Biochemical Pharmacology, Vol. 35, No. 20, pp. 3457–3464, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

THE ACUTE *IN VITRO* EFFECT OF ETHANOL, ITS METABOLITES AND OTHER TOXIC ALCOHOLS ON ION FLUX IN ISOLATED HUMAN LEUCOCYTES AND ERYTHROCYTES

RACHEL J. GREEN and DENIS N. BARON
Department of Chemical Pathology and Human Metabolism, Royal Free Hospital and School of Medicine, Pond Street, London NW3 2QG, U.K.

(*Received* 23 *January* 1986; *accepted* 21 *March* 1986)

Abstract—Using isolated healthy human leucocytes and erythrocytes as model cells, we investigated the inhibitory effect of ethanol, its metabolites and of other toxic alcohols on the active fluxes of rubidium (Rb: equivalent to K) and sodium (Na), and on Na,K-ATPase activity. Ethanol (80 mmol · l^{-1}) inhibited total and ouabain-sensitive ^{86}Rb influx and ^{22}Na efflux in leucocytes, this being dose-related for total, ouabain-sensitive and ouabain-insensitive fluxes at higher concentrations. In erythrocytes inhibition occurred at 20 mmol · l^{-1} for ^{86}Rb influx, dose-related at higher concentrations as for leucocytes. ^{22}Na efflux was inhibited at 80 mmol · l^{-1} and above.

Acetaldehyde (0.1 and 0.2 mmol · l^{-1}), 1,2-propanediol (0.8 mmol · l^{-1}) and 2,3-butanediol (0.4 mmol · l^{-1}) inhibited all fractions of ^{86}Rb influx in erythrocytes, but not in leucocytes. Methanol, 2-propanol and 1,2-ethanediol (16 and 32 mmol · l^{-1}) inhibited ^{86}Rb influx in erythrocytes, but not in leucocytes. The order of potency was 2-propanol >1,2-ethanediol > methanol. Na,K-ATPase activity was inhibited in lysed leucocyte and erythrocyte preparations only at very high concentrations of the alcohols—suggesting that inhibition is due to an alteration in membrane structure and not to a direct effect on the enzyme.

Sodium and potassium-dependent (Na^+,K^+-transporting) adenosine triphosphatase EC 3.6.1.37 (Na,K-ATPase) is responsible for active transport of sodium and potassium across membranes [1]. Ethanol has been shown to suppress this enzyme activity in cell preparations *in vitro*, at concentrations which would be non-lethal *in vivo*. These cells include liver [2, 3], heart [4], brain [5], muscle [6], intestine [7] and erythrocytes [8, 9]. There is no direct evidence that ethanol inhibits Na,K-ATPase *in vivo*, but ethanol administered acutely to rats results in a rise in intracellular sodium and a fall in intracellular potassium in the liver and kidney [10]—the authors suggest that this is because ethanol inhibits active transport of cations across cell membranes. In humans there is a rapid decrease in urinary sodium, potassium and chloride excretion after ethanol consumption, despite an increased urine volume [11]; the mechanism of this change is unknown, and normal electrolyte status is rapidly restored when ethanol intake ceases. Acetaldehyde is the primary metabolite of ethanol and is highly toxic. Different techniques of measuring plasma levels provide varied results [12], but in the non-hepatic circulation the acetaldehyde concentration is unlikely to rise above 2 μmol · l^{-1} even when the ethanol concentration is as high as 30 mmol · l^{-1} [13, 14]. Acetaldehyde is reported to inhibit Na,K-ATPase at lower concentrations than does ethanol in preparations of myocardium [4], brain microsomes [15] and hepatic plasma membranes [16].

Two further metabolites of ethanol have been identified in the serum of alcoholics, but not of controls, after drinking spirits. These are 2,3-butanediol and 1,2-propanediol, and they are each found at levels of 5 μmol · l^{-1} and greater, when the serum ethanol concentration is between 10–70 mmol · l^{-1} [17]. Other alcohols such as methanol, 2-propanol (isopropylalcohol) and 1,2-ethanediol (ethylene glycol) are sometimes used as intoxicants. Their metabolic products are toxic, these being respectively mainly formic acid, acetone and oxalic acid [18]. Methanol has been shown to be less effective than ethanol in inhibiting ^{86}Rb influx in rat hepatocytes and erythrocytes [3]. In previous work we have noticed that ethanol used as a solvent for ouabain inhibits Na,K-ATPase activity in human leucocytes [19]. We decided to investigate this inhibitory effect of ethanol further, and look at the effects of its metabolites and other toxic alcohols, using easily available blood cells. In this paper we report the short-term *in vitro* effects of ethanol, its metabolites and other toxic alcohols on active ^{86}rubidium (Rb) influx, ^{22}sodium (Na) efflux and Na,K-ATPase activity, comparing human leucocytes and erythrocytes.

