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THE FATE OF THE ETHANOL ANALOGUE 1,3-BUTANEDIOL IN THE DOG

AN *IN VIVO–IN VITRO* COMPARISON

G. MÜNST, M. M. RIS-STEIGER, R. L. GALEAZZI, J. P. VON WARTBURG and J. BIRCHER
Departments of Clinical Pharmacology, Medical Chemistry and Medicine, University of Berne, Murtenstrasse 35, 3010 Berne, Switzerland

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Abstract—In view of the current interest in 1,3-butanediol as food additive or potential drug its pharmacokinetics have been investigated in the dog and compared to its oxidation *in vitro* by alcohol dehydrogenase. Plasma disappearance of i.v. doses of 5.5 mmol/kg were zero order followed by first order. Assuming Michaelis–Menten kinetics a V_{max} of 1.23 ± S.D. 0.27 μmol/min/g of liver and a K_m of 1.15 ± 0.85 mM could be calculated. The corresponding values for 1,3-butanediol metabolism by alcohol dehydrogenase *in vitro* were 1.62 ± 0.34 μmol/min/g of liver and 5.11 ± 1.45 mM. Hepatic vein catheterizations were used to measure hepatic blood flow (18.1 ± 2.8 ml/min/kg) and the fraction of butanediol disappearing in the liver, which was only 34.2 ± 6.6 per cent. Compared to ethanol, V_{max} of 1,3-butanediol was 15 per cent smaller *in vitro*, 45 per cent smaller *in vivo*, K_m was 3 times larger *in vitro* and 60 per cent smaller *in vivo*. The splanchnic elimination fraction of 1,3-butanediol was about 1/2 the one of ethanol. These data are consistent with the concept, that oxidation by alcohol dehydrogenase is the major route of butanediol elimination. The differences between 1,3-butanediol and ethanol metabolism, however, render different pharmacological and toxicological effects likely.

1,3-Butanediol has gained increasing interest in the past few years, because it may be used as an alternative source of calories or may serve as bacteriostatic food additive [1]. Compared to ethanol (Table 1) 1,3-butanediol is less lipophilic and thus may have more difficulties in penetrating cell membranes. Moreover, on a weight basis butanediol is only about half as toxic as ethanol. In rats, butanediol metabolism is inhibited by pyrazole, the classical alcohol dehydrogenase (ADH) inhibitor, and its primary metabolite is β-hydroxybutyraldehyde [2]. As in ethanol [3], butanediol causes an increase in the

Table 1. Characteristics of ethanol and 1,3-butanediol

Parameter	Ethanol	1,3-Butanediol	Ref.*
Physico-chemical properties:			
Molecular composition	C_2H_6O	$C_4H_{10}O_2$	
Molecular weight	46.07	90.12	
Density (d^{20})	0.789	1.004	
Boiling point	78.5°	207.5°	
Miscible with:	Water, acetone, hexene, benzene, toluene	Water, acetone	†
Not miscible with:	–	Hexane, benzene, toluene	†
Partition coefficient membrane/buffer	0.096	0.060	8
Pharmacological properties:			
Toxicity:			
LD_{50} mg/kg rat			
p.o.	13,660	29,420	9
i.p.	5000	10,000	9
Anesthetic dose mg/kg rat	3000	7000	9
Nutritional properties:			
Calories/g	7.0	6.5	4

* Where no reference is given, data were obtained from Merck Index, Ninth Edition, 1976.
† Data confirmed in our laboratory.

cytosolic NADH/NAD ratio in liver [4, 5] and concomitantly a rise in the concentration ratios of β-hydroxybutyrate/acetoacetate as well as lactate/pyruvate [6]. Furthermore, in the rat the acute ethanol withdrawal syndrome could be counteracted by butanediol [7, 8]. In dogs after oral butanediol doses of 1–2 g/kg no clinical signs of ill health, apart from a slight decrease of blood pressure, could be observed [9].

In view of these slightly different properties, 1,3-butanediol seems to be an interesting model substance to study alcohol metabolism, particularly since its metabolic endproduct is β-hydroxybutyrate, a naturally occurring metabolite in fatty acid oxidation, and since its lower toxicity seems to make a therapeutic application of relatively large doses possible, e.g. as an antiwithdrawal agent. The fate of pharmacological amounts of butanediol in the intact organism and its pharmacokinetics, however, have not as yet been investigated. Experiments were, therefore, designed to follow plasma concentrations after single intravenous injections in dogs. Since liver ADH is presumably involved in butanediol disposition, its activity was measured in liver homogenates with this substrate. Ethanol was taken as reference compound. The results show that butanediol is metabolized by hepatic ADH, but that other routes of metabolism, such as extrahepatic elimination, may be more important than in the case of ethanol. Preliminary reports of these studies have been presented [10, 11].

MATERIALS AND METHODS

Animals

Female dogs (3 boxers, 2 mongrels, 1 labrador and 1 shepherd) of 21–34 kg body weight were used. They were apparently in good health and had normal plasma alkaline phosphatase and aspartate aminotransferase. They had ordinary dog food consisting of "Flatazor soupe" three times and Hodog twice weekly from Hokovit (Bützberg, Switzerland), in addition alternating Tapsy Rondellen from A.G. für Tiernahrung (Hinwil), Crocan from Nährkosan (Reitnau) and Hundebiskuit from Nugat Kraftfutterwerke, Heider (Elgg, Switzerland). Throughout the 12 month period of the experiments one dog (Filax) gained 6 kg but the rest maintained a constant body wt. For the experiments the dogs were fasted over night but water was allowed *ad lib.* An interval of at least 2 weeks was allowed between experiments.

