbitone (Table 1): the *in vitro* *N*-demethylation of aminopyrine by mouse liver preparations occurs at a much faster rate than with most other small experimental animals [19]. It is conceivable that in the mouse this cytochrome P450 dependent hydroxylation reaction occurs at a rate similar to or faster than the rates of the two oxidations which formaldehyde undergoes subsequently. So any further increase in the rate of hepatic *N*-demethylation of aminopyrine is not capable of producing an increase in the rate at which drug derived CO_2 appears with the breath, because such an increase affects the metabolic step which appears not to be the one which determines the rate of the overall oxidation of aminopyrine to CO_2. In accordance with this view, agents which decrease the rate of aminopyrine *N*-demethylation, like proadifen, could conceivably inhibit it to the extent of rendering it rate determining for the appearance of CO_2. Indeed, the pretreatment of mice with proadifen caused a decreased $^{14}CO_2$ exhalation rate (Table 1).

Both phenobarbitone and proadifen influenced the $^{14}CO_2$ plot after administration of [^{14}C]-HMM (Table 1). Induction and inhibition of the metabolism of HMM has been shown only *in vitro* previously [20]. These results indicate that the disposition of HMM and its metabolites is also affected by agents influencing drug metabolising enzymes *in vivo*. As there is good evidence that HMM requires metabolic activation [13] this finding may have therapeutic implications. HMM has been suggested to exert its antineoplastic activity via stable carbinolamine metabolites [12] and *N*-hydroxymethylpentamethylmelamine (Fig. 7), unlike HMM, is directly cytotoxic [21]. It has been identified as a major *in vitro* metabolite of HMM [22]. A *N*-hydroxymethyl metabolite of procarbazine has also been reported [23]. Little is known of the biochemical reactions which stable *N*-hydroxymethyl metabolites of *N*-methyl drugs can undergo distinguishing them from *N*-hydroxymethyl metabolites which decompose spontaneously to formaldehyde. Considering the multitude of reactions potentially operating in the disposition of the C_1 moiety derived from *N*-methyl drugs it is more than speculative to draw any general conclusions from the result presented here that CO_2, an ultimate metabolite of the *N*-methyl groups of HMM, PMM and procarbazine, appears with the breath in a different fashion than the CO_2 derived from aminopyrine. Probably the quantitative and qualitative evaluation of the fate of drug derived C_1 units *in vivo* which are not exhaled as CO_2 together with the data presented here may shed more light on the difference in the biodisposition and biochemistry of *N*-methyl moieties of xenobiotic origin.

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