MATERIALS AND METHODS

Reagents

^{86}RbCl (specific activity 37–296 MBq/mg Rb) and ^{22}NaCl (specific activity 3.7–37 GBq/mg Na) were obtained from Amersham. Ethanol, acetaldehyde, ouabain and all buffer constituents were obtained from BDH Chemicals Ltd; 2-propanol from May & Baker; 1,2-propanediol, 2,3-butanediol, methanol,

1,2-ethanediol and dextran (average molecular weight 249,000) from Sigma.

Buffers

Buffer I: $NaHCO_3$ (24 mmol·l^{-1}), NaCl (115 mmol·l^{-1}), KCl (6 mmol·l^{-1}), $CaCl_2$ (1.8 mmol·l^{-1}), $MgSO_4$ (0.8 mmol·l^{-1}), NaH_2PO_4 (1.0 mmol·l^{-1}) and glucose (5.5 mmol·l^{-1}) pH 7.4 at 37°.

Buffer II: $NaHCO_3$ (24 mmol·l^{-1}), NaCl (104 mmol·l^{-1}), KCl (5 mmol·l^{-1}), NaH_2PO_4 (1.65 mmol·l^{-1}), Na_2HPO_4 (9.35 mmol·l^{-1}) and glucose (10 mmol·l^{-1}) pH 7.4 at 37°.

Leucocytes

Isolation of leucocytes. Mixed leucocytes were obtained from 60 ml of heparinised venous blood from young healthy volunteers, using the dextran sedimentation method [20], modified by replacing TC 199 with buffer I and warming all buffers to 37° before use. The blood was diluted 1:1 with buffer I before adding dextran solution. All subjects had refrained from drinking ethanol for 24 hr before venepuncture.

Measurement of Na,K-*ATPase activity.* This was assayed as described previously [19]. Enzyme activity is assayed as the release of P_i from ATP in the presence and absence of ouabain in lysed leucocyte preparations. The effects of ethanol (17–109 mmol·l^{-1}, 80–500 mg·dl^{-1}); acetaldehyde (0.1–11 mmol·l^{-1}); 1,2-propanediol (0.8–4 mmol·l^{-1}); 2,3-butanediol (0.4–4 mmol·l^{-1}); 2-propanol (16–53 mmol·l^{-1}); methanol (16–63 mmol·l^{-1}) and 1,2-ethanediol (16–50 mmol·l^{-1}) were investigated.

Measurement of ^{86}Rb *influx.* Intact leucocytes were suspended in buffer I and equilibrated at 37° for 10 min; 40 kBq ^{86}RbCl was added per ml of buffer. The suspension was immediately added to test tubes in the presence or absence of ouabain (final concentration 1 mmol·l^{-1}). Experimental tubes also contained one of the following: ethanol (8–320 mmol·l^{-1}), acetaldehyde (0.1 and 0.2 mmol·l^{-1}), 2,3-butanediol (0.4 mmol·l^{-1}), 1,2-propanediol (0.8 mmol·l^{-1}), methanol, 2-propanol or 1,2-ethanediol (16 and 32 mmol·l^{-1}). Paired control and experimental samples were incubated at 37° for 12 min in a shaking water bath. The samples were immediately centrifuged in an Eppendorf 5414 centrifuge for 4 sec, and 100 μl of supernatant was decanted for counting. The cells were washed twice in buffer I, and both cells and supernatant counted in a γ-counter.

Rubidium influx rate is calculated according to the method of Hilton and Patrick [21]. Ouabain-sensitive ^{86}Rb influx is the amount of Rb (K) entering the leucocytes in mmol per kg protein per hr; it is the difference between the total influx measured in the absence of ouabain, and the ouabain-insensitive influx measured in its presence.

Measurement of ^{22}Na *efflux.* Intact leucocytes were suspended in 4 ml buffer I and incubated with 320 kBq ^{22}NaCl for 30 min at 37° in a shaking water bath. After ^{22}Na loading the cells were washed twice in buffer I. Paired control and experimental samples were resuspended in buffer I and incubated in the presence or absence of ouabain (final concentration 1 mmol·l^{-1}) and ethanol (80–120 mmol·l^{-1}). 1 ml samples were taken at 0, 7 and 15 min and immediately centrifuged in an Eppendorf 5414 centrifuge for 4 sec. The samples were washed once in buffer I, and counted in a γ-counter. Intracellular sodium concentration was measured as described by Baron and Ahmed [20] with minor modifications. Sodium efflux rate and efflux rate constant were calculated according to the method of Hilton and Patrick [21].

Erythrocytes

Following dextran sedimentation the remaining erythrocytes were washed twice in buffer II.

Measurement of Na,K-*ATPase activity.* The activity of Na,K-ATPase was measured as the difference in P_i released from ATP by frozen and thawed haemolysates in the presence and absence of ouabain. The assay is as described by Swaminathan *et al.* [22], with minor modifications. Na,K-ATPase activity was assayed in the presence of ethanol (20–120 mmol·l^{-1}) only.

