Metabolic Alterations Produced in the Liver by Chronic Ethanol Administration

CHANGES RELATED TO ENERGETIC PARAMETERS OF THE CELL

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1. Chronic ethanol administration to rats for 21–27 days increases the rate of O_2 consumption as measured in liver slices. The extra respiration can be abolished by inhibition of the active transport of Na^+ and K^+ . Dinitrophenol activates the respiratory rate in the liver of the treated animals only in the presence of ouabain. 2. Active (ouabain-sensitive) transport of ^{86}Rb and $(Na^+ + K^+)$ -stimulated adenosine triphosphatase activity were increased in the livers of the ethanol-treated animals. 3. Chronic ethanol administration also led to a decrease in the phosphorylation potential $([ATP]/[ADP][P_i])$ in the liver cell owing to a decrease in $[ATP]$ and an increase in $[P_i]$. 4. It is suggested that an increased sodium pump activity is responsible for the increased oxidative capacity and for the insensitivity to dinitrophenol observed in the livers of ethanol-treated animals.

It has been previously demonstrated (Videla & Israel, 1970) that the rate of ethanol metabolism by normal rat liver slices is increased in the presence of mitochondrial uncouplers. This fact suggested that in the rat liver the rate-limiting step in the metabolism of ethanol is the reoxidation of NADH by the mitochondria. Liver slices from animals chronically treated with ethanol metabolized ethanol at a greater rate that could not be further increased by dinitrophenol or arsenate. Work reported in the preceding paper (Videla *et al.*, 1973) showed that when O_2 consumption was measured, dinitrophenol stimulated the respiration of liver slices from control animals but not from chronically ethanol-fed animals in which the rate of O_2 consumption was already increased. These findings indicate that after chronic ethanol treatment the increased oxidative capacity of the liver is probably due to an increased ability of mitochondria to handle reducing equivalents.

These changes could conceivably be caused by mitochondrial uncoupling or by a shift in the cell from a state close to state 4 towards state 3 respiration (for definition of mitochondrial states see Chance & Williams, 1956). Sardesai & Walt (1969), Rubin *et al.* (1970) and Videla *et al.* (1973) have shown that isolated mitochondria prepared from animals chronically treated with ethanol appear not to be uncoupled. Results in the present paper indicate that after chronic treatment with ethanol the liver cell is in a state close to state 3 respiration, apparently because of an increased extra mitochondrial utilization of ATP. An important cellular mechanism that utilizes ATP is the $(Na^+ - K^+)$ -activated ATPase.* Previous work has shown that this activity is increased after chronic ethanol administration in rat, mouse and cat brain

and also in erythrocytes from human alcoholics (Israel *et al.*, 1970; Sun, 1970; Knox *et al.*, 1972), although in the mouse the results are not consistent and may depend on the method of ethanol administration (Goldstein & Israel, 1972). We have therefore studied, in the liver of ethanol-treated rats, the activity of the $(Na^+ - K^+)$ -activated ATPase and the active ion transport known to be dependent on this enzymic activity (Skou, 1965; Whittam & Wheeler, 1970). We have also examined, in animals chronically treated with ethanol and in control animals, the effects of inhibitors of the active transport of Na^+ and K^+ on the rate of O_2 consumption by liver slices.

Materials and Methods

Chronic ethanol treatment

Complete details of the type of diet used, pairing schedule, time of decapitation, etc. have been described in the preceding paper (Videla *et al.*, 1973). Basically, pair-fed rats were given liquid diets providing 35% of the total energy (cal) as ethanol (experimental group) or sucrose (control group) for 21–27 days. The average daily ethanol intake of animals in the experimental group was 14.3 ± 0.2 g/day per kg.

Active transport of ^{86}Rb by liver slices

The animals were killed by decapitation and liver slices of approx. 0.4 mm thickness and weighing about 40–50 mg (fresh wt.) were prepared by means of a Stadie–Riggs microtome. During the preparation the slices were kept on an ice-cold inverted petri-dish until all the slices were cut. Slices were distributed randomly among the incubation vessels in each experiment so that each vessel contained 120–150 mg of tissue in 10 ml of an anaerobic preincubation

*Abbreviation: ATPase, adenosine triphosphatase.

