Effects of Lower Alcohols on Potassium Transport and Microsomal Adenosine-Triphosphatase Activity of Rat Cerebral Cortex

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1. Slices of rat cerebral cortex, incubated anaerobically at 37°, lost K^+ from an initial concentration of 102 m-equiv./kg. to a concentration of 57 m-equiv./kg. after 10 min. On subsequent aerobic incubation they regained K^+ rapidly at a rate that varied with the K^+ concentration of the medium. 2. Lower aliphatic alcohols, present at equal thermodynamic activity, produced approximately equal degrees of inhibition of K^+ uptake during the aerobic incubation. This inhibition was reduced by an increase in K^+ content of the medium. Ethanol did not affect the rate of K^+ loss during anaerobic incubation. 3. Li^+, in concentrations of 1–10 mM, also inhibited K^+ uptake by brain-cortex slices, the degree of inhibition varying with the Li^+ concentration. Ouabain also inhibited K^+ uptake. 4. The same series of alcohols, at equal thermodynamic activity, produced comparable degrees of inhibition of Na^+,K^+,Mg^{2+}-stimulated adenosine-triphosphatase activity in brain microsomes. 5. It is suggested that inhibition of cation transport is an important, but not a primary, mechanism in the production of central nervous depression by alcohols and other substances.

It was observed previously that ethanol, in concentrations which produce non-lethal poisoning *in vivo*, inhibits the active transport of Na^+ by isolated frog skin (Israel & Kalant, 1963), and the active transport of K^+ by human erythrocytes (Streeten & Solomon, 1954) and in slices of rabbit kidney cortex and guinea-pig cerebral cortex *in vitro* (Israel-Jacard & Kalant, 1965). Ethanol also inhibits the Na^+,K^+,Mg^{2+}-stimulated ATPase* activity of microsomal preparations from mammalian brain and eel electric organ, apparently by competing with K^+ (Israel, Kalant & Laufer, 1965). Since this enzyme plays a major role in the active translocation of Na^+ and K^+ in intact cells (Skou, 1957, 1964; Post, Merritt, Kinsolving & Albright, 1960; Bonting & Caravaggio, 1962, 1963; Chan, Calabrese & Theil, 1964), it is logical to postulate that inhibition of it is the means by which ethanol inhibits active transport in intact cell systems. In support of this hypothesis, the present work demonstrates an antagonism between K^+ and ethanol with respect to active transport in brain slices, analogous to that shown previously with microsomal ATPase.

Wallgren & Kulonen (1960) showed that 0·087 M-ethanol causes approx. 40% inhibition of the excess of respiration in electrically stimulated brain slices. As shown by Keesey, Wallgren &

* Abbreviation: ATPase, adenosine triphosphatase.

McIlwain (1965), electrical stimulation increases the active transport of Na^+ and K^+. Inhibition of this process by ethanol would decrease the breakdown of ATP to ADP, and thus decrease the stimulation of respiration (Aldridge, 1957; Wallgren & Kulonen, 1960; McIlwain, 1963). Lindbohm & Wallgren (1962) also reported that different aliphatic alcohols, when used at the same thermodynamic activity (Brink & Posternak, 1948), produced comparable degrees of inhibition of the electrically stimulated respiration of rat brain-cortex slices. The present work demonstrates that the same group of alcohols, at the same thermodynamic activity, have comparable effects on the active transport of K^+ in brain slices stimulated by previous depletion of K^+. These alcohols are also shown to inhibit the microsomal Na^+,K^+,Mg^{2+}-stimulated ATPase of rat brain, and this inhibition, like that produced by ethanol, can be reduced by increasing the concentration of K^+ in the medium.

As supporting evidence for the relationship between inhibition of active transport in brain and the poisoning produced by ethanol *in vivo*, the effect of Li^+ on active transport was also explored, since some of the effects of Li^+ *in vivo* are comparable with those of ethanol (Schou, 1957).

