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Metabolism and pharmacokinetics of vinyl acetate

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Abstract. The hydrolysis of vinyl acetate (formation of acetic acid) has been studied in vitro with rat liver and lung microsomes, rat and human plasma and purified esterases (such as acetylcholine esterase, butyrylcholine esterase, carboxyl esterase). Characterization of the kinetic parameters revealed that rat liver microsomes and purified carboxyl esterase (from porcine liver) displayed the highest activity.

In order to establish the rate of metabolism of vinyl acetate in vivo, rats were exposed in closed desiccator jar chambers, and gas uptake kinetics were studied. The decay of vinyl acetate was dose-dependent, indicating possible saturation of metabolic pathway(s). The maximal clearance (at lower concentrations) of vinyl acetate from the system (30 000 ml/h per kg body weight) was similar to the maximal ventilation rate in this species. This indicated that under conditions when metabolic enzymes are not saturated the metabolic rate is mainly determined by pulmonary uptake.

The exposure of rats to vinyl acetate resulted in a transient exhalation of significant amounts of acetaldehyde into the closed exposure system. This indicates the presence of this metabolic intermediate of vinyl acetate in the organism in vivo.

Key words: Vinyl acetate – Acetaldehyde – Esterases – Gas uptake kinetics

Introduction

Contradictory reports on possible carcinogenicity of vinyl acetate (Maltoni 1977; Lijinski and Reuber 1983) have caused concern about metabolism and toxicity of this compound (Deutsche Forschungsgemeinschaft 1983).

By contrast to vinyl halides, the metabolism of which involves epoxide intermediates (Bolt et al. 1982) vinyl acetate is split by esterases to acetaldehyde and acetic acid (see Deutsche Forschungsgemeinschaft 1983). Because of the obvious importance of this deactivating mechanism, studies on biotransformation of vinyl acetate by different esterases were conducted as part of a vinyl acetate research program. These studies were performed with human and rat blood, rat tissues and commercially available esterase preparations.

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Materials and methods

Materials. Male Wistar rats (250 g) were obtained from Lippische Versuchstieranstalt, Extertal, Germany. Human blood was donated by male and female volunteers. Commercial esterase preparations were purchased from Sigma, Munich, or Boehringer, Mannheim, as indicated in Table 1.

Vinyl acetate (synthesis grade), stabilized with 0.01% hydroquinone, was from Merck-Schuchardt, Munich. For titrations a pH-stat unit (Schott, Mainz) was used, which consisted of an electronic unit (TR 156) and an automatic burette (T 100). The titrations were performed at a fixed pH. The amounts of 0.1 N NaOH which were needed to neutralize the developing acetic acid were recorded by a titration recorder (TS 165).

Experiments in vitro. Erythrocytes and plasma were separated from heparinized blood by centrifugation. The erythrocytes were washed three times with saline (0.9% NaCl). For the experiments the undiluted plasma (supernatant of first centrifugation) or a suspension of 45 parts erythrocytes in 55 parts (v/v) saline was taken.

Liver and lung tissues of rats were taken for preparation of mitochondria, microsomes and cytosol, following standard procedures (Remmer et al. 1967).

The incubation experiments were carried out at 37 °C, in a total volume of 4.5 ml. NBS buffer was used; this buffer contained KH_2PO_4 (8.7 mM) and Na_2HPO_4 (30 mM). The final pH (7.4 or 8.0, as indicated in Table 1, Table 2 and Fig. 1) was adjusted with NaOH or H_3PO_4. The concentrations of vinyl acetate varied between 1.2 mM and 30 mM in the experiments designed for determination of K_m and V_{max} (as stated in Fig. 1 and Table 1). In all other experiments a constant substrate concentration of 6 mM was used (see Results section, Table 2). After a preincubation period (adjustment of the temperature) of 0.5 min the source of enzyme was added (30 µl blood, erythrocyte suspension, plasma, or as indicated in Tables 1 and 2).

The amount of 0.1 N NaOH needed to neutralize the developing acetic acid was continuously recorded over 15 min. The initial slope of this curve (extrapolation to $t=0$) was obtained graphically and served for the calcula-

tion of micromoles vinyl acetate split per min and per mg (μl) enzyme source.

In separate experiments the spontaneous hydrolysis of vinyl acetate (in absence of any enzyme source) was determined at both pHs employed (pH 7.4 and 8.0).

