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THE REVERSIBILITY OF *N*-OXIDATION *IN VIVO*

THE FATE OF ^{14}C-*N*-HYDROXYCHLORPHENTERMINE AND ^{14}C-NITROCHLORPHENTERMINE IN THE RAT*

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Abstract—The interrelationships between primary amines and their *in vivo* *N*-oxidized metabolites are unclear. We have therefore synthesized ^{14}C-*N*-hydroxychlorphentermine and ^{14}C-nitrochlorphentermine and examined their metabolism and excretion in the rat. ^{14}C-*N*-hydroxychlorphentermine was excreted slowly in the urine (66 per cent of dose in 6 days) with a further 8 per cent in the faeces (3 per cent) and as $^{14}CO_2$ (5 per cent), and the only urinary metabolites were the unchanged hydroxylamine and its glucuronic acid conjugate. ^{14}C-Nitrochlorphentermine was eliminated more rapidly (92 per cent of dose in 4 days), with 41 per cent in the urine, 1 per cent in the faeces and 50 per cent as $^{14}CO_2$. The only urinary metabolites were the reduction product *N*-hydroxychlorphentermine and its glucuronide but the large amount of $^{14}CO_2$ found indicated that side chain oxidation was a major metabolic route. The results are discussed with reference to the possible reversibility of *N*-oxidation *in vivo* and putative mechanisms for the oxidation of the side chain.

A number of the metabolic transformations undergone by foreign compounds *in vivo* are potentially reversible reactions. Among Phase I reactions, the oxidation of propan-2-ol to acetone is reversible [1], while the body possesses a range of hydrolytic enzymes able to cleave conjugates of several types, including sulphates, glucuronides and *N*-acetyl conjugates. The array of metabolites of some compounds found in the tissues and body fluids will thus reflect the balance of forward and reverse reactions where these latter can occur.

In recent years, there has been much interest in the oxidative metabolism of nitrogen centres in foreign compounds. The interrelationships of such *N*-oxidized metabolites can be difficult to define, since these reactions are frequently reversible. Fuller *et al.* [2] have shown that *N*-hydroxy-4-chloroamphetamine is extensively reduced to 4-chloroamphetamine in the rat, and Cho *et al.* [3] similarly report the reduction of both *N*-hydroxy-amphetamine and -phentermine in the rat to the respective parent amines. More recently, Sum and Cho [4] have characterized a microsomal reductase from rat liver which reduces *N*-hydroxyphentermine to phentermine. Beckett and Bélanger [5] have proposed a complex scheme for the metabolism of phentermine in rabbit liver which involves both the oxidation of the nitrogen atom and subsequent reduction of the products.

The *N*-oxidation of a primary amine may give rise variously to nitro and nitroso compounds, hydroxylamines, nitrones and nitroxides, but the extent to which these possible products are interconvertible *in vivo* has not been studied. This paper presents results of an investigation of the possible interconversion *in vivo* of two of the *N*-oxidized metabolites of chlorphentermine, namely *N*-hydroxychlorphentermine and its nitro analogue, nitrochlorphentermine [1-(4′-chlorophenyl)-2-methyl-2-nitropropane], in the rat. These studies were performed using ^{14}C-labelled material in order to examine the total fate of the compounds, and the rat was chosen since it does not appear to metabolize chlorphentermine by *N*-oxidation [6] and thus any reduction which might occur would not be obscured by subsequent *N*-oxidation.

MATERIALS AND METHODS

Compounds. Chlorphentermine hydrochloride, mp 225°, was a gift from H. Lundbeck & Co., Copenhagen, Denmark. ^{14}C-Chlorphentermine hydrochloride, labelled in the α-methyl groups and of specific activity 0.07 μCi/mg, mp 225°, was the gift of Warner–Lambert Research Institute, Morris Plains, NJ, U.S.A., research affiliate of Warner–Chilcott Laboratories. *N*-hydroxychlorphentermine hydrochloride, mp 145°, and 1-(4′-chlorophenyl)-2-methyl-2-nitropropane (nitrochlorphentermine), an oil, were samples synthesized previously in this laboratory [6]. Purified beef liver β-glucuronidase (Ketodase) was purchased from General Diagnostics, Eastleigh, Hants., U.K.

