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Disposition of [1,2-^{14}C] Vinyl Chloride in the Rat

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Abstract. Rats were exposed to [1,2-^{14}C] vinyl chloride in a closed system at initial concentrations below 100 ppm. When the system was occupied by 3 rats, a half-life of vinyl chloride in the system's atmosphere of 1.13 ± 0.12 h was observed. The volume of the system was 10.3 l. Calculation of the clearance of vinyl chloride from the system revealed that about 40% of inspired vinyl chloride is absorbed by lung. Therefore, changes in respiration did not influence uptake of vinyl chloride.

Uptake of vinyl chloride by the rats was completely blocked by acute pretreatment with potent inhibitors of cytochrome-P-450-dependent microsomal drug metabolism (i.e., by 35 mg/kg 3-bromophenyl-4(5)-imidazole or 50 mg/kg 6-nitro-1,2,3-benzothiadiazole in 0.6 ml/kg DMSO). A weaker inhibition was observed after dosing SKF 525 A or 5,6-dimethyl-1,2,3-benzothiadiazole (50 mg/kg in 0.6 ml/kg DMSO). Metyrapone did not cause inhibition.

Uptake of vinyl chloride was increased by pretreatment with DDT and, to a lesser extent, with clotrimazol. No significant stimulation of uptake was observed after pretreatment with phenobarbital, 3-methylcholanthrene, rifampicin, or chronic ethanol treatment.

Immediately after exposure, highest radioactivity levels were observed in liver and kidney. The radioactive metabolites of ^{14}C-vinyl chloride were rapidly excreted, largely by the kidneys. Excretion of radioactivity in the urine was 69.4 ± 2.6% within 24 h.

Key words: Vinylchloride – 3-Bromophenyl-4(5)-imidazole – 6-Nitro-1,2,3-benzothiadiazole – Inhibition of vinyl chloride metabolism – Induction of vinyl chloride metabolism.

Zusammenfassung. In einem geschlossenen System wurden Ratten initialen Konzentrationen an [1,2-^{14}C] Vinylchlorid von unter 100 ppm ausgesetzt. Bei einer Besetzung des Systems durch 3 Ratten wurde in der Atmosphäre eine

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Halbwertszeit des gasförmigen Vinylchlorid von 1,13 ± 0,12 Std gemessen. Bei einem Volumen des Systems von 10,3 l ergab die Berechnung der Vinylchlorid-Clearance, daß nur ca. 40% des von den Ratten eingeatmeten Vinylchlorid resorbiert wurde. Aus diesem Grunde führten Änderungen der Atemtätigkeit nicht zu Änderungen der Aufnahmegeschwindigkeit von Vinylchlorid.

Durch sehr wirksame Inhibitoren von Cytochrom-P-450-abhängigen mikrosomalen Oxidationen (3-Bromphenyl-4(5)-imidazol und 6-Nitro-1,2,3-benzothiadiazol) konnte die Aufnahme von Vinylchlorid vollständig verhindert werden. SKF 525 A und 5,6-Dimethyl-1,2,3-benzothiadiazol waren in dieser Hinsicht weit weniger wirksam.

Durch Vorbehandlung der Ratten mit DDT und, zum geringeren Maße, mit Clotrimazol wurde die Aufnahme von Vinylchlorid gesteigert. Keine signifikante Steigerung trat auf nach Vorbehandlung mit Phenobarbital, 3-Methylcholanthren, Rifampicin und nach chronischer Alkoholgabe.

Unmittelbar nach Beendigung der Exposition wurden die höchsten Radioaktivitätswerte in Leber und Niere festgestellt. Die Metabolite von ^{14}C-Vinylchlorid wurden sehr schnell ausgeschieden. Bereits nach 24 Std wurden im Urin 69,4 ± 2,6% der inkorporierten Radioaktivität gemessen.

Schlüsselwörter: Vinylchlorid – 3-Bromphenyl-4(5)-imidazol – 6-Nitro-1,2,3-benzothiadiazol – Induktion des Vinylchlorid-Stoffwechsels – Hemmung des Vinylchlorid-Stoffwechsels.