Chemicals. 1,3-butanediol was purchased from Fluka, AG (Buchs, Switzerland); ethanol and *n*-propanol from Merck (Darmstadt, West Germany), NAD (nicotinamide adenine dinucleotide) and horse liver alcohol dehydrogenase from Boehringer GmbH (Mannheim, West Germany), Indocyanine green (ICG) from Hynson, Westcott & Dunning (Baltimore, MD), ^{125}I human serum albumin from Tschäppeler AG (Zürich, Switzerland). All additional chemicals were purchased from Fluka or Boehringer.

In vivo *experiments*

Butanediol and ethanol plasma disappearance curves. The unanesthetized dog was placed in a sling and a polyethylene catheter was inserted in a leg vein. Isotonic saline was infused at a rate of about 1.5 ml/min to a total amount of about 500 ml in order to maintain urine flow and to keep the venous catheter open. The urinary bladder was catheterized for the time of the experiments.

1,3-butanediol or ethanol in a dose of 5.5 mmoles/kg body wt (=0.5 and 0.25 g/kg respectively) at a concentration of about 50 per cent in normal saline was given through the catheter over a period of 5 min. No pharmacological effect was experienced with either alcohol. Blood samples (2 ml) for the determination of butanediol and ethanol concentrations were drawn from the catheter into heparinized tubes at time zero and at 30 min intervals for 6 hr. They were immediately centrifuged. Butanediol and ethanol concentrations were also determined in urine collected during the experimental period.

To test the effect of fructose a priming dose of 10 g (55.5 mmoles) of fructose was given i.v. within 10 min and followed by an infusion of fructose at a rate of about 1.5 mmol/min by means of a constant infusion pump to a total volume of about 500 ml. Thirty minutes after the onset of fructose administration the same experimental protocol was applied as above except for the dose of the test compounds which was 8.3 mmoles/kg b.w. (0.75 and 0.38 g/kg b.w.) of butanediol or ethanol, respectively.

In two experiments blood samples were also drawn from a second venous catheter for the determination of fructose in blood, at 60, 120, 180 and 240 min.

Estimation of splanchnic elimination of butanediol and ethanol. Through a polyethylene catheter placed in a leg vein the dogs were anesthetized with sodium pentobarbital (20–30 mg/kg b.w. in an aqueous solution of 2.5 per cent). Supplements of about 1 mg/kg were given when signs of restlessness appeared. A polyethylene catheter was then inserted into a femoral artery. A left or right hepatic vein was also catheterized by passing a nylon cardiac catheter (Cournand No. 7 or 8) from a jugular vein through the right atrium and the inferior vena cava under X-ray control. The catheter was advanced to wedge position, then withdrawn just enough to permit free sampling of blood, thereby preventing accidental sampling from the inferior vena cava. Indocyanine green (ICG) (Cardio Green®) 12.9 nmoles (10 mg) stabilized in a solution of about 30 ml of dog serum, and made up to 200 ml with isotonic saline, was administered i.v. by means of a constant infusion pump at a rate of 35 to 50 ng/min. After an equilibration period of 60 min 2 ml blood samples were drawn simultaneously at 10 min intervals from the femoral artery and the hepatic vein for the determination of plasma concentrations of ICG and of butanediol or ethanol. With the start of the ICG infusion butanediol or ethanol (5.5 mmol/kg) were administered i.v. over a period of 5 min diluted to about 50 per cent with saline. To determine the plasma volume ^{125}I human serum albumin (0.5 μCI) was injected and three arterial blood samples were drawn at 10 min intervals.

Assessment of alcohol dehydrogenase activity and autopsy. At the end of the *in vivo* studies all dogs were anesthetized with sodium pentobarbital as above. A laparotomy was performed and a 3–5 g

specimen of liver was excised, immediately cooled in an ice bath and analysed the same day. Thereafter the dogs were killed by an overdose of pentobarbital, the whole liver was removed and weighed. In addition to the liver, the other internal organs were autopsied macroscopically. No disease was found in any of the dogs.

Analytical procedures

In vitro *determination of alcohol dehydrogenase (ADH) activity*. One part of dog liver (1 g) and 2 parts of sodium pyrophosphate buffer 20 mM pH 8.8 were homogenized in a glas-glas Potter Elvehjem homogenizer and centrifuged for 1 hr at 40,000 rpm corresponding to 100,000 *g*. Dog liver ADH activity was measured in the clear supernatant by a spectrophotometric assay of the rate of NADH formation at 334 nm and 37°. The standard assay mixture contained: 30 µl NAD 50 mM, 850 µl sodium pyrophosphate buffer pH 6.8 100 mM and 100 µl of water or one of the alcohols in a concentration varying from 0.5 to 13.5 mM for ethanol and from 0.5 to 33.3 mM for butanediol, respectively. The reaction was initiated by adding 20 µl of the supernatant. The activity was then calculated as the difference in the absorption A/min of the two reactions, with and without one of the alcohols.