1-(4′-*Chlorophenyl*)-2,2-*di*[^{14}C]-*methyl*-2-*nitroethane* (^{14}*C-nitrochlorphentermine*). ^{14}C-Chlorphentermine hydrochloride (1 g; 70 μCi) was dissolved

* Abstracts of preliminary reports of some of these data appear in *Proc. Eur. Soc. Toxic.* **18**, 241 (1976) and *Biochem. Soc. Trans.* **5**, 1006 (1977).

in water (10 ml), adjusted to pH 14 with 10 M NaOH and the solution extracted with ether (3 × 5 ml). After drying over anhyd. Na_2SO_4, the ether was evaporated and the ^{14}C-chlorphentermine base so obtained dissolved in acetone (40 ml) and water (10 ml). $MgSO_4$ (1.4 g) was added with stirring, and $KMnO_4$ (8.6 g) added in portions over 1 hr. The mixture was left at 40–50° with stirring for 16 hr, after which it was filtered and the excess $KMnO_4$ destroyed by the careful addition of 100 vol. H_2O_2. After a second filtration, the acetone was removed on the rotary evaporator and the solution extracted with chloroform (80 ml). The chloroform was washed with 1 M HCl (2 × 50 ml), dried over anhyd. Na_2SO_4 and removed on the rotary evaporator, leaving ^{14}C-nitrochlorphentermine as a pale green oil, which crystallized at 4°. The yield was 655 mg (68 per cent), and specific activity 0.072 μCi/mg. Radio t.l.c. showed the compound to be at least 96 per cent pure. The chemical identity of the product was confirmed by comparison of its g.l.c., t.l.c. and g.c.–m.s. properties with those of an authentic sample.

1-(4′-*Chlorophenyl*)-2,2-*di*[^{14}C]*methyl*-2-*hydroxyaminoethane* (*^{14}C-N-hydroxychlorphentermine*). ^{14}C-Nitrochlorphentermine (300 mg; synthesized as above) was dissolved in tetrahydrofuran (10 ml) and added dropwise over 30 min to aluminium amalgam (prepared from 1 g aluminium foil, see Caldwell *et al.* [6]) in tetrahydrofuran (25 ml), kept in an ice bath. After the 30 min had elapsed, the aluminium foil was filtered, the filtrate dried over anhyd. Na_2SO_4 and the tetrahydrofuran removed by evaporation. The resulting oil was treated with a solution of HCl gas in ether (2.5 ml), whereupon white crystals of ^{14}C-*N*-hydroxychlorphentermine hydrochloride separated, which were filtered and recrystallized from acetone, mp 145°. The yield was 180 mg (54 per cent), and specific activity 0.065 μCi/mg. Radio t.l.c. showed the product to have a radiochemical purity > 98 per cent, and comparison of m.p., g.l.c., t.l.c. and g.c.–m.s. properties with those of an authentic sample provided confirmation of the chemical identity of the compound.

Animals and drug treatment. Female Wistar albino rats (body weight 250–300 g) were used. ^{14}C-Nitrochlorphentermine, dissolved in 90 per cent propane-1,2-diol, was injected intraperitoneally (100 mg/kg; 0.72 μCi/kg). ^{14}C-*N*-Hydroxychlorphentermine hydrochloride, in aqueous solution, was given by i.p. injection (100 mg/kg; 0.65 μCi/kg). The animals were housed in Metabowls (Jencons Ltd.) to permit the separate collection of urine, faeces and $^{14}CO_2$ in the expired air. The air from the cages was drawn through a $CaCl_2$ drying tube and then through two scrubbing bottles containing ethanolamine-2-methoxyethanol (1 : 2, v/v). In some experiments, rats were bile-duct cannulated after anaesthesia with pentobarbitone (60 mg/kg i.p.), and allowed to recover prior to i.p. injection of ^{14}C-nitrochlorphentermine (15 mg/kg).