medium containing 118mM-NaCl, 5mM-RbCl, 2.5mM-CaCl₂, 1.2mM-NaH₂PO₄, 1.1mM-MgSO₄, 25mM-NaHCO₃ and 10mM-glucose. This medium was saturated with N₂+CO₂ (95:5) and the resulting pH was 7.3–7.4. After 30min preincubation at 37°C, during which the slices were shaken at 70–80 strokes/min, the slices were transferred to an aerobic medium with identical composition. Carrier-free ⁸⁶RbCl (New England Nuclear Corp., Boston, Mass., U.S.A.) was added to this medium to give a final specific radioactivity of 2000–3000c.p.m./μmol of Rb⁺. Slices were incubated as before for different times during which a mixture of O₂+CO₂ (95:5) was continuously bubbled into the incubation medium. When used, ouabain (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was added to the medium to a final concentration of 10mM. Preliminary experiments, in agreement with results of Bakkeren & Bonting (1968), showed that at this concentration the active transport of ⁸⁶Rb is completely abolished. At the end of the incubation period the slices were gently blotted on Whatman no. 54 filter paper and were dried at 106°C for 24h. The slices were then weighed and transferred to γ-counter tubes for the determination of radioactivity. Uptakes are expressed as mmol of Rb⁺/kg dry wt.

ATPase activity in liver homogenates

A 300mg sample of liver taken from the median lobe was homogenized in a Teflon–glass homogenizer in 4.0ml of ice-cold 0.32M-sucrose containing 1mM-EDTA–Tris buffer, at pH7.4. The homogenate was diluted fourfold with water and ATPase activity was determined for 4min at 37°C by using 0.1ml of this suspension. The incubation medium contained in a final volume of 1.0ml:100μmol of Tris–HCl buffer, pH7.4; 2μmol of ATP Tris, 4μmol of MgCl₂; 100μmol of NaCl; 20μmol of KCl; and 5μmol of NaN₃ added to decrease the activity of the Mg²⁺-stimulated ATPase (Schwartz & Laseter, 1963). When used ouabain was added to a final concentration of 1mM in the absence of Na⁺ and K⁺.

The reaction was initiated by addition of the diluted homogenate, previously warmed for 5min at 37°C, and stopped by addition of 0.5ml of silicotungstic acid reagent (Martin & Doty, 1949). The P_i liberated was extracted and determined as described by Gibbs *et al.* (1965). Since the (Na⁺+K⁺)-stimulated ATPase activity corresponds to only a small proportion of the total ATPase, all assays were done in quadruplicate. Liberation of P_i was linear with time up to at least 6min, but linearity was not perfect with respect to the amount of tissue incubated. Therefore care was taken always to incubate the same amount of tissue for both the control and treated animals. (Na⁺+K⁺)-stimulated ATPase activity corresponds to the total ATPase activity minus that in the presence

of ouabain and in the absence of added NaCl and KCl. Results are expressed as nmol of P_i/h per mg of fresh tissue or μmol of P_i/h per mg of protein. Protein was determined by the method of Lowry as described by Millar (1959).

O₂ consumption by liver slices

Slices were prepared as described for the active-transport experiments, except that each slice was cut into three to four smaller pieces and 20–30mg of tissue/flask was used to estimate O₂ consumption. Two series of experiments were performed; in the first the effect of chronic treatment with ethanol and the effects of ouabain and dinitrophenol were determined exactly as described in the preceding paper (Videla *et al.*, 1973). In the second series O₂ consumption was determined in a medium in which 5mM-Rb⁺ was used instead of K⁺ and 5mM-Tris buffer instead of NaHCO₃. For this purpose, 5mM-Tris base was added to the medium and adjusted to pH7.4 with HCl while saturated with O₂+CO₂ (95:5). To estimate the relative proportion of O₂ uptake dependent on the sodium pump, NaCl was replaced iso-osmotically by sucrose (Elshove & van Rossum, 1963; Whittam, 1964). The slices were pre-incubated under O₂+CO₂ (95:5) for 10min at 37°C in the sodium-containing buffer and were then transferred to the O₂-electrode vessel containing 3ml of either the NaCl buffer or the sucrose buffer equilibrated with air. O₂ consumption was determined from the slopes during the interval between 4 and 6min after transfer to the O₂-electrode vessel. Results are expressed as μmol of O₂/min per g of fresh tissue.