Evidence in the literature suggests that electrical stimulation of brain slices produces relatively small changes in total K^+ content, and requires a rather

elaborate procedure. It was therefore desirable to have a simpler technique that would give larger changes in K^+ content, together with a high rate of K^+ uptake. Pappius & Elliott (1956) had observed that a brief period of anaerobic incubation causes a large fall in the K^+ content of slices, which is followed by rapid net gain of K^+ during subsequent aerobic incubation. Their technique was employed in the present work, and proved to be satisfactory for studying the actions of inhibitors of cation transport.

METHODS

Preparation and incubation of brain-cortex slices. Male albino rats of the Wistar strain, weighing 425 ± 50 g., were employed. The technique for preparing the brain slices was as described by Israel-Jacard & Kalant (1965), except that two slices rather than one were cut from each hemisphere after discarding the first thin slice bearing the meninges. The slices were weighed immediately after cutting, and floated on some of the incubation buffer in a Petri dish.

Depending on whether the experiment involved an initial anaerobiosis, the incubation medium was saturated with either $N_2 + CO_2$ (95:5) or $O_2 + CO_2$ (95:5), and the pH was adjusted to 7·4. Two incubation media were used, which varied essentially only in their K^+ content (6 mM and 3·6 mM). The composition of the media were as follows. 6 mM-Medium: NaCl, 120 mM; KCl, 4·8 mM; KH_2PO_4, 1·2 mM; $MgSO_4$, 1·3 mM; $CaCl_2$, 2·8 mM; glucose, 10 mM; $NaHCO_3$, 26 mM. 3·6 mM-Medium: NaCl, 122·4 mM; KCl, 2·4 mM; other components as in the 6 mM-medium. Slices were incubated in 25 ml. Erlenmeyer flasks containing 5 ml. of incubation medium previously gassed as required, stoppered and kept at 37° in a Dubnoff shaker bath until used. The time from decapitation until the last slice was introduced into the appropriate medium was 8 ± 1 min.

In experiments not involving anaerobic treatment, two slices from the same hemisphere were placed together in the oxygenated medium (6 mM-K^+) and bubbled with the $O_2 + CO_2$ mixture for a maximum of 15 min. The Erlenmeyer flasks were then stoppered and the slices were further incubated as required for various periods up to a maximum of 30 min. At the end of incubation, the slices were rinsed for 2–3 sec. in 0·34 M-sucrose and then digested for K^+ determination.

In experiments involving anaerobiosis, the slices were preincubated at 37° for 10 min. in stoppered flasks containing 5 ml. of medium previously gassed with $N_2 + CO_2$ (95:5). After this the flasks were opened, aerated gently for 10 min. and then restoppered and incubated aerobically for the desired time as described above.

The alcohols and other substances to be tested were introduced at the start of aeration, dissolved in 0·1 ml. of water. An equal amount of water was added to the controls. This addition does not materially affect the concentration of the medium.

Determination of K^+ by flame photometry. The slices were treated with 0·5 ml. of conc. HNO_3 and heated at 75° until completely digested. In most experiments, $LiNO_3$ was added and the sample was diluted to give a final Li^+ concentration of 100 p.p.m. and a final volume of 10 ml.

Potassium concentration in the digests was determined by flame photometry by the Li^+ internal-standard method, with a Baird DB-2 flame photometer. Preliminary studies confirmed White's (1952) observation that Na^+ has negligible effect on K^+ determinations by this apparatus. Potassium concentration was expressed as m-equiv. of K^+/kg. of fresh tissue.

In experiments in which Li^+ was added to the incubation medium, the direct method of flame photometry was used.

Preparation and assay of the microsomal Na^+,K^+,Mg^{2+}-*stimulated ATPase.* The cerebral hemispheres of one or two rats were removed as rapidly as possible and frozen in liquid N_2 for 6–8 min., then kept at $-20°$ until used. Preliminary experiments indicated that rapid freezing of the tissue did not modify the microsomal Mg^{2+}-stimulated ATPase activity appreciably, but greatly increased the Na^+,K^+,Mg^{2+}-stimulated ATPase activity. This increase resulted from (i) an approximately fivefold increase in yield of microsomal fraction/g. of tissue and (ii) a doubling of activity/mg. of microsomal N. This phenomenon is presently under further study. Similar values for ATPase activity were obtained whether the tissue was thawed immediately after the liquid N_2 treatment or kept for up to 3 weeks at $-20°$.

