REFERENCES

1. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
2. R. McCauley and E. Racker, *Molec. cell. Biochem.* **1**, 73 (1973).
3. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187**, 365 (1973).
4. J. A. Roth and C. N. Gillis, *J. Pharmac. exp. Ther.* **194**, 537 (1975).
5. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
6. J. A. Roth, *Gen. Pharmac.* **7**, 381 (1976).
7. M. D. Houslay and K. F. Tipton, *Biochem. J.* **139**, 645 (1974).
8. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **189**, 733 (1974).
9. C. Braestrup, H. Anderson and A. Randrup, *Eur. J. Pharmac.* **34**, 181 (1975).
10. P. C. Waldmeier, A. D. Stula and L. Maitre, *Naunyn–Schmiedebergs Arch. Pharmac.* **292**, 9 (1976).
11. V. Glover, M. Sandler, F. Owen and G. J. Riley, *Nature, Lond.* **265**, 80 (1977).
12. K. F. Tipton, M. D. Houslay and N. J. Garrett, *Nature, New Biol.* **246**, 213 (1973).
13. H. Y. Meltzer and S. M. Stahl, *Schizophrenia Bull.* **2**, 19 (1976).
14. E. D. Bird and L. L. Iverson, in *Essays in Neurochemistry and Neuropharmacology* (Eds. M. B. H. Youdim, W. Lovenberg, D. F. Sharman and J. R. Langnado), Vol. 1, pp. 177–95. Wiley-Interscience, New York (1977).
15. J. A. Roth, *J. Neurochem.* **27**, 1107 (1976).
16. J. A. Roth, X. O. Breakefield and C. M. Castiglione, *Life Sci.* **19**, 1705 (1976).
17. H. L. White and J. C. Wu, *J. Neurochem.* **25**, 21 (1975).
18. D. J. Edwards and S. S. Chang, *Biochem. biophys. Res. Commun.* **65**, 1018 (1975).
19. H. L. White and A. T. Glassman, *J. Neurochem.* **29**, 987 (1977).
20. D. S. Robinson, T. L. Sourkes, A. Nies, L. Harris, S. Spector, D. L. Bartlett and I. S. Kaye, *Archs gen. Psychiat.* **34**, 89 (1977).

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Metabolism *in vivo* of carbon disulfide to carbonyl sulfide and carbon dioxide in the rat

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Carbon disulfide (CS_2) is metabolized to carbonyl sulfide (COS) by rat hepatic microsomes [1]. The reaction requires NADPH, is inhibited by carbon monoxide, and is stimulated by pretreatment of the rats with phenobarbital. Thus, it appears that the metabolism *in vitro* of CS_2 to COS is catalyzed by the cytochrome P-450 containing mono-oxygenase systems. Additional studies have indicated that COS is metabolized *in vitro* to CO_2, again in a reaction catalyzed by the rat hepatic cytochrome P-450 containing mono-oxygenase system [2]. Studies by DeMatteis and Seawright [3] have shown that $[^{14}C]CS_2$ is metabolized *in vivo* in rats to $[^{14}C]CO_2$. The purpose of the present studies was to determine if $[^{14}C]CS_2$, administered *in vivo* to rats, was also excreted in the breath as $[^{14}C]COS$.

In these experiments, untreated or phenobarbital-pretreated male Sprague–Dawley rats (250–275 g) were given $[^{14}C]CS_2$ dissolved in corn oil by i.p. injection and placed individually in a 3-l. metabolic apparatus. The phenobarbital-pretreated rats received i.p. injections of 50 mg/kg of sodium phenobarbital in distilled water for 5 days followed by administration of the $[^{14}C]CS_2$ 24 hr after the last injection. The expired air was drawn through a trap containing 200 ml of 1 N NaOH and, in turn, through two traps, each containing 35 ml of a 95% ethanol–diethylamine mixture (1:1, v/v). The airflow through the system was approximately 1.5 l./min. Three doses of $[^{14}C]CS_2$ were given; these were 0.0625, 0.125 and 0.250 m-mole/kg, representing 5.64, 11.28 and 22.55 μCi/kg respectively. There were three animals at each dose. The expired air was collected for 4 hr. Preliminary experiments indicated that expiration of radioactivity after administration of $[^{14}C]CS_2$ was essentially complete in 3 hr. DeMatteis and Seawright [3] reported that the exhalation of intraperitoneally administered $[^{14}C]CS_2$ was virtually complete in 4 hr.

Experiments in which $[^{14}C]CS_2$ was introduced into the metabolic apparatus indicated that only a trace of the $[^{14}C]CS_2$ was retained in the first trap (1 N NaOH). The majority was retained in the second trap (95% ethanol–diethylamine, 1:1, v/v) with a small amount appearing in the third trap, which contained the same solution as the second. Similar experiments using $[^{14}C]COS$ showed that about 28 per cent was retained by the first trap and the remainder was found in the second trap. No attempt was made to determine the distribution of $[^{14}C]CO_2$ among the various traps. However, the NaOH trap worked very efficiently for CO_2, and the possibility of spillover of $[^{14}C]CO_2$ into the second and third traps appeared remote. $[^{14}C]CO_2$ formation after administration *in vivo* of $[^{14}C]CS_2$ was determined by liquid scintillation counting of

Table 1. $[^{14}C]CO_2$ and COS content of expired air of untreated rats administered $[^{14}C]CS_2$*

Dose $[^{14}C]CS_2$ (m-mole/kg)	Amount $[^{14}C]CS_2$ administered (μmoles)	Amount $[^{14}C]CO_2$ excreted (μmoles)	Amount $[^{14}C]COS$ excreted (μmoles)	Amount $[^{14}C]CS_2$ excreted (μmoles)	Total recovery of administered dose (%)
0.0625	16.54 ± 0.93	1.82 ± 0.06	4.20 ± 0.19	4.94 ± 0.79	66.8 ± 11.2
0.125	32.52 ± 3.24	2.23 ± 0.08	4.32 ± 0.38	12.59 ± 1.78	59.5 ± 11.4
0.250	60.57 ± 4.86	4.06 ± 0.25	4.48 ± 0.20	43.99 ± 6.89	86.5 ± 4.42

* Each value is the mean ± S.D. of the data obtained from three rats.