Determination of ATP, ADP, AMP and P_i

Animals were killed by a blow to the back of the head, so that the heart remained beating and blood circulation continued. About 1g of liver was removed as rapidly as possible and frozen in liquid N₂. Two persons collaborated on this operation to ensure that the liver sample was always completely frozen within 10s of the stunning of the animal. This is substantially less than the 30s calculated by Brosnan *et al.* (1970) as the time required after cessation of circulation to produce an anoxic state in the liver cell. A dry frozen liver powder was prepared in a mortar with liquid N₂ and was deproteinized in cold HClO₄ by the procedure of Adam (1965a). ADP and AMP were measured enzymically in a single operation as described by Adam (1965b). However, the values were obtained by allowing the reactions to reach complete equilibrium thus avoiding the extrapolation recommended by Adam (1965b). Only by this procedure were nearly quantitative recoveries obtained for internal standards of added ADP and AMP. ATP was determined with phosphoglycerate kinase accord-

ing to the Sigma Tentative Technical Bulletin (1967). P_i was determined by the method of Martin & Doty (1949). A Gilford 2400 recording spectrophotometer was utilized for all these determinations. The concentration of HPO_4^{2-} was taken to be 60% of the total phosphate concentration (Veech *et al.*, 1970). Recovery of AMP, ADP, ATP and P_i added to the frozen powder in amounts within the ranges found in the tissue was 90–100% for the adenine nucleotides and 99% for P_i .

Results

Table 1 shows the effect of ouabain, dinitrophenol and a combination of these two substances on the rate of O_2 utilization by liver slices of the control and chronically ethanol-treated animals. As previously

reported (Videla *et al.*, 1973) pretreatment with ethanol increased the rate of O_2 consumption by about 50%. Ouabain did not decrease the rate of O_2 consumption in liver slices of control animals but almost completely abolished the increase in those of the animals treated with ethanol. Further, dinitrophenol activated the O_2 consumption in slices from control animals but did not change that in slices from ethanol-treated animals. However, in slices from treated animals the effect of dinitrophenol could be shown again if ouabain was present.

Table 2 shows the effect of Na^+ on the rate of O_2 consumption by liver slices. In the presence of Na^+ in the incubation medium, chronic treatment with ethanol increased the rate of O_2 consumption by about 50%. Incubation in the Na^+ -free medium did not affect the rate of respiration of liver slices from

Table 1. *Effect of dinitrophenol and ouabain on the respiration of liver slices after chronic treatment with ethanol*

Animals kept on semi-synthetic liquid diets for 21–27 days, then starved for 18 h, were killed by decapitation and liver slices were prepared. Slices were preincubated with the inhibitors for 15 min before the polarographic determination of O_2 consumption. The values represent the means $\pm$ S.E.M. with the numbers of animals in parentheses. Statistical analysis was done by Student's *t* test for unpaired results: (a) versus (b), $P < 0.02$; (a) versus (d), $P < 0.001$; (b) versus (c), $P < 0.01$; (c) versus (d), $P < 0.001$; (d) versus (h), $P < 0.05$; (e) versus (g), $P < 0.001$; (f) versus (g), $P < 0.02$; (f) versus (h), $P < 0.02$; (g) versus (h), $P < 0.001$; (a) versus (e), $P < 0.001$; (a) versus (c), (a) versus (g), (b) versus (d), (e) or (f), (c) versus (g), (d) versus (e), (e) versus (f) or (h) were not statistically different. Dinitrophenol (0.1 mM) and ouabain (1 mM) were used.

	Additions		O_2 consumption (μ mol of O_2 /min per g of liver)
	Dinitrophenol	Ouabain	
Sucrose-control (a)	—	—	0.82 ± 0.04 (14)
(b)	+	—	1.11 ± 0.11 (9)
(c)	—	+	0.78 ± 0.05 (12)
(d)	+	+	1.25 ± 0.06 (7)
Ethanol-treated (e)	—	—	1.25 ± 0.08 (14)
(f)	+	—	1.09 ± 0.08 (9)
(g)	—	+	0.87 ± 0.06 (12)
(h)	+	+	1.41 ± 0.08 (7)

Table 2. *Effect of the omission of Na^+ from the incubation medium on respiration of liver slices after chronic treatment with ethanol*

Liver slices were prepared as in Table 1. Incubation in the Na^+ -free medium was for between 4 and 6 min. In this medium sucrose replaced NaCl iso-osmotically. The values represent the means $\pm$ S.E.M. with the numbers of animals in parentheses. *P* values were calculated by Student's *t* test for unpaired results. N.S., not significant.