1,3-butanediol was determined enzymatically with horse liver alcohol dehydrogenase (ADH): 20 µl of plasma were added to 0.5 ml of the following assay mixture: 0.84 ml NAD 50 mM, horse liver ADH adjusted to an activity of 10 µmol/min, sodium pyrophosphate buffer 100 mM pH 8.8 containing 1.5 g semicarbazide/100 ml made up to a volume of 25 ml. Differences in absorption at 334 nm were measured with an Eppendorf photometer before and after incubation for 60 min at 37°. Standard curves were linear between 0–8 mM. Urine samples were determined under identical conditions.

Ethanol was determined by gas–liquid chromatography using the headspace technique. One ml of plasma together with 1 ml of NaCl (0.9 per cent) containing *n*-propanol as internal standard and perchloric acid (0.1 ml, 70 per cent) were added to 20 ml vials and sealed. After heating the vials for 30 min at 60° 5 ml of the headspace were injected into a Perkin–Elmer 900 gas chromatograph.

Operating conditions: glass column, 2 mm I.D., 9 ft., loaded with Porpak Q 80/100 mesh (Waters Ass. Inc., Milford, MA), flame ionisation detector; temperatures: inlet 190°, column 150°, detector 250°; gasflow rates: nitrogen (carrier): 40 ml/min, hydrogen: 30 ml/min, air: 350 ml/min. A linear calibration curve in the range of 1–20 mM was obtained by adding appropriate amounts of ethanol to 1 ml samples of dog plasma.

To measure indocyanine green blood samples (3 ml) were centrifuged and plasma concentrations were determined photometrically against a preinfusion blank at 800 nm on a Beckman DU spectrophotometer.

^{125}I human serum albumin radioactivity was counted in plasma with a Gamma-Counter (Packard).

Fructose was determined enzymatically in blood by means of a kit from Boehringer GmbH (Mannheim, West Germany).

Calculations

Plasma disappearance curves. The extrapolated volume of distribution ($V_{d,extr}$) of butanediol and ethanol was calculated as the ratio of the i.v. dose to the venous concentrations obtained by linear extrapolation to time zero. Saturation kinetics were assumed to explain the plasma disappearance curves of both alcohols according to the following formula

$$v = \frac{V_{max} \cdot C_p}{K_m + C_p},$$

where C_p represents the peripheral venous concentration at time t and v the rate of removal of the alcohol from the volume of distribution at time t, V_{max} the maximal rate and K_m the apparent Michaelis constant. For each period of observation v was calculated as follows

$$v = \frac{\Delta c}{\Delta t} \cdot V_{d,extr}.$$

V_{max} and K_m were then computed non-linearly on a Hewlett Packard 9810 A desk calculator according to Wilkinson [12] in an iterative way until stability of both parameters was attained. The elimination rates (v) were weighted by the reciprocal of their variances.

Computerized non-linear least squares best fit for plasma disappearance curves. Many of the concentration vs time curves of both, ethanol and 1,3-butanediol, showed an apparent "distribution phase" at the early time points. We, therefore, tried to fit the data to a set of two differential equations representing a two compartment body model with first order intercompartmental rate constants (k_{12} and k_{21}) and Michaelis–Menten type elimination from the central compartment.

$$\frac{dC_p}{dt} = \frac{V_{max} \cdot C_p}{K_m + C_p \cdot V_1} - k_{12}C_p + k_{21}\frac{T}{V_1},$$

$$\frac{dT}{dt} = + k_{12}P - k_{21}T,$$

where C_p = plasma concentration of ethanol or butanediol; T = amount of drug in the peripheral compartment; P = amount of drug in the central compartment; V_1 = volume of distribution of the central compartment; $V_{d,extr}$ could then be calculated as

$$V_{d,extr} = V_1\left(1 + \frac{k_{12}}{k_{21}}\right).$$

The parameters (V_1, V_m, K_m, k_{12}, k_{21}) were estimated using a non-linear regression program for two differential equations kindly supplied by C. Peck [13].

Splanchnic elimination of butanediol and ethanol. The estimated hepatic blood flow (EHBF) was calculated with ICG according to the constant infusion and extraction procedure and appropriate correction for the hematocrit [14].

The plasma volume (PV) was estimated as the volume of distribution of ^{125}I human serum albumin. The splanchnic elimination rate (ER) of the alcohols was calculated by multiplying the arterio-hepatic venous plasma concentration differences with the

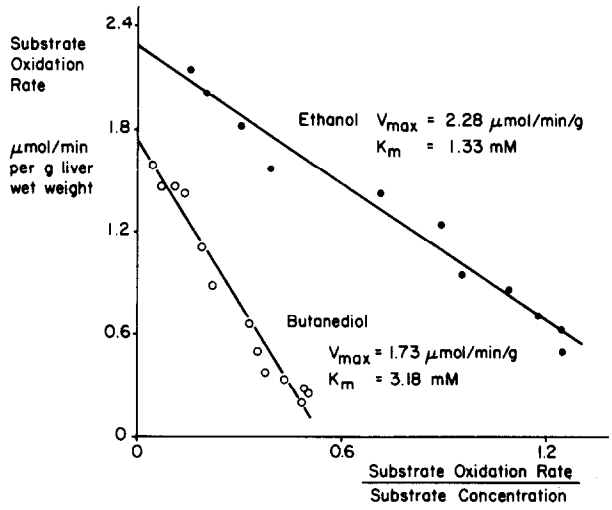

Fig. 1. Assessment of hepatic ADH activity in one dog (Filax) with butanediol and ethanol as substrates. The velocity of the reaction has been plotted against the ratio of the velocity to the corresponding substrate concentration. Therefore, the intercept on the ordinate represents V_{max} and the slope K_m. The lines have been calculated from the raw data according to Wilkinson as detailed in methods.

estimated hepatic blood flow. This procedure appeared justifiable, since the blood to plasma ratio of butanediol and ethanol was found to be almost unity.