The frozen tissue was thawed at room temperature and homogenized in 10 vol. of 0·25 M-sucrose in 0·05 M-tris-HCl buffer, pH 7·5 (Schwartz, Bachelard & McIlwain, 1962). The crude homogenate was centrifuged at 0–2° for 10 min. at 770g and immediately afterwards for 15 min. at 10000g. By this procedure, the loosely-packed nuclear fraction was covered by a well-packed mitochondrial precipitate, which permitted easy removal of the supernatant with minimal loss of microsomal material. The supernatant was centrifuged at 100000g for 30 min. The pellet obtained was gently resuspended in some of the original medium, in a loosely-fitting glass homogenizer, and used for ATPase assays.

For this purpose, 1·0 ml. of the incubation medium contained: tris-ATP (Sigma), 5 mM; $MgCl_2$, 5 mM; tris-HCl buffer, pH 7·4, 75 mM; NaCl, 150 mM; KCl, 1 or 10 mM; enzyme corresponding to 11 mg. of original brain tissue. To determine the Mg^{2+}-stimulated ATPase activity, Na^+ and K^+ were omitted and ouabain was added in a final concentration of 0·5 mM. Tubes were preincubated for 15 min. The reaction was started by the addition of ATP, and stopped after 20 min. by the addition of 2 ml. of cold 5% (w/v) trichloro-acetic acid. A sample was used to determine phosphate by the method of Fiske & Subbarow (1925).

RESULTS

K^+ *content of incubated slices.* Rat brain cortex slices incubated entirely aerobically showed a minimum K^+ content of approx. 57 m-equiv./kg. after 10 min. With further incubation, some of the lost K^+ was recovered and the K^+ content stabilized at $72·4 \pm 2·0$ m-equiv./kg. at 30 min. This concentration remained fairly constant up to 45 min. (Fig. 1). This is appreciably higher than other values in the literature for the same species (Leaf, 1960; Pappius & Elliott, 1956).

When slices were first incubated anaerobically for 10 min. the K^+ content at the end of this time
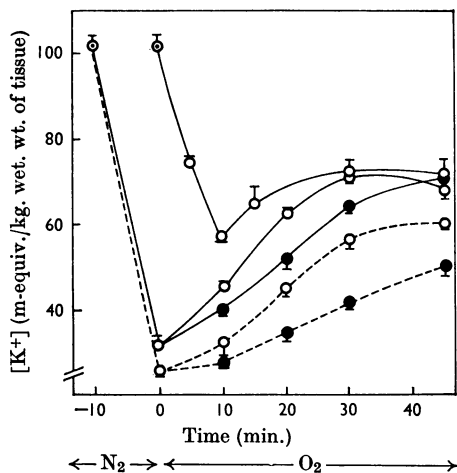
Fig. 1. Effect of ethanol on K+ content of rat brain-cortex slices incubated in modified Krebs–Ringer bicarbonate media. Anaerobic preincubation, where used, took place between −10 and 0min.; aerobic incubation between 0 and 45min. Continuous lines indicate the effect of incubation in a medium containing 6mM-K+; broken lines indicate effects in 3·6mM-K+. Mean initial value for 57 unincubated samples, ⊙; samples incubated without ethanol, ○; samples incubated in the presence of 108mM-ethanol, added at zero time, ●. Each vertical bar represents one standard deviation.

Table 1. K+ *content of rat brain-cortex slices after* 10 *min. anaerobiosis*

Differences between controls and ethanol-incubated slices in each medium were not significant ($P < 0.20$). Results are means $\pm$ s.d. (with numbers of experiments in parentheses), except where otherwise indicated.

[K+] in medium (mM)	[K+] of slices (m-equiv./kg. fresh wt.)	
	Control	Ethanol
6·0	31·5 ± 1·6 (13)	30·9 ± 0·7 (5)
3·6	25·9 ± 1·5 (15)	24·1, 25·4 (2)*

* Two individual values.