Measurement of ^{86}Rb *influx.* 750 μl of packed erythrocytes was suspended in 12 ml buffer II. 40 kBq ^{86}RbCl was added per ml buffer and 2 ml of this suspension was added to each of 6 tubes in the presence or absence of ouabain (final concentration 1 mmol·l^{-1}). Experimental tubes contained one of the following: ethanol (8–320 mmol·l^{-1}), acetaldehyde (0.1 and 0.2 mmol·l^{-1}), 2,3-butanediol (0.4 mmol·l^{-1}), 1,2-propanediol (0.8 mmol·l^{-1}), methanol, 2-propanol or 1,2-ethanediol (16 and 32 mmol·l^{-1}). Paired control and experimental tubes were incubated at 37° in a shaking water bath. One-millilitre samples were taken at 10 and 70 min. They were centrifuged immediately in an Eppendorf 5414 centrifuge for 30 sec and 100 μl supernatant decanted for counting. The samples were washed twice in buffer II and the cells and supernatant counted in a γ-counter. The calculation is basically the same as for leucocyte ^{86}Rb influx, with the exception that the influx is calculated at 10 and 70 min. The difference is the ^{86}Rb influx occurring in 60 min.

Measurement of ^{22}Na *efflux.* ^{22}Na efflux in erythrocytes and erythrocyte sodium content were calculated as described by Cumberbatch and Morgan [23] with minor modifications. The ouabain-sensitive efflux rate constant was calculated from the ouabain-sensitive efflux rate and the erythrocyte sodium content. The effects only of ethanol (80–120 mmol·l^{-1}) were investigated.

The protein concentration of each sample was measured by the technique of Lowry *et al.* [24]. Protein was measured in the erythrocyte samples in order to express the data in the same units as for leucocytes.

Statistical analysis

The Wilcoxon matched pairs signed ranks test was used because control and experimental samples from each subject were incubated simultaneously, and could be compared with each other.

RESULTS

Effects of ethanol

At concentrations up to 40 mmol·l^{-1} ethanol there

was no significant change in ^{86}Rb influx in leucocytes (Fig. 1). The apparently anomalous increase in activity at 240 mmol·l^{-1} compared to 160 mmol·l^{-1} is explained by the fact that the paired controls for these particular samples were all much higher than those for 160 mmol·l^{-1} ethanol. At 80 mmol·l^{-1} ethanol both total and ouabain-sensitive ^{86}Rb influx were decreased compared to controls ($P < 0.05$). There was no significant change in ouabain-insensitive ^{86}Rb influx. Higher concentrations of ethanol, 120–320 mmol·l^{-1}, produced a dose-related inhibition of total, ouabain-sensitive and ouabain-insensitive ^{86}Rb influx compared to paired controls. Mechanisms other than active Rb (K) entry into leucocytes may also be inhibited by ethanol.

Both 80 and 120 mmol·l^{-1} ethanol produced significant inhibition of both total and ouabain-sensitive efflux rate constant and efflux rate of sodium in leucocytes. There was no significant decrease in ouabain-insensitive sodium efflux (Table 1).

The results of Na,K-ATPase activity are summarised in Table 2. At concentrations of 17–43 mmol·l^{-1} ethanol there was no significant change in the activity of the enzyme: 65 mmol·l^{-1} ethanol and greater gave a dose-related inhibition of Na,K-ATPase.

^{86}Rb influx into erythrocytes was inhibited at much lower concentrations of ethanol than in leucocytes. 20 mmol·l^{-1} ethanol resulted in significant decreases in both total, ouabain-sensitive and ouabain-insensitive ^{86}Rb influx ($P < 0.05$) as shown in Fig. 2. Total, ouabain-sensitive and oubain-insensitive ^{86}Rb influx were all inhibited at 40 mmol·l^{-1} and greater.

Compared to controls, 40 mmol·l^{-1} ethanol did not alter any aspect of ^{22}Na efflux. This is surprising since this concentration significantly inhibited ^{86}Rb influx. 80 and 120 mmol·l^{-1} ethanol inhibited ^{22}Na efflux in erythrocytes (Table 3). Significant decreases could only be demonstrated for total and ouabain-sensitive sodium efflux rate constant and efflux rate.

Twenty and 40 mmol·l^{-1} ethanol did not significantly alter Na,K-ATPase activity; 80 ($P < 0.02$) and 120 ($P < 0.01$) mmol·l^{-1} ethanol inhibited enzyme activity as shown in Table 4.

Effects of acetaldehyde

In our experiments we used 0.1 and 0.2 mmol·l^{-1} acetaldehyde, which are far higher than the concentrations found after drinking. In leucocytes (Table 5) these concentrations did not significantly inhibit ^{86}Rb influx, though higher concentrations inhibited Na,K-ATPase (Table 2).