The total elimination rate (ER) was calculated from the pseudo-linear part of the elimination curve as

$$ER = \frac{\Delta C_p}{\Delta t} \cdot aV_{d,extr} \ (\mu moles/min),$$

where C_p represents the slope of the plasma disappearance curve and $aV_{d,extr}$ the apparent volume of distribution which was obtained by dividing the dose by the theoretical concentration at time zero obtained by extrapolation assuming zero order kinetics.

STATISTICS

Depending on the type of comparison the paired or the two sample student *t*-test was applied [15]. $P < 0.05$ was taken as the level for statistical significance.

RESULTS

Assessment of hepatic alcohol dehydrogenase (ADH) in vitro

ADH activity could be measured in 100,000 *g* supernatant of liver homogenates equally well with butanediol as substrate as with ethanol. Figure 1 shows a typical example of substrate saturation curves presented in the Eadie-Hofstee plot. Tables 2 and 3 show that V_{max} was somewhat higher for

Table 2. Apparent maximal rates (V_{max}) of butanediol and of ethanol metabolism *in vitro* and elimination *in vivo*. (Although the liver is not the only site of alcohol elimination, the values were corrected for liver weight in order to allow a direct *in vivo–in vitro* comparison. The corresponding values per kg body weight are given in Table 4)

		In vitro V_{max} (μmol min^{-1} g^{-1})		*In vivo* V_{max} (μmol min^{-1} g^{-1})			
				Without fructose*		With fructose†	
Dog	Liver weight (g)	Butanediol	Ethanol	Butanediol	Ethanol	Butanediol	Ethanol
Trüs	563	1.04	1.54	1.07			
Meta	595	1.44	1.61	1.22	2.06	1.97	
Mädi	330	1.54	1.81	1.43	1.91	2.61	4.39
Mara	590	1.56	1.44	1.72	1.34	2.36	3.23
Filax	631	1.78	2.28	0.96	1.81	1.73	2.07
Anita	576	1.95	2.00	0.99			
Rex	592	2.04	2.38	1.24			
$\bar{x}$ ± S.D.	554 ± 101	1.62 ± 0.34	1.87 ± 0.37‡	1.23 ± 0.27‖	1.78 ± 0.31‡	2.17 ± 0.39§‖	3.23 ± 1.16

* The dose of both alcohols was 5.5 mmol/kg body wt.
† Continuous fructose infusion of 1.4 mmol/min. The dose of both alcohols was 8.3 mmol/kg b.w.
‡ Significantly different from corresponding experiment with butanediol ($P < 0.05$).
§ Significantly different from experiment without fructose ($P < 0.01$).
‖ Significantly different from experiment with butanediol *in vitro* ($P < 0.05$).

Table 3. Apparent half saturation constants (K_m) for butanediol and ethanol metabolism *in vitro* and elimination *in vivo*

	K_m (mM)					
	In vitro		*In vivo*			
			Without fructose*		With fructose*	
Dog	Butanediol	Ethanol	Butanediol	Ethanol	Butanediol	Ethanol
Trüs	4.95	1.67	0.94			
Meta	6.89	2.52	1.69	2.48	1.67	
Mädi	4.87	1.79	0.43	1.60	1.45	4.05
Mara	7.24	1.46	2.82	0.95	2.80	2.99
Filax	3.48	1.32	0.41	2.41	1.40	2.32
Anita	3.71	1.39	0.78			
Rex	4.63	1.84	0.98			
$x \pm$ S.D.	5.11 ± 1.45†	1.71 ± 0.40	1.15 ± 0.85	1.86 ± 0.73‡	1.83 ± 0.66‡	3.11 ± 0.88

* Details as in Table 1.

† Significantly different from corresponding experiment with ethanol and from experiments with butanediol *in vivo* ± fructose ($P < 0.001$).

‡ Not significantly different from *in vivo* experiments with butanediol without fructose ($P > 0.05$).

ethanol than for butanediol with one exception (Mara). The interindividual variations in V_{max} were larger with butanediol than with ethanol. The K_m values were about three times higher for butanediol than for ethanol ($P < 0.001$).

Butanediol and ethanol loading tests in vivo

When 5.5 mmoles/kg b.w. of butanediol or ethanol were injected i.v. the plasma concentrations of butanediol generally were lower than those of ethanol. Nevertheless, both alcohols seemed to disappear from plasma as a zero order function until the plasma concentrations approached 1 mmol. Then the disappearance curves seemed to change to a first order process (Fig. 2).