Pharmacokinetic experiments. Rats were exposed to vinyl acetate in closed static all-glass exposure chambers as previously described (Filser and Bolt 1979, 1981). Usually two animals (250 g each) were placed in a chamber of 6.4 l gas volume. The exposure time was 1.4 h or less, and the system did not contain soda lime (as CO_2 absorbant) because vinyl acetate is rapidly decomposed in the presence of alkali. An injection of oxygen (8 ml) into the exposure chamber after each withdrawal (8 ml) of a gas sample provided sufficient oxygen supply. The initial concentrations of vinyl acetate in the system varied between 200 ppm and 2000 ppm (see Figures). The chemical was injected as vapour into the exposure system; its concentration in the gas phase was monitored at short intervals by gas-liquid chromatography. A 5 m (⅛″) stainless-steel column with Tenax GC was operated at 154 °C. Vinyl acetate showed a retention time of 2.5 min.

Results

Spontaneous hydrolysis in vitro

In several experiments the *non-enzymic* hydrolysis rate of vinyl acetate in aqueous solution (4.5 ml NBS buffer) was determined at pH 7.4 and 8.0 and at different concentrations of substrate (vinyl acetate). This reaction always followed first-order kinetics. Under our experimental conditions the $t_{½}$ was 270 min at pH 7.4 and 180 min at pH 8.0. Accordingly, the first order rate constant (k^-) amounted to 0.0026 min^{-1} (pH 7.4) or 0.0038 min^{-1} (pH = 8.0).

In further experiments to determine the kinetic parameters (K_m and V_{max}) of *enzyme-mediated* hydrolysis the spontaneous hydrolysis rate was always checked in parallel and subtracted from that observed in presence of enzyme (see below).

Kinetic parameters of enzyme-mediated hydrolysis

The enzyme-mediated hydrolysis of vinyl acetate with microsomes from rat liver and lung, rat and human plasma and three different purified esterases was examined, depending on the concentration of the substrate (vinyl acetate). Lineweaver-Burk plots were obtained for determinations of K_m and V_{max} as exemplified in Fig. 1 (rat liver and lung microsomes).

At high substrate concentration (i.e. 10 mM and above) a deviation occurs from the Michaelis-Methen kinetics observed at lower concentrations (see Fig. 1). From the experiments with rat liver microsmes (Fig. 1, bottom panel) a second K_m (4 mM as opposed to 0.73 mM) can be deduced for the high concentration range. This has already been observed by previous investigators (Arndt 1973; Mentlein and Heyman 1984). For the determination of K_m and V_{max} with other enzyme sources, as indicated in Table 1, the deviation from Michaelis-Menten kinetics at the unphysiologically high concentrations was not taken into account. This means that the data in Table 1 are valid for substrate concentrations up to about 10 mM vinyl acetate.

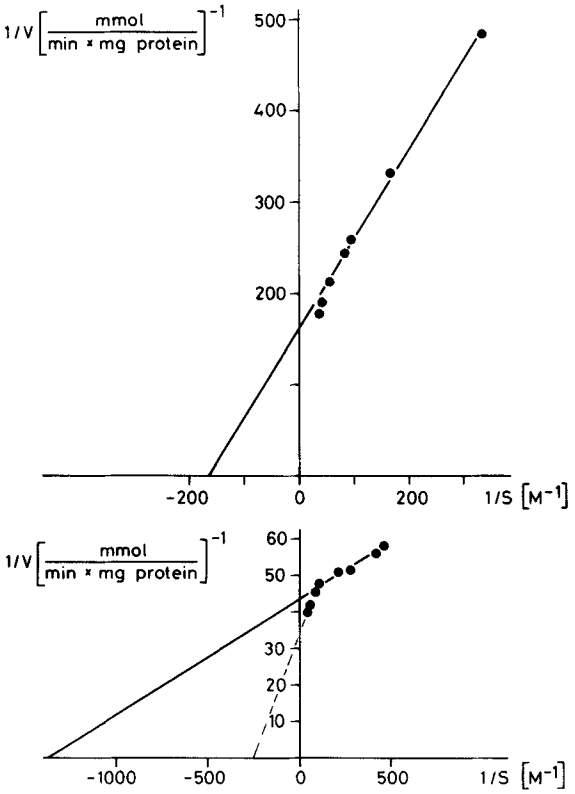

Fig. 1. Lineweaver-Burk plots of the hydrolysis of vinyl acetate at pH 8 by microsomes from rat liver *(bottom panel)* and lung *(top panel)*. At high substrate concentrations (> 10 mM) a deviation from simple Michaelis-Menten kinetics is noted: with rat liver microsomes *(bottom panel, dotted line)* a second K_m can be calculated (see text)