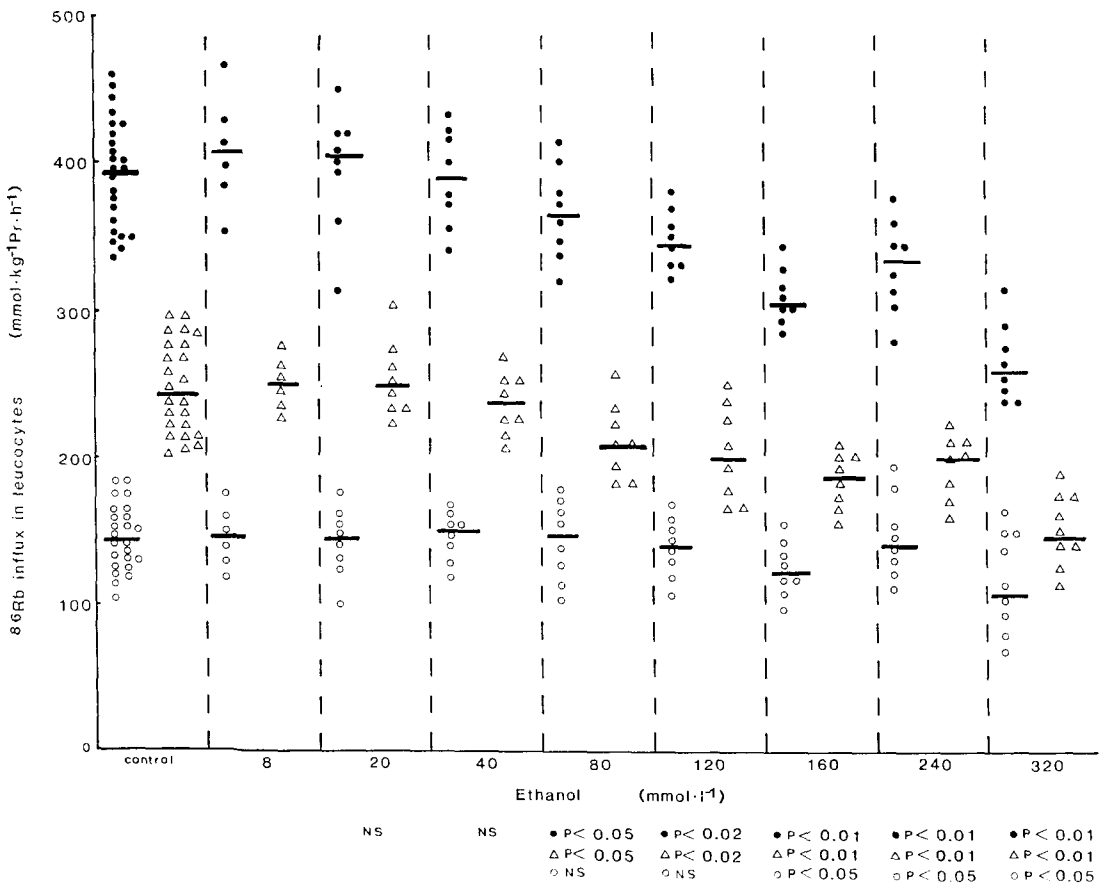

Fig. 1. Effects of ethanol on ^{86}Rb influx in isolated human leucocytes under the conditions described in the text. The results shown are (●) total influx rate, (△) ouabain-sensitive influx rate and (○) ouabain-insensitive influx rate. The horizontal bars represent the median values. NS, not significant.

Table 1. Sodium efflux rate constants and efflux rates in leucocytes with ethanol

		Total	Ouabain insensitive	Ouabain sensitive
Sodium efflux rate constant (hr^{-1})	Control	3.82 (3.16–4.48)	1.57 (1.00–2.14)	2.25 (2.16–2.34)
	80 mmol·l^{-1} Ethanol	3.65* (3.07–4.13)	1.59 (1.03–2.17)	2.06* (2.00–2.11)
	120 mmol·l^{-1} Ethanol	3.32† (2.65–4.00)	1.52 (0.95–2.09)	1.80† (1.70–1.91)
Sodium efflux rate (mmol·l^{-1} cell water·hr^{-1})	Control	107 (88–125)	44 (28–60)	63 (60–65)
	80 mmol·l^{-1} Ethanol	100* (86–116)	45 (29–61)	54* (56–59)
	120 mmol·l^{-1} Ethanol	93† (74–112)	43 (27–59)	50† (48–53)

Results are given as median and range (N = 8). There is a significant difference (*$P < 0.02$, †$P < 0.01$) for total and ouabain-sensitive sodium efflux.