The mean urinary output of butanediol and of ethanol were below 2 and 1 per cent of the dose, respectively. The volumes of distribution were, therefore, obtained from the dose and the extrapolated concentrations at time zero. As shown in Table 3 they tended to be higher for butanediol than for ethanol, where a figure of 0.74 ± 0.19 l/kg was found which is in agreement with published data [16–18].

In order to compare the data obtained *in vivo* with ADH activity *in vitro*, actual elimination rates were estimated for each period of observation by taking the product of the volume of distribution and the difference in concentration. Evaluating these rates together with the mean plasma concentrations for each period V_{max} and apparent K_m were computed according to Wilkinson [12]. Furthermore, the non-linear repression of the data to the differential equations representing a two compartment body model with Michaelis–Menten type of elimination

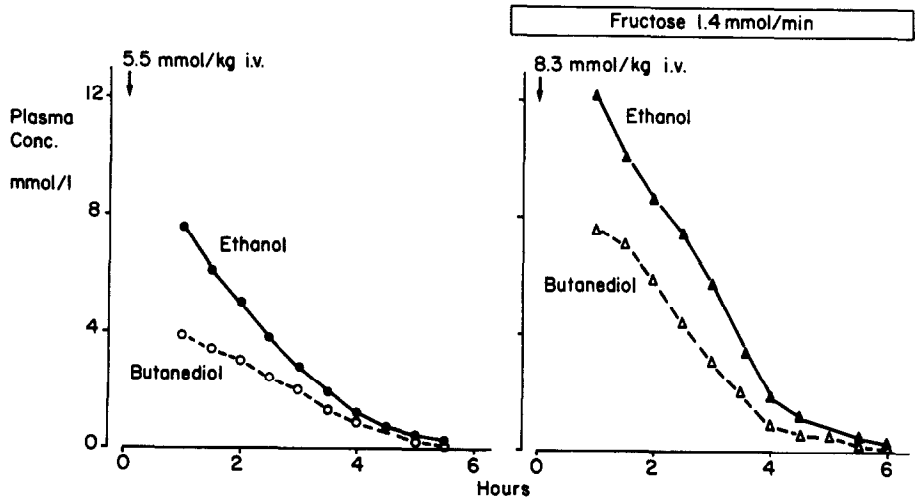

Fig. 2. Plasma concentration time curves in one dog (Filax). The left panel shows the experiments without fructose and the right panel with fructose infusion which had been started 30 min before the administration of the alcohols.

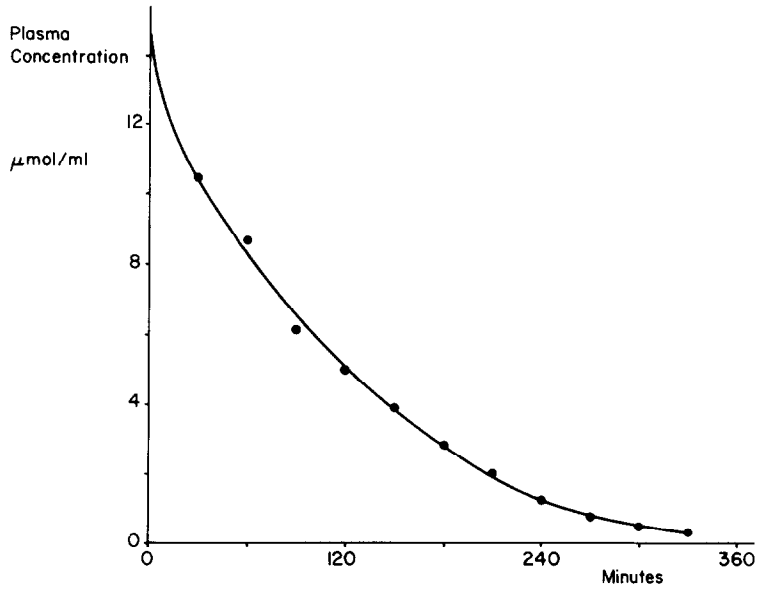

Fig. 3. Computerized non-linear least squares best fit for plasma disappearance curve in one dog (Filax) after i.v. administration of 188.89 mmoles ethanol. For details see Methods.

kinetics gave excellent results, judged by the method of residuals [19]. An example of this analysis is shown in Fig. 3. This approach fully confirmed the estimates for the relevant kinetic parameters obtained by the procedure of Wilkinson [12]. Data presentation, therefore, has been limited to the latter method (Tables 3 and 4).

If the liver weight is used as standard of reference (Table 2) V_{max} values obtained *in vivo* were of the same order of magnitude as those found for ADH *in vitro*. Again V_{max} was slightly lower for butanediol than for ethanol, with one exception (Mara). Interestingly, *in vivo* there was no difference in apparent K_m between ethanol and butanediol, the latter tending to be even lower than the former. Thus, the apparent K_m for butanediol calculated from the *in vivo* experiments was significantly lower ($P < 0.05$) than the one resulting from liver ADH determination.

Effect of fructose on butanediol and ethanol elimination

When fructose was infused, blood fructose concentrations ranged between 6.71 and 3.35 mmol/l. In each instance the maximal rates of butanediol and of ethanol elimination were increased by fructose ($P < 0.01$ for butanediol). It is noteworthy that the effect of fructose appeared to be more pronounced for ethanol than for butanediol and one dog (Mara) had a higher V_{max} with ethanol than with butanediol. As shown in Table 3, also the apparent K_m values tended to be higher during the fructose studies, although the differences were not significant.