Radiochemical techniques. ^{14}C in urine, bile, faeces, other solutions and on thin-layer chromatograms was determined by scintillation counting and radiochromatogram scanning (Packard Tri-Carb liquid scintillation spectrometer model 3385 and Chromatogram scanner model 7201) as described by Caldwell *et al.* [6]. Reverse isotope dilution analysis for chlorphentermine was performed as described by Caldwell *et al.* [6].

Table 1. Chromatographic properties of chlorphentermine and related compounds*

	R_f value in solvent 1	2
Chlorphentermine	0.50	0.00
N-Hydroxychlorphentermine	0.69	0.07
Nitrosochlorphentermine†	0.88	0.67
Nitrochlorphentermine	0.77	0.70

* Thin layer chromatography was performed using Silica gel $60F_{254}$ aluminium sheets, layer thickness 0.2 mm (E. Merck, Darmstadt, West Germany). Solvent systems were 1: methanol–chloroform (1 : 1, v/v), 2: *n*-hexanediethyl ether–dichloromethane (4 : 3 : 1, v/v), run to 12–14 cm from the origin. All compounds were visualized as dark quenching spots under u.v. light (254 nm) and *N*-hydroxychlorphentermine gave a red colour with the tetrazolium spray of Caldwell *et al.* [6] and a black colour when plates were sprayed with a saturated solution of $AgNO_3$ in 0.88 SG ammonia. Chromatograms of urine and bile were also sprayed for glucuronides with naptharesorcinol [17].

† Data provided by Dr. B. G. A. Lindeke, Biomedical Center, Uppsala, Sweden.

Chromatography. The chromatographic properties of chlorphentermine and related compounds are shown in Table 1. Portions of urine or bile, before and after treatment with β-glucuronidase, sulphatase or acid hydrolysis as described by Caldwell *et al.* [6], containing 10^3–10^5 d.p.m., were chromatographed on Silica gel $60F_{254}$ aluminium backed sheets, layer thickness 0.2 mm (E. Merck, Darmstadt, West Germany).

Concentration of urinary metabolite for chromatography. Metabolites present in urine were concentrated by the use of columns of Amberlite XAD-2 resin as described by Caldwell *et al.* [6], the concentrated methanol eluates being used for chromatography.

Gas chromatography (*g.l.c.*). A Packard–Becker Model 417 gas chromatograph with flame ionization detection was used. The column was of glass, 1.2 m long × 3 mm i.d. packed with 3 per cent OV-1 on Chromosorb G AW DMCS (80–100 mesh). The conditions used were injection port temperature 200°, oven temperature 180°, detector temperature 205°, N_2 air and H_2 pressures, 30, 28 and 30 lb/in^2 respectively, flow rate of N_2 30 ml/min. The retention times (*min*) in this system were: chlorphentermine 2.0, *N*-hydroxychlorphentermine trimethylsilyl ether 8.0 and nitrochlorphentermine 4.8.

Gas chromatography–mass spectrometry (*g.c.–m.s.*). A Varian CH5 mass spectrometer (m.s.) was used, coupled to a Varian Aerograph 1700 g.l.c. with a two-stage Biemann–Watson separator. The g.l.c. had a glass column, 2 m long × 2 mm i.d., packed with 1.5 per cent OV-1 on Chromosorb G AW-DMCS (80–100 mesh). The g.l.c. oven temperature was 190°, the helium flow rate 30 ml/min and the electron energy 70 eV, all other conditions being as

described by Caldwell *et al.* [7]. In this system, the retention times (min) were: chlorphentermine, 1.9, nitrochlorphentermine, 5.2 and *N*-hydroxychlorphentermine trimethylsilyl ether, 8.0.

Procedures for oxidation and reduction of urinary metabolites. Urine (5 ml) was treated with β-glucuronidase as described by Caldwell *et al.* [6] and metabolites concentrated using XAD-2 columns. The methanol eluate was evaporated in a stream of N_2, the residue taken up in water (5 ml) and this treated as follows:

Reduction. Zn dust (100 mg) and 2 M HCl (3 ml) were added and the suspension stirred at room temp. for 1 hr, after which it was adjusted to pH 14 (10 M NaOH), extracted with ether (15 ml), the ether evaporated and the residue taken up in methanol (100 μl) for examination by t.l.c. and g.l.c.