Table 2. *Inhibition by ethanol of* K+ *reaccumulation in anaerobically preincubated rat brain slices*

[K+] in medium (mM)	Inhibition (%)			
	10 min.	20 min.	30 min.	45 min.
3·6	78	56	47	27
6·0	37	34	25	0*

* The group means with and without ethanol, in the medium with 6mM-K+, were not significantly different at 45min.

was 25·9 ± 1·8m-equiv./kg. for slices incubated in 3·6mM-K+ and 31·5 ± 1·6m-equiv./kg. for those in 6mM-K+. In subsequent aerobiosis, the slices actively reaccumulated K+, the rate of recovery depending on the concentration of K+ in the incubation medium (Fig. 1). After 30min. of aerobic treatment, slices in 6mM-K+ regained K+ to give concentrations similar to those of slices incubated entirely aerobically ($P > 0.30$), suggesting that the short period of anaerobiosis had no deleterious effects on the active transport mechanism. After the beginning of aeration, there was a short lag period before K+ reaccumulation achieved its maximal rate. This possibly represents the time of diffusion of oxygen into the tissue, and of ATP production. After this lag period the maximum rates of K+ reaccumulation in 3·6mM-K+ and 6mM-K+ media were 1·14 and 1·70m-equiv./kg. of tissue/min. respectively. The maximal rate of reaccumulation in 6mM-K+ is about one-half as fast as that reported by Keesey et al. (1965) for slices recovering K+ after electrical stimulation, but of the same order as that reported by Pappius & Elliott (1956) after anaerobiosis.

Ethanol effect. Ethanol was added in amounts sufficient to give a concentration of 108mM (500mg./100ml.) in the incubation medium. Four

measurements of ethanol concentration in the medium at the end of incubation, by the alcohol-dehydrogenase method (Kaplan & Ciotti, 1957; Smith & Newman, 1959), gave values ranging from 76 to 102mM. At this concentration ethanol did not greatly modify the initial passive outflow of K+ produced by anaerobiosis (Table 1). This is in agreement with the previous reports (Streeten & Solomon, 1954; Israel-Jacard & Kalant, 1965) that ethanol does not modify the passive efflux of K+. In any case, in subsequent experiments ethanol was added after the anaerobiosis treatment.

Ethanol (108mM) inhibited the active reaccumulation of K+ during aerobic incubation (Fig. 1). The inhibition was always greater in the medium with 3·6mM-K+ than with 6·0mM-K+. The percentage inhibition was calculated as $100(1 - \Delta[K^+]_i/\Delta[K^+]_c)$ where $\Delta[K^+]_i$ and $\Delta[K^+]_c$ are the amounts of K+ accumulated in the presence and absence of ethanol respectively. As shown in Table 2, the degree of inhibition produced by ethanol became progressively less with longer times of incubation.

Effect of other alcohols. Other alcohols tested were added in concentrations calculated to give equal thermodynamic activities (Brink & Posternak, 1948; Lindbohm & Wallgren, 1962). The K+ content of the slices, determined after 20min. of aerobic incubation, was not significantly different in the presence of the various alcohols (Table 3).

Table 3. *Effects of several lower aliphatic alcohols on active reaccumulation of* K^+
by rat brain-cortex slices

The medium contained 6mM-K^+ in all cases. Activity coefficients are those given by Brink & Posternak (1948). Concentrations shown in column 3 are those calculated to give equal thermodynamic activity of the various alcohols (Lindbohm & Wallgren, 1962). K^+ contents of slices (column 4) are those after 10 min. anaerobic incubation, followed by 20 min. of aerobic incubation. Alcohols were added after completion of the period of anaerobiosis. The K^+ reaccumulation (column 5) is the K^+ content after 20 min. of aerobiosis minus the K^+ content after 10 min. of anaerobiosis [$31 \cdot 5 \pm 1 \cdot 6$ (13); from Table 1]. The percentage inhibition produced by the various alcohols at the concentrations used did not differ significantly from each other.