Estimation of splanchnic elimination of butanediol and of ethanol

A representative study carried out in dog Filax is shown in Fig. 4. After equimolar doses of butanediol and of ethanol the plasma concentrations of butanediol were again lower than those of ethanol. In view of the general anesthesia and the limited duration of those studies, total elimination of the alcohols had to be calculated from the pseudolinear phase of the disappearance curve. Splanchnic removal was estimated as product of blood flow and arterio-hepatic venous concentration difference which averaged 0.42 mmol/l for butanediol and 0.95 mmol/l for ethanol in this dog. Hepatic blood flow estimated with the ICG infusion and extraction technique amounted to 18.4 and 16.8 ml/min/kg, respectively and, therefore, could not explain the smaller arterio-hepatic venous concentration difference observed with butanediol.

As shown in Table 5, also the other dogs showed analogous results. Despite a similar total elimination rate for butanediol and ethanol (27.5 and 33 μmol/min/kg, respectively, $P > 0.05$), splanchnic removal was significantly smaller in the case of butanediol (9.2 μmol/min/kg) than in experiments with ethanol (22.1 μmol/min/kg). Thus, the fraction of butanediol being eliminated by the splanchnic organ (34.2 per cent) was smaller than the corresponding fraction of ethanol (66.4 per cent, $P < 0.01$).

In vivo–in vitro *comparison*

Although removal of butanediol or ethanol by the liver has not been assessed in these studies it was felt that V_{max} corrected for the extrahepatic alcohol removal represents the most appropriate value to be compared with ADH assays obtained from the same liver *in vitro*.

As shown in Fig. 5 maximal splanchnic ethanol removal was similar to V_{max} for ADH only in one dog (Meta) (*in vivo* 1.77, *in vitro* 1.6 μmol/min/g of liver), whereas all other experiments with butanediol and ethanol showed that V_{max} *in vivo* was 2–7 times smaller than maximal ADH activity *in vitro*. This

Table 4. Apparent maximal rates (V_{max}) of butanediol and ethanol elimination and volumes of distribution (V_d) *in vivo** (All values are expressed per kg of body weight)

Dog	Body weight (kg)	V_{max} (μmol min $^{-1}$ kg^{-1})				V_d (l. kg^{-1})			
		Without fructose		With fructose		Without fructose*		With fructose*	
		Butanediol	Ethanol	Butanediol	Ethanol	Butanediol	Ethanol	Butanediol	Ethanol
Trüs	25	24.2				0.95			
Meta	21	34.6	58.5	55.7		0.86	0.82	1.12	
Mädi	21	23.0	30.1	41.1	69.0	1.05	0.59	0.76	0.62
Mara	21	48.3	36.2	66.3	90.6	0.90	0.97	1.12	0.89
Filax	28–34†	21.6	33.5	38.9	38.4	1.12	0.59	0.80	0.65
Anita	28	20.4				0.79			
Rex	31	23.6				0.90			
$\bar{x}$ ± S.D.	25.9 ± 5.3	28.0 ± 10.1	39.6 ± 12.9§	50.5 ± 12.9‡	66.0 ± 26.2§	0.94 ± 0.11	0.74 ± 0.19§	0.95 ± 0.20	0.72 ± 0.15§

* Experimental conditions as in Table 1.
† Changes in body weight within 10 months. The weight of the day was used for each experiment.
‡ Significantly different from experiments with butanediol, without fructose ($P < 0.02$).
§ Not significantly different from corresponding experiment with butanediol.

Table 5. Data from experiments with liver vein catheterization which were used to calculate splanchnic and total elimination rates (ER) for butanediol and for ethanol

	Indocyanine—green						Butanediol			
Dog	Extraction ratio (%)	$\bar{a}$† μg/100 ml	Hepatic removal μg/100 ml	Clearance ml min^{-1} kg^{-1}	Plasma volume ml kg^{-1}	EHBF* ml min^{-1} kg^{-1}	$\bar{a} - h\bar{v}$ Difference† μmol ml^{-1}	Splanchnic ER μmol min^{-1} kg^{-1}	Total ER μmol min^{-1} kg^{-1}	Splanchnic elimination fraction (%)
Rex	34.0	64	50	2.46	49	14.4	0.75	10.8	27.3	39.5
Mädi	21.6	84	41	2.37	42	21.1	0.46	9.2	26.0	39.6
Anita	30.5	62	51	2.84	37	18.4	0.46	8.5	26.6	31.9
Filax	18.1	87	43	1.78	40	18.4	0.42	7.8	30.2	25.9
$x \pm$ S.D.		74 ± 13	46 ± 5	2.38 ± 0.46	42 ± 5	18.1 ± 2.8	0.52 ± 0.15	9.23 ± 1.34	27.5 ± 1.86	34.2 ± 6.6
							Ethanol			
Meta	22.2	74	50	3.18	53	28.2	1.28	36.1	44.2	85.7
Mara	13.4	85	53	2.96	53	34.1	0.54	18.4	33.5	55.0
Mädi	14.2	106	61	2.76	43	33.0	0.54	17.8	30.1	59.3
Filax	15.8	106	50	1.39	44	16.8	0.95	16.0	24.4	65.5
$x \pm$ S.D.		93 ± 16‡	53 ± 5‡	2.57 ± 0.81‡	48 ± 6‡	28.0 ± 7.9‡	0.83 ± 0.36‡	22.1 ± 9.4§	33.1 ± 8.3‡	66.4 ± 13.6‖