Recent reports of angiosarcoma of the liver due to occupational exposure to vinyl chloride (Creech and Johnson, 1974; Lee and Harry, 1974; Buchter and Bolt, 1974; Thomas et al., 1975) had focused attention on metabolism and disposition of this compound, particularly since it has been suggested (Jaeger et al., 1974; Bartsch et al., 1975) that hepatotoxicity and carcinogenicity of vinyl chloride may be attributed not to the compound itself, but to a hitherto unknown reactive metabolite. However, due to the gaseous nature of vinyl chloride, metabolic studies require some special techniques.

Recently, Hefner et al. (1975) exposed rats to vinyl chloride in a closed system and monitored concentration in the atmosphere by infrared absorption. The observed decline could be described by a logarithmic function. Uptake of vinyl chloride from air by the rats was inhibited by acute high doses of ethanol or pyrazole. This indicated that uptake was dependent on metabolism: apparently the organism takes up vinyl chloride only to that extent which is metabolized. At concentrations of vinyl chloride above 200 ppm the metabolizing system was saturated and metabolism therefore was much slower than at low atmospheric concentrations of vinyl chloride. In these studies, Hefner et al. (1975) were faced with considerable difficulties of minimizing losses of vinyl chloride from their exposure system, apparently due to the solubility of vinyl chloride in rubber and diffusion through rubber membranes.

The present report aims at further extending knowledge of the fate of vinyl chloride in vivo and deals with the inhibition of uptake of vinyl chloride by specific and potent inhibitors of cytochrome-P-450-dependent mixed function oxidations. Furthermore, the effect of agents known to induce the cytochrome P-450 system has been examined. The use of an all-glass exposure system and avoidance of rubber

material prevented losses of vinyl chloride from the exposure system. [^{14}C] Vinyl chloride was used which also permitted determination of distribution of radioactive metabolites in the rat organism. In order to avoid saturation of the metabolizing enzymes, in these experiments, concentrations below 100 ppm have been applied.

Materials and Methods

Materials. [1,2-^{14}C] Vinyl chloride, specific radioactivity 10.7 mCi/mmol, was synthesized by the Radiochemical Department of Farbwerke Hoechst, Frankfurt/Main. Synthesis started from [1,2-^{14}C] acetylene. The purity of the product was 99.9%, as checked by gas-liquid chromatography (stationary phase: Tenax; carrier gas: He, 70–75 ml/min; temperature 70–130° C; FID). For storage, the preparation was diluted with helium (3 pts.) to prevent polymerization. A sample, also diluted with helium, which was stored for 2 months at room temperature, contained about 8% of vinyl chloride oligomers. The rest was unchanged vinyl chloride monomer (Farbwerke Hoechst, personal communication). In our laboratory, the radioactive preparation was stored in the dark at 4° C and used within 1 month after synthesis.

3-Bromophenyl-4(5)-imidazole, 6-nitro-1,2,3-benzothiadiazole, and 5,6-dimethyl-1,2,3-benzothiadiazole were kindly donated by Dr. C. F. Wilkinson, Cornell University, Ithaca, N.Y.

SKF 525 A (2-diethylaminoethyl-2,2-diphenylvalerate · HCl) was obtained from Smith, Kline, & French, Philadelphia, Pa., and metyrapone was obtained from Ciba, Basel, Switzerland. Pyrazole was a commercial product of Fluka, Buchs, Switzerland.

Pretreatment of Animals. Male Wistar rats (200–250 g) from Ivanovas, Kisslegg, Allgäu were used for the experiments.

Rats were pretreated with phenobarbital by a single i.p. injection of 80 mg/kg, followed by 5 days of 0.1% phenobarbital as a constituent of the drinking water. 3-Methylcholanthrene was given as a single i.p. dose of 40 mg/kg in olive oil, 4 days prior to the experiment. Di-(p-chlorophenyl)β-trichloroethane (DDT) was given 1 week before the experiment by a single i.p. dose of 200 mg/kg in olive oil. Clotrimazol (Bay b 5097; Canesten® of Bayer, Leverkusen) was administered at a daily i.p. dose of 20 mg/kg in olive oil for 5 days, followed by 2 days without treatment. Rifampicin (20 mg/kg) was given orally for 5 days; rats were used 1 day after cessation of treatment. Chronic ethanol treatment of rats was achieved by adding 30% (v/v) ethanol and 30% (w/v) sucrose to the drinking water for 3 weeks. Treatment ceased 1 day before the experiment.