Oxidation. To the above solution was added acetone (10 ml) and $MgSO_4$ (20 mg) and five portions of $KMnO_4$ (20 mg) were added over 1 hr with stirring. After stirring at room temperature overnight, the mixture was filtered, 100 vol. H_2O_2 added dropwise to destroy excess $KMnO_4$, the mixture filtered and the acetone removed by rotary evaporation. The aqueous residue was extracted with $CHCl_3$ (10 ml), this dried (anhyd. Na_2SO_4), evaporated and the residue taken up in methanol (100 μl) for examination by t.l.c. and g.l.c.

Identification of urinary and biliary metabolites

N-*Hydroxychlorphentermine.* Thin layer chromatograms of partially purified (XAD-2 resin) urine developed in solvent 1 showed on radiochromatogram scanning two ^{14}C peaks with R_f values 0.69 (minor) and 0.25 (major). The peak at R_f 0.69 corresponded with *N*-hydroxychlorphentermine, and gave a red colour with the tetrazolium spray and a black colour with ammoniacal silver nitrate. On treatment of the urine with β-glucuronidase, but not sulphatase, the peak at R_f 0.25, which gave a blue colour with naphtharesorcinol, disappeared with a concomitant increase in that at R_f 0.69, and the peak at R_f 0.25 was thus assigned to *N*-hydroxychlorphentermine glucuronide. Similar chromatograms developed in solvent 2 showed ^{14}C peaks at R_f 0.00 (major) and 0.07 (minor), the former, which gave a positive reaction with naphtharesorcinol, disappearing on β-glucuronidase treatment, and the latter having the colour reactions of *N*-hydroxychlorphentermine.

G.l.c. examination of extracts of urine prepared and derivatized as described revealed a peak with retention time 8.0 min, identical with that of authentic *N*-hydroxychlorphentermine trimethylsilyl ether and not present in blank extracts. G.c.–m.s. of these extracts showed that the mass spectrum of the peak corresponding in retention time to *N*-hydroxychlorphentermine trimethylsilyl ether was identical with that of the authentic compound (Table 2).

Treatment of urine with oxidizing or reducing agents as described converted the *N*-hydroxychlorphentermine present to nitrochlorphentermine and chlorphentermine, respectively, as shown by comparison of the t.l.c. and g.l.c. properties of the products with those of authentic samples. Reverse isotope dilution analysis for chlorphentermine was negative.

Table 2. G.c.–mass spectra of *N*-hydroxychlorphentermine trimethylsilyl ether extracted from body fluids*

Ion (m/e)	Relative intensity (%) Standard	Sample I†	II‡	III§
271	1	0.5	2	1
256	8	5	6	5
146	100	100	100	100
130	79	86	81	91
125	50	44	48	50
116	28	24	20	29

* Mass spectra were recorded by subjecting an authentic sample of *N*-hydroxychlorphentermine and extracts of urine (prepared as described in the text) to g.c.–m.s. analysis as described in the text. In all four cases recorded above, the retention time of the g.c. peak was 8.0 min.

† I Extracted from urine after administration of *N*-hydroxychlorphentermine.

‡ II Extracted from urine after administration of nitrochlorphentermine.

§ III Extracted from bile after administration of nitrochlorphentermine.

Nitrochlorphentermine. Chromatographic investigation of urine from rats given nitrochlorphentermine showed the same picture described above for *N*-hydroxychlorphentermine, with two compounds present, *N*-hydroxychlorphentermine and its glucuronide, demonstrated using the methods described above. Reverse isotope dilution analysis for chlorphentermine was negative. Similar analysis of bile showed the presence of one naphtharesorcinol-positive peak, with R_f 0.25 in solvent 1 and 0.00 in solvent 2, which disappeared upon treatment with β-glucuronidase, being replaced by a new peak of R_f 0.69 and 0.07 in solvents 1 and 2, respectively, which corresponded in R_f and colour reactions with *N*-hydroxychlorphentermine. The identity of the compound resulting from β-glucuronidase treatment of bile was confirmed by g.l.c. and g.c.–m.s. of its trimethylsilyl derivative, the m.s. of which is shown in Table 2.