Incubation medium	O_2 consumption (μ mol of O_2 /min per g of liver)		<i>P</i>
	Sucrose-control	Ethanol-treated	
Ringer	0.58 ± 0.03 (9)	0.82 ± 0.05 (9)	< 0.01
	N.S.	< 0.01	
Na^+ -free	0.58 ± 0.04 (9)	0.58 ± 0.05 (9)	N.S.

control animals but completely abolished the increase found in those from ethanol-treated animals. Absolute values of O_2 consumption are about 30% lower in this series than those in Table 1; however, this probably reflects the differences in experimental techniques (see the Materials and Methods section).

Fig. 1 shows that the active transport of ^{86}Rb , an analogue of K^+ (Bakkeren & Bonting, 1968; Bernstein & Israel, 1970), was significantly increased by 70% as a result of the chronic ethanol treatment. Total and passive transport (ouabain-insensitive) were slightly increased and decreased respectively. Table 3 shows that the activity of the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase, which is believed to represent the mechanism for the active transport of Na^+ and K^+ (Whittam & Wheeler, 1970), was increased by

180–190% when expressed as either units/mg of protein or units/mg of tissue. The Mg^{2+} -activated ATPase activity was decreased by 15% but this decrease was significantly different only when expressed as units/mg of protein.

Table 4 shows that phosphorylation potential is decreased in the livers of animals chronically treated with ethanol. The decrease is the result of a decrease in the cellular concentration of ATP and of an increase in P_i concentration. No change was found in the concentration of ADP nor in the adenylate kinase mass-action ratio, $[\text{ATP}][\text{AMP}]/[\text{ADP}]^2$, indicating that the nucleotides remain in equilibrium in this reaction. The concentrations of ADP and AMP obtained in control animals on the liquid diets are higher than those obtained by other workers also

Fig. 1. Rb^+ uptake by liver slices of rats after chronic treatment with ethanol

Liver slices were prepared as described in Table 1 and preincubated in Ringer solution for 30 min under $\text{N}_2 + \text{CO}$ (95:5) and then transferred to an oxygenated Ringer medium containing ^{86}Rb . Differences in active transport were analysed by comparing the slopes of the lines by means of analysis of variance. $F = 6.66$ ($P < 0.02$). The values are means, the bars represent $\pm \text{S.E.M.}$ and the numbers in parentheses represent the numbers of animals for each point. ●, Control animals; ○, ethanol-treated animals. (a) Active transport; (b) total transport; (c) passive transport.

Table 3. ATPase activity in liver homogenates after chronic treatment with ethanol

Experimental animals were treated as described in Table 1. Livers were homogenized in 0.32M-sucrose containing 1 mM-EDTA-Tris buffer, pH 7.4. The values represent the means $\pm \text{S.E.M.}$ with the numbers of animals used in parentheses. P values were calculated by Student's t test for paired results. N.S., not significant.

	Sucrose-control	Ethanol-treated	<i>P</i>
$(\text{Na}^+ + \text{K}^+)$-activated ATPase			
(μmol of P_i /h per mg of protein)	0.32 ± 0.05 (8)	0.89 ± 0.09 (8)	<0.001
(nmol of P_i /h per mg of tissue)	60.32 ± 10.49 (6)	175 ± 28.77 (6)	<0.01
Mg^{2+}-activated ATPase			
(μmol of P_i /h per mg of protein)	4.40 ± 0.22 (8)	3.66 ± 0.13 (8)	<0.02
(nmol of P_i /h per mg of tissue)	945 ± 45.1 (6)	840 ± 45.7 (6)	N.S.

Table 4. *Effect of chronic ethanol treatment on the amounts of adenine nucleotides and P_i of the liver*

Animals kept for 21–26 days on semi-synthetic liquid diets were starved for 18h then killed by cervical dislocation and a portion of liver was frozen in liquid N_2 in less than 10s. Nucleotides and P_i were determined as described in the Materials and Methods section. The values represent the means $\pm$ S.E.M. of eight animals in each group. The P values were calculated by Student's t test for paired results. N.S., not significant.

	Amount of compound ($\mu\text{mol/g}$ of liver)		P
	Sucrose-control	Ethanol-treated	
ATP	2.43 ± 0.27	1.70 ± 0.20	<0.01
ADP	2.31 ± 0.16	2.16 ± 0.10	N.S.
AMP	1.11 ± 0.08	1.19 ± 0.16	N.S.
Total nucleotides	5.85 ± 0.36	5.05 ± 0.25	N.S.
HPO_4^{2-}	2.85 ± 0.26	3.72 ± 0.35	<0.01
$[\text{ATP}]/[\text{ADP}][\text{HPO}_4^{2-}]$ (M^{-1})	410 ± 60	250 ± 50	<0.001
$[\text{ATP}][\text{AMP}]/[\text{ADP}]^2$	0.53 ± 0.08	0.43 ± 0.05	N.S.

using the stop-freeze technique. Similar results were found, however, for ATP and P_i (Brosnan *et al.*, 1970; Stubbs *et al.*, 1972; Veech *et al.*, 1972).