* Estimated hepatic blood flow.
† Mean plasma concentration in arterial ($\bar{a}$) or hepatic venous ($h\bar{v}$) blood.
‡ Not significantly different from experiments with butanediol ($P > 0.05$).
§ Significantly different from experiments with butanediol ($P < 0.05$).
‖ Significantly different from experiments with butanediol ($P < 0.01$).

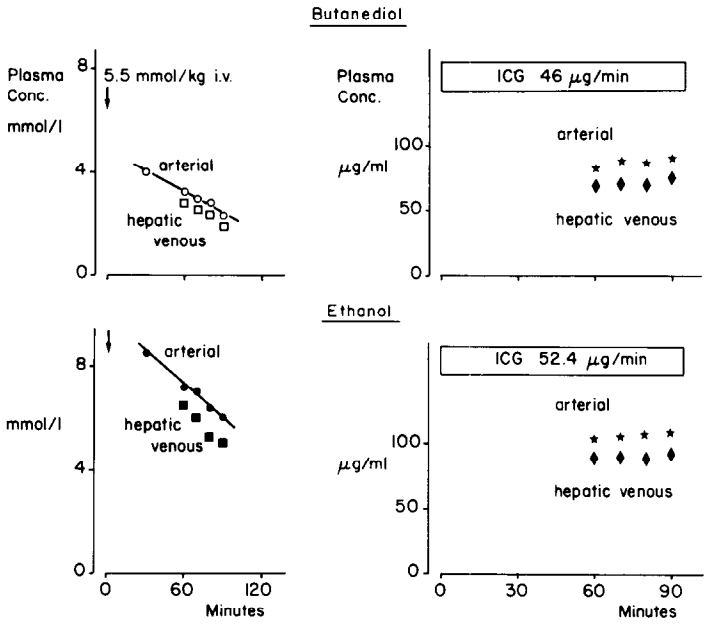

Fig. 4. Determination of splanchnic removal of butanediol and ethanol in one dog (Filax). On the left panel arterial (○ ●) and hepatic venous (□ ■) plasma concentrations of butanediol and of ethanol are represented against time. On the right side arterial (★) and hepatic venous (◆) concentrations of indocyanine green are shown, which were used to estimate hepatic blood flow.

discrepancy was much reduced in the experiments with fructose. Although few in numbers, they show a better *in vivo–in vitro* correspondence for ethanol and one of the studies with butanediol. The other study with butanediol in one dog (Filax) showed a maximal ADH activity which was four times larger than the maximal splanchnic butanediol removal *in vivo*.

DISCUSSION

These studies confirm that butanediol can serve as a substrate for liver ADH. Compared to ethanol the *in vitro* V_{max} of dog liver ADH tends to be smaller for butanediol and K_m larger. Nevertheless, the order of magnitude of both values suggests that also *in*

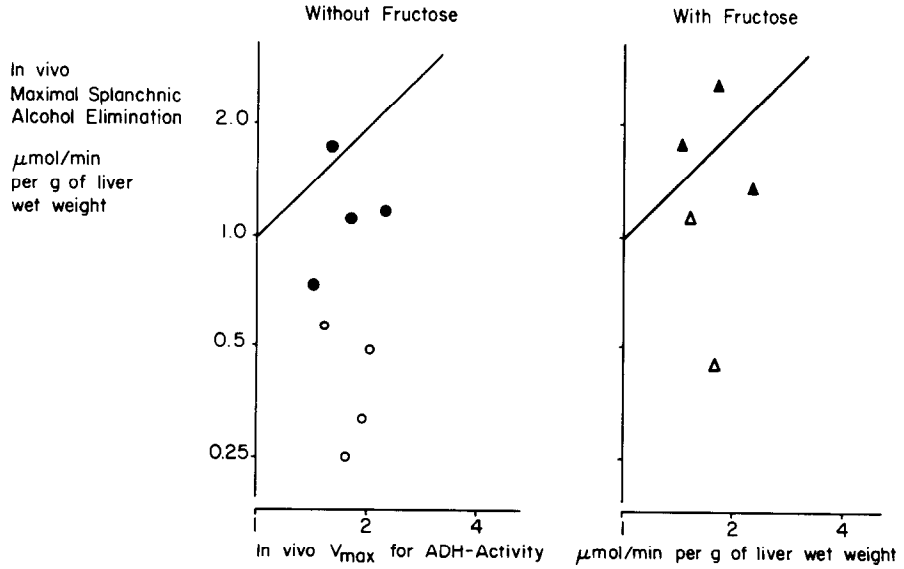

Fig. 5. *In vivo–in vitro* comparison of butanediol and ethanol metabolism. The abscissa shows maximal rates of ADH activity measured in 100,000 *g* supernatants. The ordinate represents maximal alcohol removal obtained from Table 1, but reduced to the splanchnic fraction indicated in Table 4. The straight lines are identity lines. In experiments without fructose only one experiment with ethanol showed a satisfactory *in vivo–in vitro* agreement. Despite fructose infusion one dog (Filax) metabolized butanediol in splanchnic organs only at about 1/4 of the rate he could theoretically achieve with the ADH content of his liver.

vivo butanediol metabolism could at least in part be mediated by ADH.