RESULTS

Elimination of ^{14}C. The pattern of elimination of ^{14}C by rats dosed with ^{14}C-*N*-hydroxychlorphentermine was very different from that after administration of ^{14}C-nitrochlorphentermine (see Tables 3 & 4). With *N*-hydroxychlorphentermine, the urine was by far the most important route of elimination, with 66 per cent of dose being excreted in 6 days, and small amounts of ^{14}C were found in the faeces (3 per cent) and in the expired air as $^{14}CO_2$ (5 per cent). In the case of nitrochlorphentermine, however, the major route of excretion was as $^{14}CO_2$ in the expired air (50 per cent of dose in 2 days) with a further 41 per cent in the urine and 1 per cent in the faeces over 4 days.

Metabolism of N-*hydroxychlorphentermine.* The metabolism of ^{14}C-*N*-hydroxychlorphentermine is shown in Table 3. Two ^{14}C-labelled compounds could be detected in the urine, free *N*-hydroxychlorphen-

Table 3. Fate of ^{14}C-*N*-hydroxychlorphentermine in the rat*

Compound	% Dose excreted as:
N-Hydroxychlorphentermine free	24 (23–27)
glucuronide	42 (37–46)
total	66 (60–73)
Nitrosochlorphentermine	n.d.
Nitrochlorphentermine	n.d.
Chlorphentermine	0 (0–0)
Total metabolites	66 (60–73)
% ^{14}C dose excreted in 0–6 day urine	66 (60–73)
faeces	3 (3–3)
% ^{14}C dose excreted as $^{14}CO_2$ in 3 days	5 (4–6)
Total ^{14}C excreted in 6 days	74 (67–82)

* Values given are means of six animals with ranges in parentheses. ^{14}C-*N*-Hydroxychlorphentermine was administered as an aqueous solution of the hydrochloride salt by intraperitoneal injection (100 mg/kg; 0.65 μCi/kg). Urine, faeces and expired $^{14}CO_2$ were collected as described in the text. Analysis of urinary metabolites was as described in the text. n.d. = not detected.

termine and its glucuronide. Chromatographic studies failed to reveal the presence of either nitroso- or nitro-chlorphentermine, while reverse isotope dilution analysis showed the absence of chlorphentermine. A small amount of side chain degradation, leading to the elimination of $^{14}CO_2$ in the expired air, also occurred.

Metabolism of nitrochlorphentermine. Table 4 shows the metabolism of ^{14}C-nitrochlorphentermine in the rat. The principal route of metabolism involves the oxidation of the side chain methyl groups to $^{14}CO_2$ excreted in the expired air. Two urinary metabolites were detected, *N*-hydroxychlorphentermine and its glucuronide, but neither nitroso- or nitro-chlorphentermine were present as adduced by t.l.c. analysis. No chlorphentermine could be detected by reverse isotope dilution.

Fate of nitrochlorphentermine in bile-duct cannulated rats. Urinary and biliary metabolites of ^{14}C-nitrochlorphentermine are shown in Table 5. About twice as much ^{14}C was recovered in the bile (22 per cent of dose) as in the urine (10 per cent), and the urine contained the same two metabolites as in intact animals, namely *N*-hydroxychlorphentermine and its glucuronide. As in the other studies, neither chlorphentermine nor its nitro and nitroso analogues were present in urine or bile. $^{14}CO_2$ in the expired air was not collected in this experiment.