Table 2. The effect of ethanol, acetaldehyde, diols and toxic alcohols on Na,K-ATPase activity in lysed leucocytes

Inhibitor	Concentration (mmol·l^{-1})	Ouabain-sensitive Na,K-ATPase (μmol Pi·g Pr^{-1}·hr^{-1})
Control		189 (126–250)
Ethanol	17/21/33/43	185 (122–241)
	65	172 (138–236)
	87	167 (120–228)
	109	156* (115–219)
Acetaldehyde	0.1/0.2	180 (130–248)
	2	170 (131–235)
	5	152* (111–213)
	11	121† (68–174)
2,3-Butanediol	0.4	183 (128–239)
	4	143* (103–191)
1,2-Propanediol	0.8	189 (134–241)
	4	144* (110–189)
2-Propanol	16/32	186 (135–254)
	53	149* (92–209)
Methanol	16/32	181 (121–240)
	63	155 (105–220)
1,2-Ethanediol	16/32	178 (130–232)
	50	149 (89–199)

Results are expressed as medians and ranges: *$P < 0.02$; †$P < 0.01$.

In erythrocytes (Table 6) all fractions of ^{86}Rb influx were inhibited by both 0.1 and 0.2 mmol·l^{-1} acetaldehyde.

Effects of 1,2-propanediol and 2,3-butanediol

In leucocytes (Table 5), neither 1,2-propanediol (0.8 mmol·l^{-1}) nor 2,3-butanediol (0.4 mmol·l^{-1}) inhibited ^{86}Rb influx. In erythrocytes (Table 6) both diols inhibited total, ouabain-sensitive and ouabain-insensitive ^{86}Rb influx at these concentrations ($P < 0.01$). Neither diol inhibited Na,K-ATPase in lysed leucocytes at these concentrations, though inhibition was observed at 4 mmol·l^{-1} (Table 2).

Effect of toxic alcohols

In intact leucocytes, neither methanol, 2-propanol nor 1,2-ethanediol (16 and 32 mmol·l^{-1}) inhibited ^{86}Rb influx (Table 5). In the case of 1,2-ethanediol ^{86}Rb influx actually appeared to be stimulated by the alcohol, though statistical analysis did not reach significance at the 5% level. Higher concentrations inhibited Na,K-ATPase activity in lysed leucocytes (Table 2).

Conversely erythrocyte ^{86}Rb influx was significantly inhibited by all three alcohols at these concentrations. Total, ouabain-sensitive and ouabain-insensitive ^{86}Rb influx were inhibited in all cases. The order of potency was 2-propanol > 1,2-ethanediol > methanol (Table 6).

DISCUSSION

At concentrations that produce mild to severe intoxication *in vivo*, acute exposure to ethanol has been demonstrated to inhibit Na,K-ATPase activity in a variety of tissues [2–9]. Israel *et al.* [25] found 50% inhibition of activity at 0.22 mol·l^{-1} in microsomal preparations from brain and eel electric organ, and suggest that acute ethanol inhibition is an allosteric event which alters the conformation of binding sites on the enzyme, lowering its affinity for potassium. Others suggest that it simply inhibits Na,K-ATPase by acting on the bulk lipid component of