Table 1. K_m and V_{max} of enzymes from different sources for vinyl acetate hydrolysis (pH 8.0)

Source of enzyme; mg protein or enzyme preparation per incubation (4.5 ml)	K_m [mM]	V_{max} [μmol/min] per mg protein or mg preparation
Rat liver microsomes; 0.06 mg	0.73	23
Rat lung microsomes; 0.12 mg	6.1	6.2
Rat plasma; 1.04 mg	4.0	0.56
Human plasma; 2.1 mg	7.1	0.69
Purified AChE[a]; 2 mg	55.5	1.7
Purified BChE[b]; 0.64 mg	77	4.6
Purified carboxyl esterase[c]; 0.11 mg	0.65	238

Enzyme preparations used:

[a] Acetylcholinesterase (EC 3.1.1.7) from human erythrocytes (Sigma); 1 U/mg

[b] Butyrylcholinesterase (pseudocholinesterase, EC 3.1.1.8) from human plasma (Boehringer); 9 U/mg, substrate butyryl-thiocholine iodide, 25°C; 5 U/mg, substrate acetylthiocholine iodide, 25°C

[c] Carboxyl esterase (EC 3.1.1.1) from porcine liver (Sigma); 100 U/mg, substrate ethylbutyrate, 25°C

Table 2. Specific activities for hydrolysis of vinyl acetate in different tissues at pH 7.4 and pH 8.0; substrate concentration: 6 mM

Enzyme source	Specific activity ($\bar{x} \pm SD$) [$\mu mol \times min^{-1} \times mg\ protein^{-1}$]	
	at pH 7.4	at pH 8.0
Human plasma (male)	0.49 ± 0.11 ($n = 13$)	0.51 ± 0.12 ($n = 13$)
Human plasma (female)	0.44 ± 0.10 ($n = 11$)	0.50 ± 0.12 ($n = 11$)
Rat plasma (male)	0.40 ± 0.11 ($n = 11$)	0.45 ± 0.122 ($n = 11$)
Rat liver (male)		
1000 *g* supernatant	2.95 ± 0.82 ($n = 5$)	3.05 ± 0.75 ($n = 5$)
cytosol	0.28 ± 0.10 ($n = 5$)	0.26 ± 0.10 ($n = 5$)
mitochondria	3.20 ± 1.10 ($n = 5$)	3.30 ± 0.95 ($n = 5$)
microsomes[a]	12.50 ± 2.80 ($n = 5$)	14.50 ± 2.81 ($n = 5$)
Rat lung (male)		
1000 *g* supernatant	1.21 ± 0.19 ($n = 5$)	1.335 ± 0.199 ($n = 5$)
cytosol	0.12 ± 0.05 ($n = 5$)	0.13 ± 0.05 ($n = 5$)
mitochondria	0.53 ± 0.28 ($n = 5$)	0.62 ± 0.21 ($n = 5$)
microsomes[a]	2.37 ± 0.43 ($n = 5$)	2.90 ± 0.57 ($n = 5$)

[a] Substrate concentration: 1.2 mM

The kinetic characteristics of the reaction with all enzyme sources examined are summarised in Table 1. These data show that hepatic microsomes as well as plasma enzymes are particularly capable of forming acetic acid from vinyl acetate (high V_{max}). Further experiments were carried out to compare the rates of vinyl acetate hydrolysis with rat and human blood constituents and with subcellular fractions from rat liver and lung.

Table 2 shows the capacities of preparations of different tissues to hydrolyse vinyl acetate at pH 7.4 and pH 8.0 using a substrate concentration of 6 mM (resp. 1.2 mM). The highest enzyme activities are present in microsomes from (rat) liver. This is consistent with the high V_{max} of purified carboxyl esterase from (porcine) liver (Table 1). Esterases present in rat and human plasma are somewhat less effective in metabolizing vinyl acetate.