Exposure of Rats to ^{14}C-Vinyl Chloride. Rats were exposed to ^{14}C-vinyl chloride in a closed all-glass system as shown in Figure 1. A desiccator was used as the exposure cage. It contained a center

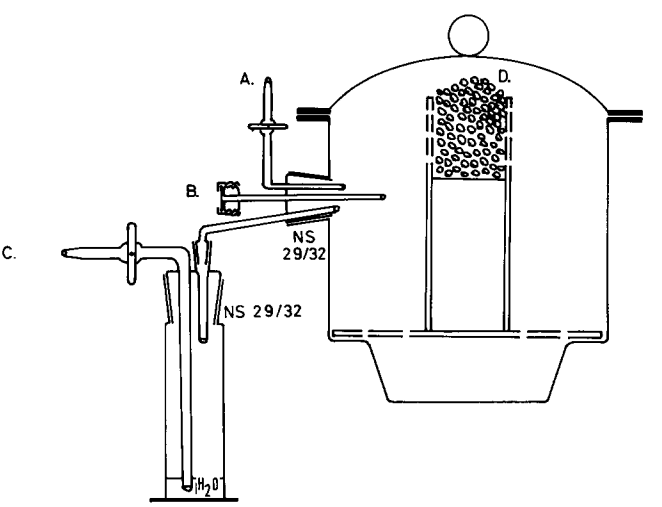

Fig. 1. All-glass exposure system. (A) inlet for ^{14}C-vinyl chloride; (B) septum (rubber, coated with teflon). For determination of concentration of ^{14}C-vinyl chloride in system's atmosphere, samples of air (2 ml) were withdrawn by means of syringe, containing toluene scintillator (see text). (C) inlet for oxygen; connected with spirometer. (D) CO_2 absorbent. Three rats were placed in chamber. Volume of system was 10.3 l

well (D) filled with the carbon dioxide absorbent ("Indikator-Atemkalk", Dräger, Lübeck). A spirometer, filled with oxygen, was connected with the oxygen inlet (C). The total volume of the system was 10.3 l. The spirometer used for these experiments was of type 336 (A. Dargatz, Hamburg), and allowed mechanical recording of the consumed gas.

When starting an experiment, 3 rats were placed in the desiccator and oxygen consumption was monitored by the spirometer. ^{14}C-Vinyl chloride was mixed with atmospheric air and the mixture was injected with a 20-ml syringe into the rat exposure cage via inlet A.

Concentration of radioactive vinyl chloride in the air of the system was measured every 30 min.

Measurement of Radioactivity. Because of the solubility of vinyl chloride in toluene, a toluene scintillation mixture (0.4% PPO plus 0.01% POPOP in toluene) could be used for determination of ^{14}C-vinyl chloride. A 5-ml syringe was filled with 3 ml of this mixture. Air of the exposure system (2 ml) was drawn by this syringe with a long needle through the rubber septum (B). This septum was coated with teflon to avoid diffusion of vinyl chloride through the rubber. After withdrawal of the air, the contents of the syringe, scintillation mixture and ^{14}C-vinyl chloride containing air, were shaken for 2 min to dissolve the vinyl chloride, and the scintillator was poured into a scintillation vial with an additional 7 ml of scintillator. The scintillation mixture was counted for radioactivity in a Berthold BF 5000 liquid scintillation spectrometer.

Determination of Radioactivity in Tissues and Urine. For determination of radioactive metabolites of vinyl chloride in tissues, organs of rats exposed to ^{14}C-vinyl chloride were homogenized with 4 vol of water by means of an Ultra-Turrax tissue homogenizer. Losses of nonmetabolized vinyl chloride due to diffusion from the tissue into air could be minimized by immediately sacrificing the rats after withdrawal from the exposure chamber, and by immediate removal of the organs. The distribution of nonmetabolized vinyl chloride in rat tissues after exposure will be reported separately.

After homogenization, ½ ml of homogenate, corresponding to 0.1 g of wet tissue, was digested with 0.5 ml 1 M hydroxide of hyamine. After achieving solution, 0.5 ml of peroxide mixture (supernatant of 12.5 g commercial benzoylperoxide plus 12.5 g $CaCl_2$ plus 100 ml toluene) was added, stored overnight at 37°, and counted for radioactivity in 10 ml of Bray's solution (Bray, 1960).