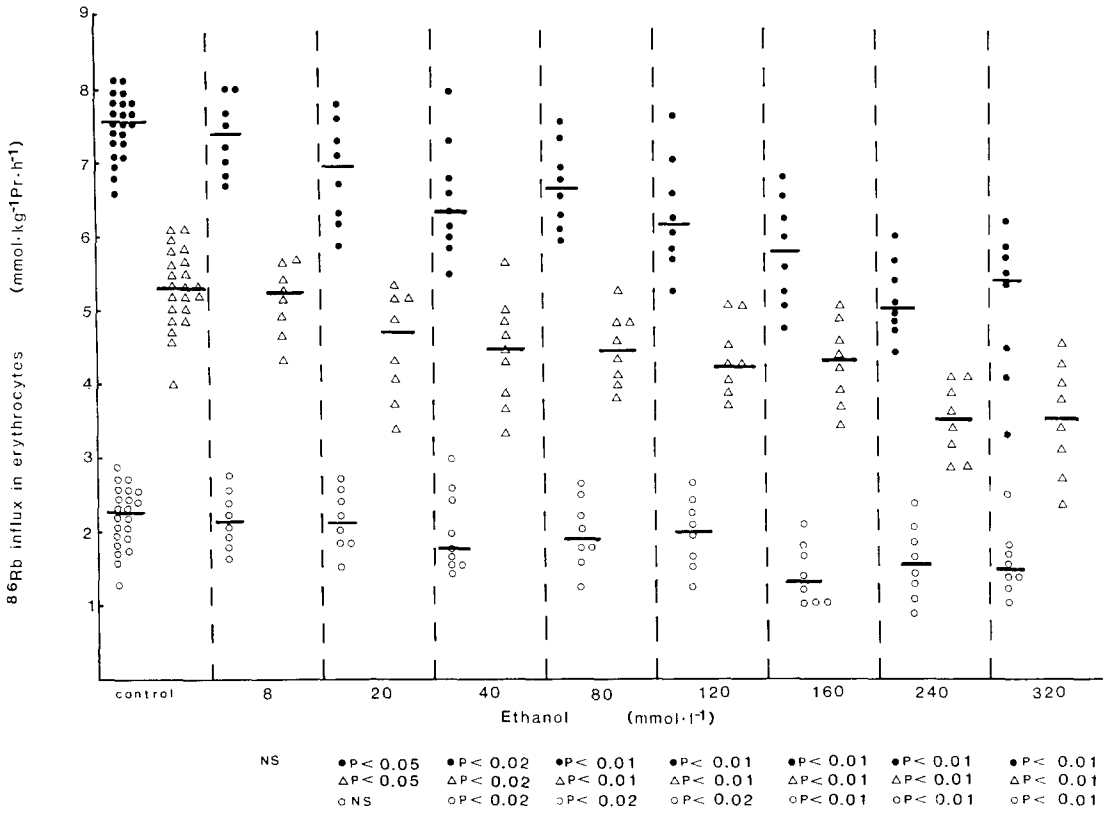

Fig. 2. Effects on ethanol on ^{86}Rb influx in isolated human erythrocytes. The symbols are as for Fig. 1.

Table 3. Sodium efflux rate constants and efflux rates in erythrocytes with ethanol

		Total	Ouabain insensitive	Ouabain sensitive
Sodium efflux rate constant (hr^{-1})	Control	0.34 (0.34–0.42)	0.10 (0.07–0.13)	0.24 (0.22–0.29)
	40 mmol · l^{-1} Ethanol	0.38 (0.29–0.44)	0.11 (0.07–0.14)	0.27 (0.22–0.30)
	80 mmol · l^{-1} Ethanol	0.28* (0.23–0.37)	0.09 (0.06–0.14)	0.19† (0.16–0.24)
	120 mmol · l^{-1} Ethanol	0.20† (0.15–0.27)	0.08 (0.06–0.12)	0.12† (0.08–0.17)
Sodium efflux rate (mmol · l^{-1} cell water · hr^{-1})	Control	3.93 (3.43–4.86)	1.16 (0.82–1.51)	2.77 (2.55–3.35)
	40 mmol · l^{-1} Ethanol	4.39 (3.35–5.08)	1.28 (0.82–1.60)	3.11 (2.57–3.47)
	80 mmol · l^{-1} Ethanol	3.23* (2.65–4.28)	1.05 (0.70–1.62)	2.19* 1.85–2.77)
	120 mmol · l^{-1} Ethanol	2.31† (1.73–3.13)	0.92 (0.70–1.40)	1.39† (0.92–1.97)

Results are given as median and range (N = 8). There is a significant difference (*$P < 0.02$; †$P < 0.01$) for total and ouabain-sensitive sodium efflux.

Table 4. The effect of ethanol on ouabain-sensitive Na,K-ATPase activity in lysed erythrocytes

Concentration of ethanol ($mmol \cdot l^{-1}$)	Ouabain-sensitive Na,K-ATPase activity ($\mu mol\ Pi \cdot g\ Pr^{-1} \cdot hr^{-1}$)
Control (N = 32)	5.94 (4.90–7.57)
20 (N = 8)	6.00 (4.81–7.43)
40 (N = 8)	5.60 (4.30–7.36)
80 (N = 8)	5.12* (4.31–5.94)
120 (N = 8)	4.37† (3.57–5.63)

Results are expressed as medians and ranges: *$P < 0.02$; †$P < 0.01$.

the membrane, and not by direct action on the enzyme [26]. Our experiments confirm inhibition of Na,K-ATPase by ethanol by measurement of active ^{86}Rb influx and coupled ^{22}Na efflux, and of Na,K-ATPase activity. One of the most interesting observations is that the concentration of ethanol required to inhibit the enzyme in erythrocytes is far less than that for leucocytes where the concentration would be potentially fatal in man [18]. In erythrocytes inhibition occurred at 20 $mmol \cdot l^{-1}$ for active ^{86}Rb influx; the U.K. driving limit is 17 $mmol \cdot l^{-1}$, and so this concentration would certainly be found in the blood after a moderate drinking bout. This difference is of particular interest because it has been demonstrated that erythrocytes behave differently from leucocytes in other aspects of ion flux, for example their response to thyroid hormones (Khan and Baron, in preparation) and in hypertension [27]. Both of these cell types, due to their easy availability, are employed as examples of human cells and as a means of studying intracellular chemical pathology. Recent work shows acetaldehyde to be about twenty times more potent than ethanol in inhibiting Na,K-ATPase in hepatic membrane and mouse microsomal preparations [15, 16]. However, the concentrations used (6–200 $mmol \cdot l^{-1}$) would never be found circulating in the plasma in living subjects. In our experiments we used 0.1 and 0.2 $mmol \cdot l^{-1}$, which though lower,