Discussion

The results show that chronic treatment of rats with ethanol produces a marked increase in the rate of O_2 consumption in the liver, as measured *in vitro*. This extra utilization of O_2 could be completely blocked by ouabain, an inhibitor of the active transport of Na^+ and K^+ and of the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase (Skou, 1965; Whittam & Wheeler, 1970), and by replacement of Na^+ by sucrose in the incubation medium, which is also known to inhibit the sodium pump (Elshove & van Rossum, 1963; Whittam, 1964; Skou, 1965; Ruscak & Whittam, 1967; Seidman, 1970; Sjodin, 1971). The extra O_2 consumption therefore appears to depend on the activity of the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase. No significant effects were found in liver slices from control animals when incubated with ouabain or when Na^+ was replaced by sucrose, indicating that in these animals the influence of the sodium pump in the overall O_2 consumption is negligible.

The active transport of cations in slices measured as uptake of ^{86}Rb , an analogue of K^+ (Bernstein & Israel, 1970), and the $(\text{Na}^+ + \text{K}^+)$ -activated ATPase activity measured in homogenates were markedly increased in the liver of animals chronically treated with ethanol. This is to be expected if an increased sodium pump activity is responsible for the stimulated respiration observed in liver slices from these animals.

It is well known that the rate of O_2 consumption by mitochondria is regulated by the relative concentrations of adenine nucleotides and P_i . When these are expressed as $[\text{ATP}]/[\text{ADP}][P_i]$, the ratio is often referred to as the phosphorylation potential. A decrease in this potential increases the rate of respiration

(Chance & Maitra, 1963; Klingenberg, 1968, 1969). Results presented in the present paper show that the phosphorylation potential of the rat liver cell was decreased after chronic treatment with ethanol. The drop in the phosphorylation potential was solely due to changes in the cellular $[\text{ATP}]$ and $[P_i]$, since no change in $[\text{ADP}]$ was found.

The ADP and AMP amounts found by us in the liver of overnight-starved animals previously kept for 21–27 days on semi-synthetic liquid diets are higher than those reported by other workers for starved animals kept on normal chow diets (Stubbs *et al.*, 1972; Veech *et al.*, 1972). This is not due, however, to anoxic conditions in our livers since the amount of ATP, the adenine nucleotide most clearly affected by ischemia, was normal in the sucrose-fed controls. Further, anoxia could not explain the high ADP amounts, because the content of ADP in the liver does not increase under anoxic conditions (Brosnan *et al.*, 1970). Amounts of AMP such as those in Table 4 are to be expected only after 5 min of anoxia (Brosnan *et al.*, 1970), which is obviously not the case in our experiments in which the liver was frozen in liquid N_2 in less than 10s (see the Materials and Methods section). A plausible explanation for these differences must therefore be sought in the diets employed, the animals used, differences in experimental procedures or a combination of these factors.

Control animals kept on chow diet showed values $\pm$ S.E.M. (numbers of animals) in $\mu\text{mol/g}$ of liver of: 2.66 ± 0.19 (3) for ATP; 1.87 ± 0.15 (3) for ADP; 0.86 (2) for AMP and 1.97 (2) for HPO_4^{2-} . These values give a phosphorylation potential of about 750 and an adenylate kinase mass-action ratio of 0.6, which are in good agreement with findings by other investigators. Thus it can be seen that the liquid diets are only partly responsible for the increases in both ADP and AMP. It should also be observed that $[\text{ATP}]$ appeared slightly decreased by

the liquid diets, in agreement with results of Walker & Gordon (1970), who reported a significant decrease in ATP content by this type of diet. The adenylate kinase equilibrium does not change with the type of diet despite a decrease in phosphorylation potential, as would be expected for a reaction that is in equilibrium in the cell. Mass-action ratios for adenylate kinase between 0.4–0.6 are those accepted as normal under physiological conditions (Brosnan *et al.*, 1970; Eggleston & Hems, 1952). Values higher than these may well be due to underestimation of ADP and AMP content since both are usually determined in a single operation. A very sensitive indicator for anoxia is the decrease in ATP content, and if a ratio is desired the $[ATP]/[P_i]$ ratio would appear to be the most reliable. Further, variations in ADP and AMP contents cannot be interpreted with certainty either in qualitative or in quantitative terms because of fast-draining reactions for these nucleotides. The commonly used $[ATP]/[ADP]$ ratio has little meaning, since it will only reflect the decrease in $[ATP]$ and there is therefore no reason to prefer it over the value of ATP content alone.