For determination of excretion of radioactivity in urine of rats exposed to ^{14}C-vinyl chloride, the rats were placed in a metabolism cage after exposure. Urine was counted for radioactivity in Bray's solution (Bray, 1960).

Results

Uptake of ^{14}C-Vinyl Chloride. Before starting the animal studies, control experiments with the unoccupied chamber showed that no measurable radioactivity was lost from the exposure system (Fig. 2, curve A). When the chamber was occupied by 3 rats, a logarithmic decline of radioactivity in the air of the exposure system was observed (Fig. 2, curve B) with a half-life of 1.13 ± 0.12 h (6 determinations, $\bar{x} \pm s_x$). This agrees with the data of Hefner et al. (1975) who measured a similar decline by means of infrared spectroscopy.

Changes in the uptake of oxygen from the spirometer (Fig. 2, curve C) do not coincide with changes of uptake of vinyl chloride by the animals from the system (Fig. 2, curve B).

Inhibiton of Uptake of ^{14}C-Vinyl Chloride. Hefner et al. (1975) had shown that inhibition of metabolism of vinyl chloride, e.g., by acute high doses of pyrazole or ethanol, results in a corresponding inhibition of uptake of vinyl chloride from air. However, they failed to show inhibition by specific inhibitors of microsomal mixed function oxidases. In their experiments SKF 525 A did not inhibit the uptake of vinyl chloride to a significant extent.

Much more potent inhibitors of microsomal oxidative drug metabolism than SKF 525 A have been synthesized recently by Wilkinson and his coworkers (Wil-

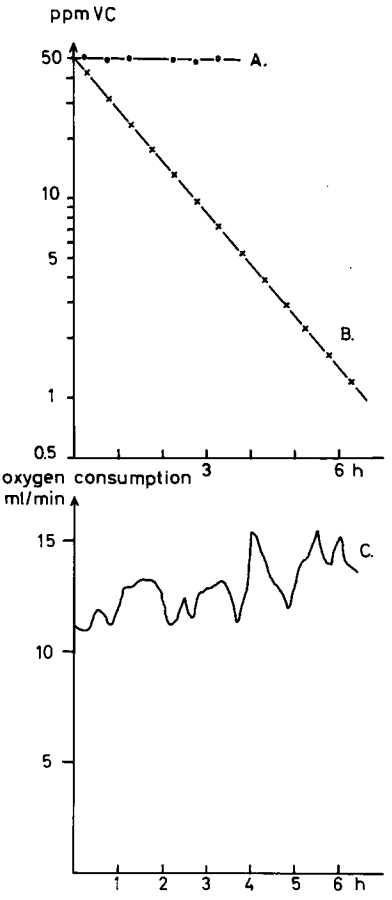

Fig. 2. Decline of ^{14}C-vinyl chloride in closed system due to uptake by 3 rats. (A) control experiment with unoccupied chamber. (B) chamber occupied by rats. Half-life of vinyl chloride = 1.1 h. (C) oxygen consumption (ml/min) of rats during experiment B. Changes in respiration do not influence uptake of vinyl chloride.

kinson, 1971; Wilkinson and Brattsten, 1972). These inhibitors were originally designed as insecticide synergists. In the present study two benzothiadiazoles (6-nitro-1,2,3-benzothiadiazole; 5,6-dimethyl-1,2,3-benzothiadiazole) and one substituted imidazole (3-bromophenyl-4(5)-imidazole) have been studied as to their inhibition of vinyl chloride metabolism. Because these compounds turned out to be insoluble in most of the solvents commonly used for application to animals in vivo it was necessary to administer them in DMSO solution.