Table 5. ^{86}Rubidium influx in leucocytes with acetaldehyde, diols, and toxic alcohols

	^{86}Rubidium influx ($mmol \cdot kg^{-1}\ Pr \cdot hr^{-1}$)		
	Total	Ouabain sensitive	Ouabain insensitive
Acetaldehyde			
Control	433 (330–463)	273 (233–307)	155 (100–185)
0.1 $mmol \cdot l^{-1}$	430 (356–481)	275 (243–315)	160 (105–190)
0.2 $mmol \cdot l^{-1}$	432 (312–483)	280 (232–295)	158 (100–198)
Diols			
Control	420 (365–460)	270 (232–300)	150 (115–188)
0.8 $mmol \cdot l^{-1}$ Propanediol	412 (375–478)	265 (217–303)	153 (120–190)
0.4 $mmol \cdot l^{-1}$ Butanediol	449 (350–480)	295 (230–320)	135 (92–160)
Methanol			
Control	440 (360–500)	318 (252–339)	122 (110–139)
16 $mmol \cdot l^{-1}$	422 (345–496)	299 (228–340)	135 (107–179)
32 $mmol \cdot l^{-1}$	431 (375–470)	304 (230–351)	137 (109–120)
1,2-Ethanediol			
Control	425 (355–520)	327 (240–405)	131 (105–140)
16 $mmol \cdot l^{-1}$	445 (363–499)	340 (235–417)	140 (112–188)
32 $mmol \cdot l^{-1}$	441 (380–536)	332 (250–406)	139 (115–177)
2-Propanol			
Control	440 (360–500)	318 (252–339)	122 (110–139)
16 $mmol \cdot l^{-1}$	422 (345–496)	299 (228–340)	135 (107–179)
32 $mmol \cdot l^{-1}$	431 (375–470)	304 (230–351)	137 (109–200)

Results are medians and ranges N = 8 per group; All P values NS.

Table 6. ^{86}Rubidium influx in erythrocytes with acetaldehyde, diols, and toxic alcohols

	^{86}Rubidium influx (mmol · kg^{-1} Pr · hr^{-1})		
	Total	Ouabain sensitive	Ouabain insensitive
Acetaldehyde			
Control	6.65 (6.17–7.28)	4.64 (4.35–5.09)	1.84 (1.50–2.88)
0.1 mmol · l^{-1}	6.16* (5.92–6.62)	4.38* (3.79–4.84)	1.84‡ (1.42–2.13)
0.2 mmol · l^{-1}	5.68† (5.02–7.16)	4.04† (3.68–4.26)	1.76* (1.25–2.17)
Diols			
Control	6.50 (5.85–7.90)	4.63 (4.00–5.30)	1.90 (1.21–2.85)
0.8 mmol · l^{-1} Propanediol	6.20† (5.42–7.63)	4.35† (3.82–5.07)	1.73† (1.10–2.81)
0.4 mmol · l^{-1} Butanediol	6.23† (5.10–7.25)	4.25† (1.25–2.84)	1.74† (1.18–2.84)
Methanol			
Control	6.85 (5.65–9.10)	4.89 (4.17–7.28)	1.82 (1.29–2.71)
16 mmol · l^{-1}	6.73‡ (5.41–9.35)	4.61‡ (3.97–7.49)	1.94 (1.44–2.71)
32 mmol · l^{-1}	6.25* (3.88–6.78)	4.43† (2.40–4.90)	1.66* (1.35–2.42)
1,2-Ethanediol			
Control	6.32 (5.48–8.05)	4.74 (4.34–6.04)	1.80 (0.99–2.14)
16 mmol · l^{-1}	6.05* (5.35–7.15)	4.28* (3.73–5.25)	1.77* (0.92–2.15)
32 mmol · l^{-1}	6.06* (5.35–7.05)	4.43* (3.57–4.83)	1.68* (0.78–2.46)
2-Propanol			
Control	6.20 (5.44–7.08)	4.30 (3.90–5.30)	1.87 (1.18–2.45)
16 mmol · l^{-1}	5.85† (4.74–6.72)	4.12† (3.51–4.97)	1.53† (1.09–2.44)
32 mmol · l^{-1}	5.21† (3.73–6.00)	3.65† (3.07–4.12)	1.40† (1.00–2.35)

Results are medians and ranges N = 8 per group: *P < 0.02; †P < 0.01; ‡P < 0.05.

are still toxic levels. No inhibition of ^{86}Rb influx in leucocytes was observed. Erythrocyte ^{86}Rb influx was significantly inhibited at both concentrations. Leucocyte Na,K-ATPase was inhibited at higher concentrations of acetaldehyde. Intoxication with methanol (methylated spirits), 1,2-ethanediol (antifreeze) and 2-propanol (cosmetic solvent) is frequently found both accidently and in suicidal and derelict people; and mortality rates are high. We find 2-propanol to be more inhibitory than 1,2-ethanediol or methanol on ^{86}Rb influx in erythrocytes. None of these alcohols affected leucocyte ion flux.