Table 4. Fate of ^{14}C-nitrochlorphentermine in the rat*

Compound	% Dose excreted as:
N-Hydroxychlorphentermine free	11 (10–13)
glucuronide	30 (27–34)
total	41 (39–43)
Nitrosochlorphentermine	n.d.
Nitrochlorphentermine	n.d.
Chlorphentermine	0 (0–0)
Total metabolites	41 (39–43)
% ^{14}C dose excreted in 0–4 day urine	41 (30–43)
% ^{14}C dose excreted in 0–4 day faeces	1 (1–1)
% ^{14}C dose excreted as $^{14}CO_2$ in 2 days	50
Total ^{14}C excreted in 4 days	91 (83–99)

*Values given are means of results from six animals with ranges in parentheses. ^{14}C-Nitrochlorphentermine was administered in 90% propane-1,2-diol by intraperitoneal injection (100 mg/kg; 0.72 μCi/kg). Urine, faeces and expired $^{14}CO_2$ were collected as described in the text. Analysis of urinary metabolites was as described in the text. n.d. = not detected.

Table 5. Fate of ^{14}C-nitrochlorphentermine in bile duct-cannulated rats*

Compound		% Dose excreted in that form in: Urine	Bile
N-Hydroxychlorphentermine	free	4 (3.2–6.0)	n.d.
	glucuronide	6 (4.3–7.7)	22 (17–28)
	total	10 (8.5–13.2)	22 (17–28)
Nitrosochlorphentermine		n.d.	n.d.
Nitrochlorphentermine		n.d.	n.d.
Chlorphentermine		0 (0–0)	0 (0–0)
Total metabolites		10 (8.5–13.2)	22 (17–28)
% ^{14}C dose in 0–24 hr excreta		10 (8.5–13.2)	22 (17–28)

* Values given are the mean results from six animals with ranges in parentheses. ^{14}C-Nitrochlorphentermine was given to bile duct-cannulated rats as described in the text. Collection of urine and bile and analysis of metabolites was as described in the text. n.d. = not detected.

DISCUSSION

This study has shown that after the administration of both *N*-hydroxychlorphentermine and nitrochlorphentermine to rats, the urine contains free *N*-hydroxychlorphentermine and its glucuronide, demonstrated by comparison of t.l.c., g.l.c. and g.c.–m.s. properties with authentic material, and by oxidation and reduction studies. The presence of the glucuronide was shown by specific enzyme hydrolysis of the major metabolite to *N*-hydroxychlorphentermine. As reported earlier in our study of chlorphentermine metabolism [6], β-glucuronidase liberated *N*-hydroxychlorphentermine from its glucuronide quantitatively, and the results with this treatment were the same as for acid hydrolysis. This is at variance with the findings of Beckett and Bélanger [8] who have noted that more free *N*-hydroxychlorphentermine was produced when urine was subjected to acid hydrolysis than when incubated with β-glucuronidase.

The principal route of metabolism of *N*-hydroxychlorphentermine was by conjugation of the free *N*-hydroxy group with glucuronic acid. No evidence was found for its oxidation to either nitroso- or nitrochlorphentermine or for its reduction to chlorphentermine. If chlorphentermine had been produced, it would have been expected to appear in the urine, since when given to rats [6] it was excreted unchanged to a major extent. In addition, there was a small amount of $^{14}CO_2$ excreted in the expired air, which presumably arose from oxidation of the two α-methyl groups in the chlorphentermine side chain, and a very small amount of ^{14}C was elimated in the faeces, the nature of which was not investigated.

By way of contrast, nitrochlorphentermine was metabolized extensively along two pathways, reduction of the nitro group and side chain oxidation. The only urinary metabolites found were *N*-hydroxychlorphentermine and its glucuronide, so that the nitro group is only partially reduced *in vivo*. Again neither chlorphentermine nor its other possible *N*-oxidation products were detected in the urine. The most important route of elimination of ^{14}C was as $^{14}CO_2$ in the expired air, arising from oxidation of the side chain α-methyl groups. Studies in bile duct cannulated rats showed that *N*-hydroxychlorphentermine glucuronide was excreted in the bile to a considerable extent (*ca.* 22 per cent of dose) which would be expected from its molecular weight (375.5) and amphipathic character, both of which would favour its biliary excretion in the rat [9]. Since intact animals excreted only 1 per cent of the administered dose in the faeces, it appears that *N*-hydroxychlorphentermine glucuronide may undergo enterohepatic circulation in the rat, probably involving hydrolysis of the β-glycosidic linkage by the gastrointestinal flora [10]. From data presented in Table 4, it is suggested that the balance of ^{14}C is accounted for by the elimination of $^{14}CO_2$, which was not collected from bile-duct cannulated animals.