The analogies between butanediol and ethanol were also evident *in vivo*. Similar rate limiting processes are suggested, because butanediol disappears from plasma by apparent zero order kinetics over a large range of butanediol plasma concentrations. While, analogously to the *in vitro* situation, the V_{max} for butanediol is lower than for ethanol, the apparent K_m's are similar for both alcohols.

Several parts of this study, however, point to important differences between butanediol and ethanol. Butanediol has a larger apparent volume of distribution. In contrast to ethanol, the apparent K_m computed from *in vivo* studies with butanediol is significantly lower than the K_m for liver ADH *in vitro*. The latter finding is difficult to interpret, because *in vivo* many biochemical and physiological factors may influence the apparent K_m thereby rendering an *in vivo–in vitro* comparison difficult. Most of these factors (e.g. diffusion limitation, flow limitation etc.) tend to increase the apparent K_m *in vivo* and, therefore, cannot be used to explain the finding that the K_m was at least 4 times lower *in vivo* than *in vitro*. If, however, it is assumed that another process than liver ADH is rate limiting for butanediol disposition *in vivo*, the K_m of this process may correspond to the apparent *in vivo* K_m found in our studies.

Since the V_{max} for butanediol *in vivo* is significantly lower than the one *in vitro*, the question arose, whether ADH could not fully display its activity. *In vivo*, the overall activity of ADH may be limited by the availability of the oxidized form of its coenzyme (NAD^+) which is present in excess, when measuring ADH activity *in vitro*. Fructose has been shown to accelerate alcohol metabolism [3, 20, 21]. Although its mode of action is not yet established in all details, it is generally agreed that by reactions of intermediary metabolites of fructose more NAD may be provided for the reaction of ADH. Accordingly, the metabolism of both alcohols was enhanced by fructose infusion although the extent of enhancement was smaller for butanediol than for ethanol. Surprisingly, the V_{max} values of both alcohols were much more elevated after fructose infusion than expected from the *in vitro* results, suggesting that acceleration of the NADH-reoxidation alone may be insufficient to explain the extent of the fructose effect.

In a living organism it is often difficult to localize the site of elimination of a foreign compound. In view of the many analogies with ethanol it was assumed that butanediol should be metabolized mostly in the liver. It was surprising, therefore, to find that only about 1/3 of the injected amount could be accounted for by splanchnic removal, whereas the corresponding fraction of ethanol was about 2/3 of the dose. In man, the splanchnic removal fraction also is estimated to amount to 60–80 per cent of the total ethanol elimination [22–24].

It should be realized that these figures are subject to some uncertainties. The hepatic vein which was catheterized may not be representative for mixed hepatic venous blood. Furthermore, the estimations of hepatic blood flow with indocyanine green may be less accurate in the dog than in man because in this species hepatic extraction of the dye is relatively low. Nevertheless, the obtained values were within the range of hepatic blood flow in the dog as assessed with other methods [25, 26]. Finally, anesthesia may have modified rates of metabolism of the alcohols. Despite these limitations the results obtained with ethanol are consistent with published similar studies [27, 28] thereby supporting the general approach.

In preliminary studies sites of extrasplanchnic butanediol removal other than the ones of ethanol have not been found. Homogenates of muscle, heart, lung, stomach, kidney, spleen and small intestine did not differ with respect to ADH activity whether ethanol or butanediol was used as substrate, neither did electrophoresis on starch gel of these homogenates reveal any differences when stained with the two substrates. Other mechanisms than oxidation by ADH, therefore, must be assumed. MEOS, the NADPH-dependent microsomal alcohol oxidizing system, is known to be capable of metabolizing butanol at about half the oxidation rate of ethanol [29, 30]. For catalase, on the other hand, butanol was not found to serve as substrate [31]. Whether MEOS and/or catalase, however, may contribute to 1,3-butanediol metabolism remains to be determined.

It is obvious that hepatic removal rates of butanediol should be compared with the results obtained using 100,000 *g* supernatant of liver homogenates. Since hepatic removal could not be measured *in vivo* the fraction disappearing in splanchnic organs was used to correct V_{max} values derived from peripheral venous plasma disappearance curves. This was thought to be the best approximation to a hepatic removal rate. Despite these efforts, the *in vivo–in vitro* correlation (Fig. 4) was satisfactory neither for butanediol nor for ethanol. Even if it is admitted that fructose infusion improved the agreement for ethanol, it corrected only one of the two experiments with butanediol. Thus the *in vitro–in vivo* comparison also suggests that the ADH may not be the rate limiting step for butanediol elimination.

If these studies are taken as a basis for clinical trials of comparable doses of butanediol in patients suffering from an alcohol withdrawal syndrome, the experimental design should allow for the apparent zero order kinetics. This is important because of the difficulties involved in the establishment of a steady state with a drug being eliminated in this manner. Dose titration by effect evaluations may be needed.

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