The finding that the chronic treatment with ethanol did not increase the ADP content of the liver while decreasing that of ATP could, however, be caused by the fact that variations in the concentration of ADP are minimized by the adenylate kinase system that appears to be in equilibrium in the cell. It is not clear why the concentration of AMP was not increased after chronic treatment with ethanol. A decrease in the ATP content of the liver after chronic administration of ethanol has also been reported by French (1966), Ammon & Estler (1967) and Walker & Gordon (1970). The latter authors have also reported an increase in the P_i content in the liver.

The decrease found in the phosphorylation potential could explain the increased rate of O_2 consumption by the livers of animals chronically treated with ethanol (Tables 1 and 2 and Videla *et al.*, 1973) and might also explain the increased rate of ethanol metabolism which we have previously reported (Videla & Israel, 1970). In agreement with this is the observation that dinitrophenol did not further increase the respiratory rate (Table 1) or the ethanol metabolism in liver slices from ethanol-treated animals (Videla & Israel, 1970). However, the dinitrophenol effect could be recovered in the presence of ouabain. This indicates that the active transport of Na^+ and K^+ is implicated not only in the increased O_2 consumption, but also in the dinitrophenol-insensitivity in the treated animals.

An increased $(Na^+ + K^+)$ -stimulated ATPase activity, as found in the ethanol-treated animals, could result in changes in the phosphorylation potential, with a concomitant change from a state close to state 4 towards state 3 respiration and loss of the dinitrophenol effect. Ouabain would block these changes.

Assuming a P/O ratio of 3 for the intact cell it can be calculated that the activity of the $(Na^+ + K^+)$ -stimulated ATPase in the ethanol-treated animals can account for all the ouabain-sensitive O_2 consumption in these animals ($46 \mu g$ -atoms of O/h per g of tissue versus $175 \mu mol$ of P_i/h per g of tissue). It should be considered, however, that the activity of this enzyme was measured in homogenates. In the treated animals ouabain-sensitive ^{86}Rb uptake by liver slices corresponds to about $46 \mu mol$ of Rb^+/h per g of tissue. The K/ATP (or Rb/ATP) coupling ratio is not known for liver. However, the number of g-atoms of K^+ transported/mol of ATP consumed varies from 2:1 in erythrocytes (Sen & Post, 1964; Whittam & Ager, 1965) to 1:1 in brain cortex and 1:3 in seminal vesicles (Whittam, 1964). If one assumes a Rb/ATP ratio of 1:3 in liver, the active transport of ^{86}Rb could also account for the increased ouabain-sensitive O_2 consumption in liver slices from ethanol-treated animals.

These results would also suggest that when the rate of active transport in liver is sufficiently high, this process becomes the pacemaker of respiration, as is known to occur in other tissues (Whittam, 1964). Our results show that in the ethanol-treated animals the $(Na^+ + K^+)$ -stimulated ATPase activity increases proportionally more than the active transport of Rb^+ (190% versus 70%). Similar observations have been made in the brain of rats chronically treated with ethanol and in erythrocytes from human alcoholics (Israel *et al.*, 1970). This could be caused by a change in the coupling ratio as a result of the chronic ethanol treatment. If this is the case an increased utilization of ATP would be required to actively transport the same number of ions. This alteration would amplify the effect on O_2 consumption produced by an increase in the active ion transport. A change in the coupling ratio is also suggested by the fact that in animals chronically treated with ethanol the ouabain-sensitive O_2 consumption increased proportionally more than the actual active ion transport.

The increase in the activity of the sodium pump could constitute a compensatory mechanism to overcome the inhibitory effect of ethanol on the pump and on the $(Na^+ + K^+)$ -stimulated ATPase. This inhibitory action of ethanol seems to be a general phenomenon since many tissues appear to be affected in the same manner (Israel, 1970). Work by Kalant *et al.* (1966) and J. Bernstein (unpublished work) suggests that it is true in liver also. The possibility that these changes could be also explained by other mechanisms that alter the sodium pump is dealt with in the following paper (Israel *et al.*, 1973).

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