It is clear from these investigations that neither of the *N*-oxidation products of chlorphentermine are reduced to chlorphentermine *in vivo*, and that this *N*-oxidation process is not fully reversible. Nitrochlorphentermine does undergo a partial reduction to *N*-hydroxychlorphentermine, but is not excreted unchanged even though small amounts of this compound are found in the urine after administration of chlorphentermine to rabbits and human volunteers [6]. Sum and Cho [4] have suggested that the inability of the rat to excrete *N*-hydroxychlorphentermine after chlorphentermine administration is due not to the absence of the appropriate *N*-oxidizing enzymes in that species but to the high hydroxylamine reductase activity which it possesses. The present study presents strong evidence that this is not the case *in vivo*, and that the observed species differences in chlorphentermine *N*-oxidation [6, 11] do not reflect real species differences in the activities of the *N*-oxidizing enzymes.

The oxidation of the side chain methyl groups of nitrochlorphentermine, which is also a minor metabolic pathway of *N*-hydroxychlorphentermine, is a reaction of considerable interest. Possible intermediates have not been identified, but it would seem likely that the nitrogen atom is lost prior to the oxidation. Three potential reaction sequences can be suggested. It is known that the denitration of nitroparaffins involves glutathione [12, 13] but in this case transformation products of *S*-alkyl glutathione are excreted in the urine [12] and this would therefore be expected to occur in the present case rather than subsequent oxidation of the arylalkyl moeity to give $^{14}CO_2$.

Two reports have recently appeared suggesting that some aliphatic nitrocompounds can undergo oxidative denitration by the hepatic microsomal

enzymes, yielding a carboxyl compound and nitrate: this has been observed with 1- and 2-nitropropane [14] and 2-nitro-1-phenylpropane (the nitro analogue of amphetamine [15]). Since nitrochlorphentermine lacks a proton on the carbon α- to the nitro group, this route of metabolism would not yield a carbonyl compound, but could perhaps result in side chain oxidation yielding $^{14}CO_2$.

It is to be assumed that in the reduction of nitrochlorphentermine to corresponding hydroxylamine, the analogous nitroso compound is an obligatory intermediate, although this has not been shown in the present *in vivo* study. Indeed, in a study of the reduction of nitrophentermine by rabbit liver 10,000 *g* supernatant, nitrosophentermine was detected by g.l.c. analysis in addition to *N*-hydroxyphentermine and phentermine itself [5]. It has been established that such nitroso compounds are liable to autoxidation, particular in the presence of Cu^{2+} ions, and lose the nitroso group to give a dimethylstyrene [16] and if a similar oxidation were to occur in the body, the 4′-chlorodimethylstyrene so formed could be further oxidized to 4-chlorobenzaldehyde and acetone. It is known that acetone is extensively metabolized to CO_2 [1] and since the α-methyl groups of nitrochlorphentermine would be in the acetone so produced, this mechanism provides an origin for the $^{14}CO_2$ liberated from this compound.

It is worthy of note that when nitrosophentermine was administered to rabbits, only 10 per cent of the dose was recovered as the sum of all *N*-oxidized metabolites and the parent amine [8], even though when *N*-hydroxyphentermine was given some 55 per cent was excreted, principally as the glucuronide of *N*-hydroxyphentermine. The discrepancy between the results with these compounds was attributed to the greater lipid solubility of the nitroso compound causing its retention in the tissues. However, in view of our findings with the *N*-oxidized metabolites of chlorphentermine, it is conceivable that nitrosophentermine undergoes side chain oxidation, thus accounting for the absence of metabolites retaining the nitrogen atom.

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