Alcohol	Activity coefficient	Concn. (mM)	K^+ of slices (m-equiv./kg.)	K^+ reaccumulation (m-equiv./kg.)	Inhibition (%)
—	—	—	$62 \cdot 3 \pm 0 \cdot 3$ (9)	$30 \cdot 8$	—
Ethanol	$3 \cdot 69$	109	$51 \cdot 9 \pm 2 \cdot 5$ (6)	$20 \cdot 4$	34
Propan-1-ol	$14 \cdot 4$	28	$51 \cdot 1 \pm 2 \cdot 3$ (4)	$19 \cdot 6$	36
Propan-2-ol	$7 \cdot 7$	52	$51 \cdot 8 \pm 4 \cdot 9$ (4)	$20 \cdot 3$	34
Butan-1-ol	$52 \cdot 9$	$7 \cdot 6$	$49 \cdot 0 \pm 3 \cdot 4$ (4)	$17 \cdot 5$	43
2-Methylpropan-2-ol	$11 \cdot 8$	34	$51 \cdot 9 \pm 2 \cdot 3$ (4)	$20 \cdot 4$	34

Table 4. *Effect of* Li^+ *on active reaccumulation of* K^+ *by rat brain-cortex slices*

The incubation medium contained 6mM-K^+. K^+ content of slices was determined by the direct method of flame photometry: initial values represent K^+ content of slices after 10 min. anaerobic incubation; 20 min. values represent K^+ contents after subsequent 20 min. aerobic incubation. Li^+ was added at the start of anaerobiosis.

Concn. of Li^+ (mM)	No. of experiments	$[K^+]$ of slices (m-equiv./kg.)		Net uptake (m-equiv./kg.)	Inhibition (%)
		Initial	After 20 min.		
0	5	$34 \cdot 3 \pm 1 \cdot 3$	$60 \cdot 8 \pm 1 \cdot 7$	$26 \cdot 5$	—
1	4	$34 \cdot 8 \pm 1 \cdot 4$	$57 \cdot 4 \pm 2 \cdot 8$	$22 \cdot 6$	15
5	5	$32 \cdot 5 \pm 2 \cdot 3$	$52 \cdot 8 \pm 2 \cdot 8$	$20 \cdot 3$	23
10	4	$31 \cdot 9 \pm 2 \cdot 7$	$45 \cdot 3 \pm 1 \cdot 4$	$13 \cdot 4$	49

Effect of Li^+ *on* K^+ *reaccumulation.* Li^+ was added from the beginning of the anaerobic incubation to allow an adequate time of penetration into the slices. The final concentrations of Li^+, added as lithium chloride, were $1 \cdot 0$, $5 \cdot 0$ and $10 \cdot 0 \text{mM}$. As shown in Table 4, Li^+ caused an inhibitory effect on the transport of K^+, in terms of the net uptake between 0 and 20 min. There also appeared to be a decrease in the K^+ concentration at zero time, related to the amount of Li^+ added, but this was not statistically significant.

Effect of ouabain on active reacucmulation of K^+. Ouabain in final concentrations of $2 \cdot 5$ and $5 \mu\text{M}$ was added after the anaerobiosis period, and slices were incubated for a further 20 min. in a medium containing 6mM-K^+ in an atmosphere of oxygen. Both concentrations produced a marked and significant inhibition of the reaccumulation of K^+. In the absence of ouabain the K^+ concentration rose from $31 \cdot 5 \pm 1 \cdot 6$ (15 samples) to $62 \cdot 3 \pm 0 \cdot 3$ (nine samples). At $2 \cdot 5$ and $5 \mu\text{M}$-ouabain, the resulting

20 min. K^+ concentrations were $53 \cdot 3 \pm 3$ and $48 \cdot 9 \pm 3$ respectively (four samples each). This represents inhibitions of $29 \cdot 2\%$ and $43 \cdot 5\%$ respectively.

Effect of different alcohols on the Na^+, K^+, Mg^{2+}-*stimulated ATPase activity.* As shown in Table 5, the different alcohols, present at equal thermodynamic activity, produced similar degrees of inhibition on the Na^+, K^+, Mg^{2+}-stimulated ATPase activity of rat-brain microsomal preparations. The range of variation of the alcohol concentrations is over 14-fold, yet the degree of inhibition varied by only 2–3·6-fold, depending on the K^+ concentration of the medium. As noted with ethanol by Israel *et al.* (1965), the effect produced by all the alcohols was decreased by an increase in K^+ concentration of the medium (Table 5).