Gas uptake pharmacokinetics of vinyl acetate

Rats were exposed in closed desiccator exposure chambers to different initial concentrations of vinyl acetate. The procedures for determining gas uptake kinetics have been previously described with a number of other volatile compounds (Filser and Bolt 1979, 1981).

Experimental decline curves of vinyl acetate in the atmosphere of the closed system (6.4 l) when occupied by two rats (250 g each) are shown in Fig. 2. The kinetics show nonlinear patterns, as individual curves are composed of apparent zero-order and first-order sections (at higher and lower concentrations, respectively) with some transition zone in between. This demonstrates that a saturable process must be involved in uptake or metabolism of vinyl acetate.

When other volatile compounds (hydrocarbons or halogenated hydrocarbons) were studied, initial phases of rapid decline in the atmospheric concentration were observed (Filser and Bolt 1979, 1981) which are indicative for an initial equilibration of the compound between gas phase and organism. Similar initial curve sections are not obtained with vinyl acetate (Fig. 2). This may be explained

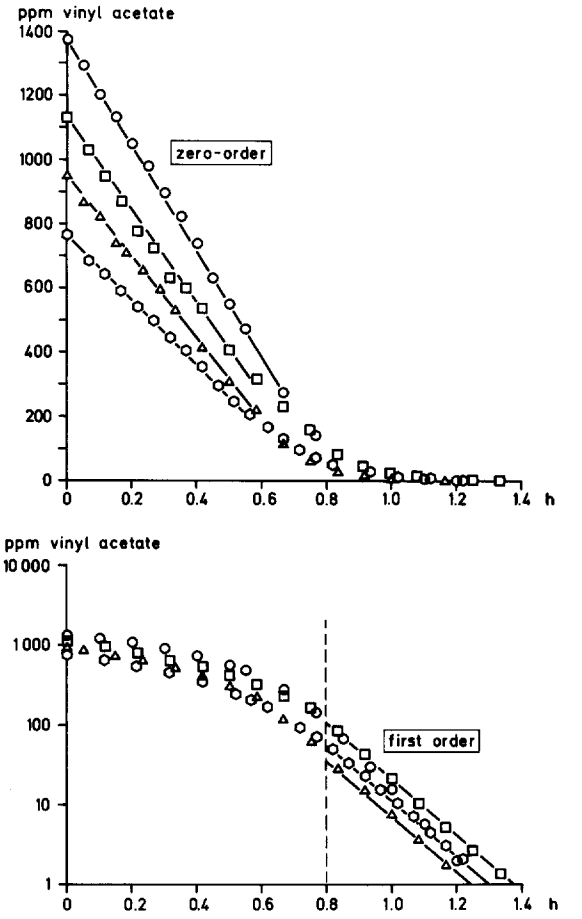

Fig. 2. Atmospheric decline of vinyl acetate in closed chambers (6.4 l), occupied by two rats (250 g each). Different experiments starting at different initial concentrations are shown by different symbols. *Top panel:* linear plot; *bottom panel*; semilogarithmic plot of the same experimental data. The curves show sections of zero-order and first-order declines as indicated

by rapid metabolic conversion by blood esterases, which prevents distribution of the compound in the tissues of the organism. This was confirmed by experiments in which we injected different amounts of vinyl acetate intraperitoneally into rats: no exhalation of vinyl acetate by these animals was observed, in contrast to similar experiments with hydrocarbons which were distributed and exhalaled (Bolt et al. 1984). This indicated that the usual two-compartment open kinetic model with consideration of the animal as a single compartment (Filser and Bolt 1979, 1981) is not appropriate for vinyl acetate. Hence, the metabolic rates could not be determined using such a model and knowledge of its kinetic microconstants. For this reason, the elimination rates for vinyl acetate were approximated based on the total clearance of the compound from the system. Details of this procedure are given in the Appendix.

In the first-order section of the decline curves in Fig. 2 (i.e. at lower atmospheric concentrations of vinyl acetate) the total clearance (dose/AUC) of vinyl acetate from the gas phase amounted to 30 000 ml/h per kg body weight. For experiments including higher concentrations, the clearance was lower because of the saturation pattern already mentioned.

The (metabolic) elimination rates were calculated from the clearance values of different experiments and the initial vinyl acetate concentrations (see Appendix). The resulting curve presented in Fig. 3 indicates that vinyl acetate, on exposure of rats to constant concentrations up to 650 ppm, is metabolized in linear correlation with the atmospheric concentration. Above this point a saturation phenomenon is visible.