The diols 2,3-butanediol and 1,2-propanediol proved to be inhibitory to ^{86}Rb influx in erythrocytes, but not leucocytes. At very high concentrations they inhibited leucocyte Na,K-ATPase.

In conclusion we can demonstrate inhibition of active cation fluxes at concentrations of ethanol which would be non-lethal *in vivo* in erythrocytes, but in leucocytes only at doses likely to be lethal. Macrocytosis (increased erythrocyte mean corpuscular volume) is a sensitive index of alcohol intake [28], though the cause is unknown. Ethanol is distributed throughout body water and because both intra and extracellular compartments contribute to free water loss, there should be no significant redistribution of fluid and electrolytes following ethanol ingestion; only small changes in plasma sodium and volume would be anticipated [11]. If our findings in erythrocytes also occurred *in vivo* in the general body cells, particularly in muscle, one would expect regularly to see gross changes in plasma electrolyte concentrations following consumption of large amounts of ethanol. We therefore envisage that the leucocyte is behaving as a model in response to ethanol; and that it is erythrocytes alone that are particularly sensitive to the effects of ethanol. We are currently undertaking experiments to try and correlate our *in vitro* results with *in vivo* experiments, by raising blood ethanol concentrations in volunteers to levels demonstrated to have effects in erythrocytes.

REFERENCES

1. K. J. Sweadner and S. M. Goldin, *New Engl. J. Med.* **302**, 777 (1980).
2. R. L. Ricci, S. S. Crawford and P. B. Miner, *Gastroenterology* **80**, 1445 (1981).

3. G. Pocock, *J. Physiol.* **367**, 92P (1985).
4. J. W. Williams, M. Tada, A. M. Katz and E. Rubin, *Biochem. Pharmac.* **24**, 27 (1975).
5. D. L. Lin, *Ann. N.Y. Acad. Sci.* **273**, 331 (1976).
6. A. M. Katz, *Fedn. Proc.* **41**, 2456 (1982).
7. A. M. Hoyumpa, S. G. Nichols, F. A. Wilson and S. Schenker, *J. Lab. clin. Med.* **90**, 1086 (1977).
8. D. H. P. Streeten and A. K. Soloman, *J. gen. Physiol.* **37**, 43 (1953).
9. Y. Israel, *Q. Jl Stud. Alcohol.* **31**, 293 (1970).
10. H. Kalant, W. Mons and M. A. Mahon, *Can. J. Physiol. Pharmac.* **44**, 1 (1966).
11. N. McIntyre, in *Contemporary Issues in Clinical Biochemistry, Clinical Biochemistry of Alcoholism*, Vol. 1 (Ed. S. B. Rosalki) p. 117. Churchill Livingstone, Edinburgh (1984).
12. C. J. P. Eriksson, *Pharmacol. Biochem. Behav.* **18**, 141 (1983).
13. H. L. Iversen and S. E. Damgaard, *Clin. chim. Acta* **135**, 51 (1983).
14. H. U. Nuutinen, M. P. Salaspuro, M. Valle and K. P. Lindross, *Eur. J. clin. Invest.* **14**, 306 (1984).
15. V. G. Erwin, J. Kim and A. D. Anderson, *Biochem. Pharmac.* **24**, 2089 (1975).
16. J. L. Gonzalez-Calvin, J. B. Saunders and R. Williams, *Biochem. Pharmac.* **32**, 1732 (1983).
17. D. D. Rutstein, R. L. Veech, R. J. Nickerson, M. E. Felver, A. A. Vernon, L. L. Needham, P. Kishore and S. B. Thacker, *Lancet* **ii**, 534 (1983).
18. P. Jatlow, *Am. J. clin. Pathol.* **74**, 721 (1980).
19. D. N. Baron and F. A. Khan, *Clin. Sci.* **68**, 143 (1985).
20. D. N. Baron and S. A. Ahmed, *Clin. Sci.* **37**, 205 (1969).
21. P. J. Hilton and J. Patrick, *Clin. Sci.* **44**, 439 (1973).
22. R. Swaminathan, G. Clegg, M. Cumberbatch, Z. Zareian and F. A. McKenna, *Clin. Sci.* **62**, 489 (1982).
23. M. Cumberbatch and B. Morgan, *Clin. chim. Acta* **89**, 221 (1978).
24. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
25. Y. Israel, H. Kalant and I. Laufer, *Biochem. Pharmac.* **14**, 1803 (1965).
26. L. S. Williamson, W. G. Wood, C. Gorka and R. Strong, *Alcoholism. Clin. exp. Res.* **9**, 187 (1985).
27. J. D. Swales, *Biosci. Rep.* **2**, 967 (1982).
28. A. Wu, *Lancet* **i**, 829 (1974).