The inhibition of uptake of vinyl chloride by 3-bromophenyl-4(5)-imidazole and its reversibility is shown in Figure 3. These experiments have been carried out on consecutive days, always using the same animals (3 male Wistar rats of 250 g). The half-life of vinyl chloride in the closed system was 1.1 h when the rats were untreated (curve A). In contrast, pretreatment with 35 mg/kg 3-bromophenyl-4(5)-imidazole in DMSO (0.6 ml/kg) by i.p. injection, 30 min prior to exposure, completely blocked the uptake of vinyl chloride for 5 h. After this period inhibition became incomplete (curve B). Twenty-four hours after application of the inhibitor, the half-life of vinyl chloride in the closed system turned to the normal value (curve C). The control experiment with DMSO showed that this solvent inhibited uptake of vinyl chloride, but to a much lesser degree than was observed in presence of the imidazole: initially,

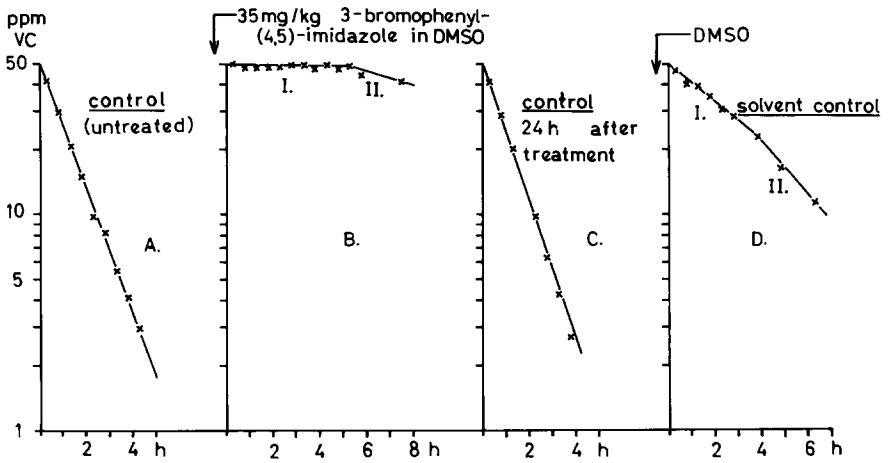

Fig. 3. Reversible inhibition of uptake of ^{14}C-vinyl chloride. Experiments performed with same 3 rats on consecutive days. (A) control experiment, $t_{1/2}$ of vinyl chloride in system = 1.1 h. (B) total inhibition of uptake of vinyl chloride by acute dosage of 35 mg/kg 3-bromophenyl-4(5)-imidazole in 0.6 ml/kg DMSO (Section I). Inhibition becomes incomplete after 5 h (Section II). (C) control experiment, 24 h after dosage of 3-bromophenyl-4(5)-imidazole. $t_{1/2}$ = 0.92 h. (C) solvent control, 0.6 ml/kg DMSO 30 min prior to experiment. Initial $t_{1/2}$ (Section I) 3.3 h, lasting for 4 h (Section II)

the half-life of vinyl chloride in the system was 3.3 h (curve D, Section I), but this initial inhibition lasted only for 3.5 h (curve D, Section II).

In order to compare the inhibitory potency of some further compounds (SKF 525 A, metyrapone, pyrazole) with that of the benzothiadiazoles and of bromophenyl-imidazole, all compounds in a similar manner were administered in DMSO solution. The results are given in Table 1. Complete inhibition of uptake of vinyl chloride was achieved by 3-bromophenyl-4(5)-imidazole (35 mg/kg) and by 6-nitro-benzothiadiazole (50 mg/kg). 5,6-Dimethyl-benzothiadiazole (50 mg/kg) showed a lesser effect, comparable to that of SKF 525 A (50 mg/kg). Metyrapone did not inhibit,

Table 1. Inhibition of uptake of ^{14}C-vinyl chloride by acute dosage of potential inhibitors. The compounds were administered 30 min prior to the experiment by i.p. injection in DMSO solution. Half-life of vinyl chloride in the closed system ($t_{1/2}$) and the corresponding rate constant of elimination from the system are shown. System was occupied by 3 rats