Further experiments in the same system after pretreating rats with dithiocarb (diethyldithiocarbamate, 200–600 mg/kg) showed that the pharmacokinetics of vinyl acetate were not influenced by this compound (detailed data not shown). This is in contrast to results with volatile chemicals which are biotransformed via monooxygenases (Siegers et al. 1978), and is additional proof for the view that monooxygenases do not play a major role in metabolism of vinyl acetate in vivo.

Simultaneously with the atmospheric decline of vinyl acetate in the experimental system, we observed an exhalation and transient increase in the system of acetaldehyde, the metabolic intermediate. Two characteristic experiments are shown in Fig. 4.

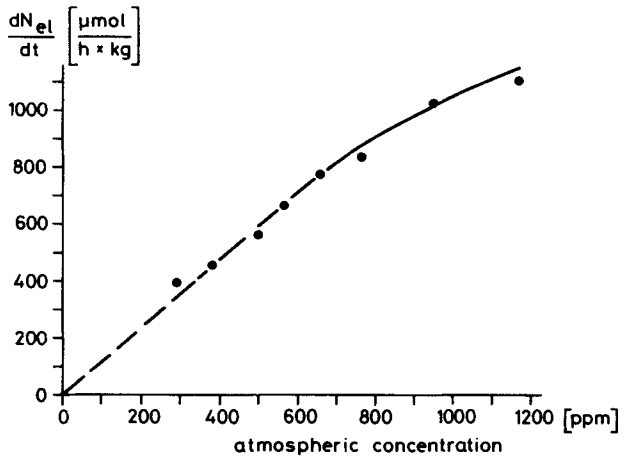

Fig. 3. Dependence of the metabolic elimination rate of vinyl acetate, dN_{el}/dt, on the atmospheric exposure concentration. The figure refers to conditions of "open exposure" to constant atmospheric concentrations of the chemical (i.e. $V_I \rightarrow \infty$, see Bolt et al. 1981)

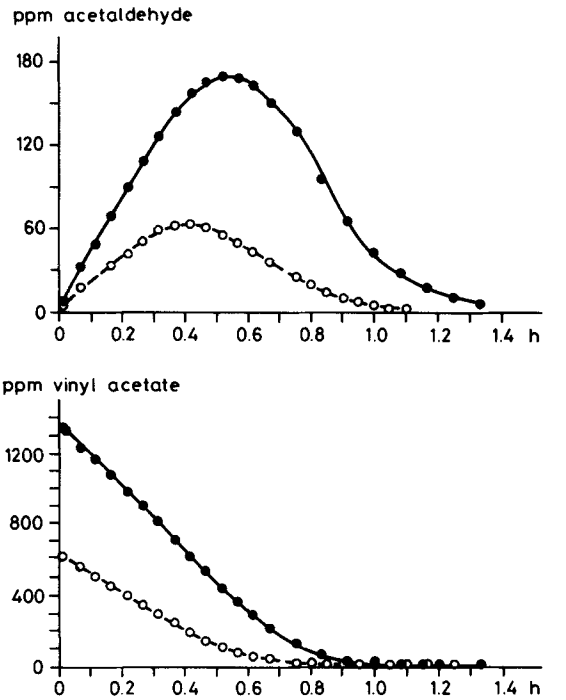

Fig. 4. Exhalation of acetaldehyde in two experiments (same conditions as in Fig. 2). *Bottom panel:* concentrations of vinyl acetate in the gas phase of the closed system (different initial concentrations). *Top panel:* concentrations of acetaldehyde in the gas phase in the same two experiments

Discussion

The pharmacokinetic studies in rats show that the metabolism of vinyl acetate, in principle, is saturable. From the V_{max} obtained in vitro with rat plasma (0.56 µmol/min per mg protein) a maximal capacity to hydrolyze vinyl acetate of the total plasma volume per kg rat body weight can be calculated to be about 14 000 µmol/h. Such a metabolic rate can practically not be reached in vivo, even at high vinyl acetate exposures (compare with Fig. 3). This means that, on one hand, the metabolic rate of vinyl acetate should not be limited in vivo by the capacity of metabolizing enzymes. On the other hand, the pulmonary uptake of the compound can be limited by the ventilation rate. This is confirmed by the maximal total clearance value (see Results section) of 30 000 ml/h per kg body wt., which is nearly identical with the maximal ventilation rate in rats, of 32 000 ml/h per kg body wt. (Guyton 1947).