DISCUSSION

The use of anaerobically preincubated brain-cortex slices appears to offer a satisfactory model for the study of effects of inhibitors upon the

Table 5. *Effects of several lower aliphatic alcohols on ATPase activities of rat-brain microsomal preparations*

Alcohols were added in concentrations calculated to give equal thermodynamic activities (Lindbohm & Wallgren, 1962). ATPase activity is expressed as μmoles of P_i liberated/vol. of microsomal suspension equivalent to 1 g. fresh wt. of tissue. Each figure for ATPase activity is the mean of two experiments with duplicate samples in each.

| Alcohol | Concn. (mM) | ATPase activities (μmoles of P_i/hr./g. fresh wt. of tissue) | | | Inhibition by alcohol (%) | | |
| | | Mg^{2+}-stimulated ATPase | Na^+,K^+,Mg^{2+}-stimulated ATPase | | Mg^{2+}-stimulated ATPase | Na^+,K^+,Mg^{2+}-stimulated ATPase | |
			1 mM-K^+	10 mM-K^+		1 mM-K^+	10 mM-K^+
—	—	110	185	536	—	—	—
Ethanol	220	101	126	472	8	32	12
Propan-1-ol	56	101	146	510	8	21	5
Propan-2-ol	104	99	137	483	10	26	10
Butan-1-ol	15·2	104	148	501	5	20	7
2-Methylpropan-2-ol	68	104	106	441	5	42	18

processes related to active transport of cations in activated tissues. Anaerobiosis caused no evident cellular damage in relation to this process, since the slices reaccumulated K^+ vigorously and reached final concentrations comparable with the best reported by other investigators (cf. Table 6 of Keesey *et al.* 1965). In addition, the maximum velocity of net K^+ uptake, although lower than that found by Keesey *et al.* (1965) in electrically stimulated slices, was at least of the same order. The difference may possibly be explained by greater selectivity of electrical stimulation; anaerobiosis would cause loss of K^+ from both neural and glial cells, whereas electrical stimulation would be expected to affect only the neurons, and these would presumably have a higher rate of K^+ uptake.

The results of the present work support the view that ethanol inhibits the active transport of K^+ in brain slices by inhibiting the microsomal $Na^+,K^+,-Mg^{2+}$-stimulated ATPase. Thus ethanol inhibits both processes to comparable degrees, and in both cases the inhibition is diminished by an elevation of the K^+ concentration of the medium. A seeming discrepancy is provided by the fact that 108 mM-ethanol causes a greater inhibition of K^+ uptakes by slices in a medium containing 6 mM-K^+ (Tables 2 and 3) than that produced by 220 mM-ethanol on the microsomal Na^+,K^+,Mg^{2+}-stimulated ATPase activity in a medium containing 1 mM-K^+ (Table 5). Moreover, the true effect of ethanol on K^+ uptake by slices is probably even slightly greater, since some loss of ethanol by evaporation occurred during incubation. However, the apparent discrepancy is possibly resolved by reference to the rates of uptake of K^+ by brain slices, and of Na^+,K^+,Mg^{2+}-stimulated ATPase activity of brain microsomes, at different K^+ concentrations in the media. From

Lineweaver–Burk plots, it can be calculated that the approximate K^+ concentration required for half-maximal velocity is 19·2 mM for active transport in brain slices, and 2·2 mM for the ATPase activity (Israel *et al.* 1965). If the rate of active transport of cations is chiefly a reflection of the Na^+,K^+,Mg^{2+}-stimulated ATPase activity, and the external K^+ concentration is a rate-determining factor, then these calculations suggest that the actual K^+ concentration at the ATPase site in the cell membrane is only one-eighth to one-ninth that in the extracellular medium. This would imply the existence of an external permeability barrier at the surface of the intact cell that is partly or wholly abolished in disrupted systems such as microsomal preparations. Such a diffusion barrier might be analogous to that postulated in frog skin (Curran & Gill, 1962).