	$t_{1/2}$ (h)	k (h^{-1})	% inhibition
DMSO control (see Fig. 3)	3.3	0.21	–
35 mg/kg 3-Bromophenyl-4(5)-imidazole in DMSO (see Fig. 3)	∞	0	100
50 mg 6-Nitro-1.2.3-benzothiadiazole in DMSO	∞	0	100
50 mg/kg 5,6-Dimethyl-1.2.3-benzothiadiazole in DMSO	13.7	0.051	76
50 mg/kg SKF 525 A in DMSO	14.0	0.050	76
50 mg/kg Metyrapone in DMSO	3.2	0.22	–
50 mg/kg Pyrazole in DMSO	9.8	0.071	66
100 mg/kg Pyrazole in DMSO	∞	0	100

and a comparatively weak inhibition was observed after administration of pyrazole (50 mg/kg). However, increase of the dose of pyrazole to 100 mg/kg in DMSO completely blocked uptake of vinyl chloride. This inhibition by pyrazole is greater than that reported by Hefner et al. (1975), but it has to be taken into account that the solvent DMSO which has been used in our study, exerts a synergistic inhibitory effect. When given in aqueous solution, 100 mg/kg pyrazole blocked uptake of vinyl chloride only by 71% (data not shown in the table).

Induction Experiments. Rats were pretreated with agents which are known to induce drug metabolizing enzymes in the endoplasmic reticulum of the liver. Table 2 compares the half-life of vinyl chloride in our system before and after pretreatment. It is surprising that phenobarbital does not shorten the half-life. After pretreatment with phenobarbital it was of no influence whether the rats were examined immediately after the treatment period or 1 day later.

No significant enhancement of uptake was observed after application of 3-methylcholanthrene. Neither rifampicin, which induces the microsomal NADPH-cytochrome-c-reductase in the rat (Otani and Remmer, 1975), nor chronic treatment with ethanol, which induces a specific species of cytochrome P-450 (Ullrich et al., 1975) showed any effect. Pretreatment with clotrimazol which has been found to increase demethylation of mestranol in rats (Bolt and Kappus, 1973) slightly increased uptake of vinyl chloride, but a marked effect was observed only after pretreatment with DDT. The well-known persistence of DDT in fat effects a long-lasting induction: even when assessed 1 month later, the uptake of vinyl chloride by the rats was enhanced.

Distribution and Excretion of Radioactivity. Rapid conversion of vinyl chloride to polar metabolites has been reported in vivo, using rats (Hefner et al., 1975), and in the isolated perfused rat liver (Radwan and Henschler, 1975). Therefore, radioactivity measured in tissues after exposure to ^{14}C-vinyl chloride should mostly represent metabolites rather than the parent compound. As demonstrated in Table 3, organs of highest content of radioactivity are liver and kidney. Radioactivity in fat and in

Table 2. Effect of pretreatment of rats with inducing agents on uptake of ^{14}C-vinyl chloride. Data give the half-life of vinyl chloride in the closed system when occupied by 3 male rats (200–250 g)

Rats pretreated with	Half-life ($t_{1/2}$) of vinyl chloride (h)	
	Before pretreatment	After pretreatment
Phenobarbital:		
1st experiment	1.1	1.2
2nd experiment[b]	1.2	1.6
3-Methylcholanthrene	1.1	1.0
Rifampicin	1.1	1.1
Ethanol (chronic treatment for 3 weeks; 1 day cessation)	1.1	1.0
Clotrimazol	1.1	0.88
DDT	1.0	0.69[a]

[a] This value was taken 1 week after a single i.p. dose of 200 mg/kg DDT. One month later, the same rats showed a $t_{1/2}$ of 0.80 h

[b] For the second phenobarbital experiment rats of 175 g were used

Table 3. Radioactivity in organs of rats after exposure to ^{14}C-vinyl chloride (initial concentration 50 ppm, 5 h exposure). Male Wistar rats, 200 g. Mean values ± standard deviation are shown; n = 3

	% of incorporated vinyl chloride radioactivity/g tissue	
	Immediately after exposure	48 h after beginning of exposure
Brain	0.171 ± 0.008	0.038 ± 0.004
Liver	1.86 ± 0.30	0.32 ± 0.02
Spleen	0.73 ± 0.05	0.37 ± 0.01
Kidney	2.13 ± 0.43	0.21 ± 0.03
Adipose tissue	0.22 ± 0.06	0.044 ± 0.004
Muscle (M. psoas)	0.32 ± 0.07	0.05 ± 0.01

Table 4. Excretion of radioactivity in urine of rats after 5 h exposure to ^{14}C-vinyl chloride (initial concentration 50 ppm)

Day after exposure	% excretion of incorporated radioactivity ($\bar{x} \pm s_x$; n = 3)
1st	69.4 ± 2.58
2nd	1.74 ± 0.48
3rd	0.49 ± 0.08
4th	0.42 ± 0.03

brain, organs which may be expected to be of affinity for the very lipid-soluble vinyl chloride, is comparably low.