Vinyl acetate, in concentrations between 0.1 and 1 mM, has been reported to induce sister-chromatid exchanges (SCE) in isolated human lymphocytes and in Chinese hamster ovary cells (Norppa et al. 1983). Because such effects were also induced by acetaldehyde, it has been suggested that the SCE observed with vinyl acetate could be elicited by acetaldehyde as metabolite (Norppa et al. 1983). The present data confirm the formation of this metabolite of vinyl acetate in vivo and in vitro.

The data demonstrate that most of the vinyl acetate entering the organism is very rapidly metabolized by ester-

ases, mainly in the blood. Monooxygenases ought to have only aminor role if any in metabolising the compound. Because their presumable product, the epoxide of vinyl acetate, has been theoretically predicted to be genotoxic (Jones and Mackrodt 1983), further studies will be focussed on this probably minor pathway to establish its metabolic and toxicological relevance.

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Appendix

Approximation of the metabolic rate from the total clearance of the compound

The integration of individual experimental concentration/time curves (as in Fig. 2) leads to "Areas Under the Curve" (AUC). The quotient D/AUC (D = dose applied) is equal to the mean total clearance of the compound which refers to the entire period between $t=0$ (when a steady-state is not yet existent) and $t=\infty$ (when all compound in the system is metabolized). This experimental mean total clearance equals the clearance obtained for the condition of exposure to the mean concentration (between $y_{l\,(0)}$ and $y_{l\,(8)}=0$ in an "open" system, i.e. $V_l \to \infty$; Filser and Bolt 1981; Bolt et al. 1981). The proof for this statement is derived by Filser (1985). This means

$$\frac{D}{AUC} = \lim_{V_l \to \infty} Cl_{tot} \tag{1}$$

with

$$D = y_{l(0)} \cdot V_l. \tag{2}$$

Clearance values obtained for dissimilar experimental conditions (as to number and weights of animals) can be adjusted, by analogy to the procedure given in the Appendix of Filser and Bolt (1983), to "standard" conditions two rats of 500 g each, i.e. $n'=2$; $V'_2 = 1000$ ml):

$$\lim_{V_l \to \infty} Cl'_{tot} = \frac{\frac{D}{AUC} \cdot n' \cdot \left(\frac{V'_2}{n'}\right)^{2/3}}{n \cdot \left(\frac{V_2}{n}\right)^{2/3}} \tag{3}$$

The product of this clearance and the mean concentration prevailing throughout the experiment gives the metabolic elimination rate (for conditions of exposure in an "open" system, $V_l \to \infty$). Practically, the mean concentration was approximated by half of the initial concentration:

$$\frac{y_{l(0)}}{2} \cdot \lim_{V_l \to \infty} Cl'_{tot} \approx \frac{dN_{el}}{dt} \tag{4}$$

The dN_{el}/dt thus obtained refers to "standard conditions" two animals, 500 g each) and conditions of continuous exposure of these animals, in an "open" system ($V_l \to \infty$) to the concentration given by $y_{l(0)}/2$.

Different sets of experiments (different $y_{l(0)}$) resulted in the values of dN_{el}/dt which are compiled in Fig. 3.

In Fig. 3 (calculated for "open" exposure) a saturation of metabolism is visible only at atmospheric concentrations of vinyl acetate higher than 750 ppm, whereas the experimental figures ("closed" system, Fig. 2) show a deviation from exponential declines above 200 ppm vinyl acetate in the system. This is due to a different accumulation of the compound in the organism under both conditions; the metabolic rate is ultimately determined by the concentration in the metabolizing tissues and not by that in the atmosphere. It has been previously shown (Filser and Bolt 1981) that the dynamic distribution ratio, K_{st}, is always higher in a "closed " than in an "open" exposure system because of the continuous decline of the concentration in the gas phase (y_l). This means that at the same actual y_l the concentration in tissues (y_2) is higher in a closed compared to an open system. Consequently, saturation phenomena become visible in a closed system at lower y_l than in an open exposure system because metabolism depends on the tissue concentration. This must be remembered when comparing Fig. 2 and 3.

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