If these considerations are valid, the rate of K^+ transport in brain slices incubated with 6 mM-K^+ should provide a suitable comparison with the rate of microsomal ATPase activity in a medium containing less than 1 mM-K^+. Under these conditions the inhibitory effects of ethanol are indeed comparable in the two systems, 108 mM-ethanol causing about 30% inhibition in both. Since this concentration of ethanol corresponds to about 400 mg./100 ml. of whole blood, whereas the lethal concentration for the rat is over 1000 mg./100 ml., the inhibition of active transport of cations would be appreciable under conditions of even relatively mild ethanol poisoning.

As shown in Tables 3 and 5, other alcohols present at equal thermodynamic activity have effects similar to those of ethanol: they produce similar degrees of inhibition of the active uptake of K^+ by brain slices and the Na^+,K^+,Mg^{2+}-stimulated ATPase activity of brain microsomes,

and their effect on the ATPase activity is diminished by an increase in the concentration of K^+ in the medium. These findings are compatible with the hypothesis that alcohols inhibit active transport by inhibiting the membrane ATPase activity, and that this inhibition is based on an allosteric modification of the K^+ site (Israel *et al.* 1965). The competitive relation between alcohols and K^+ is also in keeping with the observation that a higher concentration of ethanol is required to inhibit the respiration of stimulated brain slices when the slices are stimulated by a high K^+ concentration than by electrical pulses.

As shown in Fig. 1, the inhibition produced by ethanol decreased with longer incubation. This change is too great to be attributed to the small loss of ethanol by evaporation. It is much more reasonable to explain it in terms of Skou's (1964) theory of regulation of intracellular K^+ and Na^+ content, and of the 'safety factor' concept proposed by Kalant & Israel (1966). With increasing intracellular K^+ concentration, the active uptake of K^+ would be increasingly inhibited by competition of K^+ at the intracellular Na^+ site on the ATPase system. Under these conditions, the unused ATPase capacity would constitute a 'safety factor' against the effect of exogenous inhibitors such as ethanol, since any tendency of the latter to inhibit further K^+ uptake would be offset by a decrease in the internal inhibition. Only when all the potential capacity of the transport system is in use, as in the early period of aerobic recovery, or after electrical stimulation of the slices, will the maximum effect of ethanol of the transport, and on the associated oxygen consumption, be demonstrable.

The inhibition of ion transport in brain slices produced by Li^+ (Table 4) is similar to that observed in kidney slices (Mudge, 1951), frog skin (K. Zerahn, unpublished work cited by Schou, 1957) and goldfish gills (Sexton & Meyer, 1955). This effect of Li^+ is similar to that of ethanol, and it is noteworthy that the administration of Li^+ to humans has been reported to produce depressant effects (Schou, 1957) that are in many respects similar to those of ethanol. Such a resemblance lends credence to the suggestion that the depressant effects *in vivo* result, at least in part, from the inhibition of Na^+ and K^+ transport.

The effect of ouabain appears to be more complex. Cardiac glycosides inhibit the active transport of Na^+ and K^+ in various tissues, but at therapeutic doses *in vivo* they do not produce depression of the central nervous system such as that caused by ethanol or Li^+. Confusion, drowsiness and other central disturbances are observed as chronic toxic effects (Lyon & DeGraff, 1963); perhaps the lack of acute effects depends on relative slowness of passage across the blood–brain barrier. Repke (1961) has reported that digitoxin administered *in vivo* enters the myocardium at least 30 times as rapidly as it enters the brain. In contrast, the genin enters the brain rapidly, and has relatively much greater central nervous system effect than digitoxin.

It may be concluded that inhibition of cation transport in brain is probably an important mechanism in the production of depressant effects by alcohols. This appears to be a non-specific action, which can be produced by Li^+, cardiac glycosides and probably by numerous other drugs, and in many other tissues. As Quastel (1965) has suggested, further exploration of the mode of action of alcohols should perhaps be directed towards some more basic interaction with the cell membrane, of which the effect on ATPase activity is a consequence.

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