Metabolites of vinyl chloride are largely excreted during the first 24 h after exposure, predominantly in the urine (Table 4). Fourty-eight h after exposure, a comparatively small part of incorporated radioactivity remains in the organism (Table 3).

Discussion

The results suggest that respiration is not a rate-limiting factor for the uptake of vinyl chloride by lung. This is also reflected by calculation of the clearance of vinyl chloride in the closed system. For $k = 0.61\ h^{-1}$ (i.e., $t_{1/2} = 1.13$ h) and the volume of the system of 10.3 l the clearance of vinyl chloride for three rats, each of 250 g, amounts to $k \cdot v = 6.3$ l/h. On the other hand, the mean value of oxygen consumption has been determined to be 13 ml/min or 0.78 l/h (Fig. 2). If we assume a difference of oxygen content between inspired and expired air of about 5%, total respiration of the 3 rats averages 15.6 l/h. A comparison with the above clearance of vinyl chloride shows that only about 40% of inspired vinyl chloride is taken up by the organism. This means that normally respiration should not limit the rate of entry of vinyl chloride into the organism.

The dependence of uptake upon metabolism is clearly expressed by its total, and reversible, inhibition after dosing potent inhibitors of oxidative microsomal drug metabolism. It is currently thought that substituted imidazoles occupy the type-II binding site at the 6th ligand of heme iron in cytochrome P-450 (Wilkinson et al., 1974), whereas benzothiadiazoles may trap a reactive oxygen species involved in drug oxidation (Marshall and Wilkinson, 1973). The much lesser effect of 5,6-dimethyl-benzothiadiazole compared to 6-nitrobenzothiadiazole which is found in the present study, has also been observed in studies of microsomal drug metabolism in vitro. Using rat liver microsomes, the oxidation of aniline, ethylmorphine, and aminopyrine was markedly inhibited by 6-nitrobenzothiadiazole, but not by 5,6-di-

methylbenzothiadiazole, whereas demethylation of p-nitroanisole and hydroxylation of estrogens was inhibited by both compounds to nearly the same extent (Bolt and Kassel, 1976). It may be considered that vinyl chloride is metabolized by a distinct species of cytochrome P-450. This may also explain the lack of stimulation of metabolism of vinyl chloride by phenobarbital and methylcholanthrene which contrasts to stimulation by the chlorinated lipophilic compounds DDT and clotrimazol. The lack of stimulation by phenobarbital in the present experiments coincides with a missing inhibitory effect by metyrapone. Metyrapone is currently thought to inhibit the specific species of cytochrome P-450 which is induced by phenobarbital.

Further experiments in vitro on metabolism of vinyl chloride by rat liver microsomes also revealed no stimulation by phenobarbital pretreatment, neither of the total rate of metabolism, nor of covalent binding of vinyl chloride to macromolecules, although cytochrome P-450 was enhanced under these conditions (unpublished results). This is in contrast to recent reports on enhanced liver toxicity of vinyl chloride in phenobarbital-pretreated rats (Jaeger et al., 1974), and on increased formation of mutagenic metabolites from vinyl chloride by liver microsomes from rats pretreated with phenobarbital (Bartsch et al., 1975). However, our results agree with observations of Gehring (personal communication) who also did not find increased metabolism of vinyl chloride in rats after pretreatment with phenobarbital. We think that most probably strain differences may be responsible for these diverging results.

The present experiments further prove that vinyl chloride is metabolized very rapidly. Most of the metabolites leave the body via the kidneys within 24 h. The rest of about 30% which is not excreted in urine is partly excreted via feces and via expiration of $^{14}CO_2$ (Green and Hathway, 1975; Gehring, personal communication). However, some metabolites remain in tissues even 48 h after exposure (Table 3). This should be viewed along with the previous findings that vinyl chloride can be converted in vitro by rat liver microsomes to reactive metabolites which covalently bind to macromolecular structures (Bolt et al., 1975; Kappus et al., 1975). Hence, the possibility of long-lasting covalent binding to cellular macromolecules in vivo and its toxicologic implications will be a matter of a further survey (Bolt et al., 1976).

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