Table 2. [^{14}C]CO_2 and COS content of expired air of phenobarbital-pretreated rats administered [^{14}C]CS_2*

Dose [^{14}C]CS_2 (m-mole/kg)	Amount [^{14}C]CS_2 administered (μmoles)	Amount [^{14}C]CO_2 excreted (μmoles)	Amount [^{14}C]COS excreted (μmoles)	Amount [^{14}C]CS_2 excreted (μmoles)	Total recovery of administered dose (%)
0.0625	15.66 ± 1.05	4.27 ± 0.63	2.69 ± 0.84	1.11 ± 0.98	48.6 ± 11.0
0.125	32.98 ± 2.59	8.01 ± 1.16	4.39 ± 0.63	6.93 ± 0.53	58.9 ± 7.9
0.250	69.58 ± 3.92	9.71 ± 1.97	5.23 ± 0.40	20.32 ± 3.88	51.0 ± 9.0

* Each value is the mean ± S.D. of the data obtained from three rats.

an aliquot from the first trap; this was corrected for the amount of COS present. COS was determined by gas chromatography [1] using essentially the method described by Thornsberry [4]. CS_2 concentrations in the second and third traps were determined colorimetrically [5].

[^{14}C]CS_2 (58 mCi/m-mole) was a product of the Amersham-Searle Corp. The purity was greater than 99 per cent, determined by gas chromatography [4]. [^{14}C]COS was synthesized as described previously [2].

Table 1 shows the results of analyses of expired air of untreated rats given [^{14}C]CS_2. The recovery of the administered dose as [^{14}C]CO_2 ranged from 7 per cent at 0.25 m-mole to 11 per cent at 0.0625 m-mole/kg. [^{14}C]COS accounted for 7 per cent of the administered dose at 0.25 m-mole/kg, 13 per cent at 0.125 m-mole/kg, and 25 per cent at 0.0625 m-mole/kg. Recovery of administered radioactivity as unchanged CS_2 ranged from 30 per cent at 0.0625 m-mole/kg to 72 per cent at 0.25 m-mole/kg. Total recovery of administered radioactivity in expired air is shown in the last column of Table 1.

Table 2 lists the amounts of the various doses of [^{14}C]CS_2 administered to phenobarbital-pretreated rats which were excreted in the breath as [^{14}C]CO_2 and [^{14}C]COS. In untreated rats, the predominant metabolite was COS. In the phenobarbital-pretreated animals, CO_2 was more important, ranging from 14 per cent of the administered dose at 0.25 m-mole/kg to 27 per cent at 0.0625 m-mole/kg. The COS was 8 per cent of the administered dose at 0.25 m-mole/kg, 13 per cent at 0.125 m-mole/kg, and 17 per cent at 0.0625 m-mole/kg. The increased metabolism of CS_2 to COS and CO_2 in the phenobarbital-pretreated rats is reflected in a decreased recovery of the administered dose as unchanged CS_2. An increased metabolism of CS_2 to CO_2 in phenobarbital-pretreated rats has been noted previously [3]. Also, as noted previously [3], there is a decreased total recovery of the administered dose of [^{14}C]CS_2 in phenobarbital-treated rats as compared to untreated rats. The reason for this decreased recovery is not known.

From these experiments it is clear that CS_2 is metabolized to COS *in vivo*. In untreated rats it is the predominant metabolite excreted in the expired air. After treatment with phenobarbital, more of the administered CS_2 is metabolized to COS and CO_2, with CO_2 being the predominant metabolite. This increased rate of metabolism *in vivo* of CS_2 in phenobarbital-pretreated rats is in agreement with *in vitro* data showing an increased rate of metabolism of CS_2 to COS using microsomes from phenobarbital-pretreated rats [1]. The *in vitro* data [1, 2] indicate that the metabolism of CS_2 to CO_2 involves, first, the cytochrome P-450 mono-oxygenase-catalyzed metabolism of CS_2 to COS, followed by metabolism of COS to CO_2 by these same enzyme systems. The present experiments suggest that the same sequence of reactions is operative *in vivo*.

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REFERENCES

1. R. R. Dalvi, R. E. Poore and R. A. Neal, *Life Sci.* **14,** 1785 (1974).
2. R. R. Dalvi, A. L. Hunter and R. A. Neal, *Chem. Biol. Interact.* **10,** 347 (1975).
3. F. DeMatteis and A. A. Seawright, *Chem. Biol. Interact.* **7,** 375 (1973).
4. W. L. Thornsberry, Jr., *Analyt. Chem.* **43,** 452 (1971).
5. R. W. McKee, *J. ind. Hyg. Toxicol.* **23,** 151 (1941).