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ETHANOL METABOLISM AND LIVER OXIDATIVE CAPACITY IN COLD ACCLIMATION

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ABSTRACT

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Exposure of rats to an ambient temperature of 5°C for 4 to 6 weeks led to a 30 to 80% increase in the rate of oxygen consumption and a 50% increase in the rate of ethanol oxidation by liver slices, a 50% increase in mitochondrial α-glycerophosphate oxidase activity of liver, and a 100% increase in $Na^+ + K^+$-activated adenosine-triphosphatase, activity. Ouabain, an inhibitor of the $Na^+ + K^+$-activated adenosine-triphosphatase, completely blocked the extra respiration and ethanol oxidation. Dinitrophenol, which increases oxygen consumption and ethanol oxidation by liver slices from normal rats, was ineffective with slices from cold-exposed animals. Ethanol disappearance rate *in vivo* was also increased by cold acclimation, even though liver alcohol dehydrogenase activity was reduced. It is suggested that increased hydrolysis of ATP by the sodium pump system is responsible for the increased oxygen consumption and ethanol metabolism in the livers of cold-acclimated animals.

Work presented in the preceding paper (Israel *et al.*, 1975) indicates that the increase in the rate of ethanol metabolism by the liver after chronic ethanol administration appears to be mediated by an increase in the utilization of adenosine triphosphate (ATP) by the cell-membrane $Na^+ + K^+$-activated adenosine triphosphatase [(Na + K)-ATPase]. This enzyme and the active transport of cations have been previously reported to be increased in the liver of ethanol-treated animals. An increased utilization of ATP by this system also appears to be responsible for the marked increase in the rate of oxygen consumption of these livers (Bernstein *et al.*, 1973). An increased ATP utilization by the (Na + K)-ATPase system has recently been postulated as the mechanism of the calorigenic action of thyroid hormones in the liver (Ismail-Beigi and Edelman, 1970; 1971). Israel *et al.* (1973) suggested that the changes produced in the liver by chronic ethanol intake are similar to many of the changes after thyroid hormone administration.

Prolonged exposure of rats to cold has been shown to produce a 2- to 3-fold increase in heat production (when measured at the cold ambient temperature) which enables the animals to maintain a normal body temperature

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(Sellers and You, 1950; Sellers *et al.*, 1951). Initially this increase is related to an increased activity of skeletal muscle (shivering thermogenesis) (Sellers *et al.*, 1954; Heroux *et al.*, 1956; Hart *et al.*, 1956; Jansky, 1973) and this can be blocked by curare (Cottle and Carlson, 1956). However, after a week or two of exposure to cold, the increased muscle activity declines although heat production remains high (nonshivering thermogenesis, NST) (Sellers *et al.*, 1954; Cottle and Carlson, 1956; Sellers, 1957; Davis, 1959). In this situation, norepinephrine has been shown to mediate the increased heat production, which can be blocked by *beta* adrenergic blocking drugs (Hsieh and Carlson, 1957; Hsieh *et al.*, 1957).

It has been suggested that thyroid hormones play a permissive role in nonshivering thermogenesis; thyroidectomized animals fail to survive the cold treatment (Sellers and You, 1950). Furthermore, studies by Weiss (1957) have indicated that only those tissues that show a calorigenic response to thyroid hormones show increased rates of oxygen consumption after exposure to cold. Many of the effects elicited by epinephrine and norepinephrine are markedly enhanced (supersensitivity) in hyperthyroidism and are reduced in thyroidectomized or hypothyroid animals (Harrison, 1964). Marked supersensitivity to epinephrine and norepinephrine has also been observed in cold-acclimated animals (Hsieh and Carlson, 1957; Bartunkova *et al.*, 1971; LeBlanc, 1971; Himms-Hagen *et al.*, 1972). Thus, the calorigenic effects produced by thyroid hormones and by sympathetic stimulating agents appear to be interdependent. Furthermore, the calorigenic effects of epinephrine and norepinephrine on brown adipose tissue also appear to be mediated by an increased activity of the sodium pump (Girardier *et al.*, 1968; Herd *et al.*, 1970, 1973; Horwitz, 1971, 1973).

After cold exposure, it has been shown that both the rate of oxygen consumption by liver slices (You and Sellers, 1951; Weiss, 1954, 1957; Hannon, 1958) and the rate of ethanol metabolism *in vivo* (Forbes and Duncan, 1961; Platanow *et al.*, 1963) are increased; however, the mechanism for the increased oxidative capacity of the liver has not been elucidated. Therefore we have studied the role of the (Na + K)-ATPase system in the changes leading to an increased oxidative capacity and an increased ethanol metabolism in the livers of rats exposed to cold.

Materials and Methods

Male Wistar rats (Canadian Breeding Laboratories, Montreal, Quebec, Canada or High Oak Ranch Ltd., Toronto, Ontario, Canada) were divided into two groups and housed singly in cages in either a cold room at 5°C or a room at 23°C (age-controls) for 4 to 6 weeks. The animals were supplied with food and water *ad libitum*. In some experimental series, rats weighing 186 ± 2 g were purchased 20 to 25 days before sacrifice and were kept in the same conditions as the age-control rats until their weights were essentially the same as those of the animals exposed to cold (weight-controls). The initial average weights of the animals were 168 ± 3 g (age-control) and 173 ± 1 g (cold-exposed group). At the end of the period of exposure to cold, the average weights were 424 ± 8 g (age-control) and 326 ± 7 g (cold-exposed group).

ATPase activity in liver homogenates was determined as described by Bernstein *et al.* (1973), except that AT^{32}P (γ-labeled, New England Nuclear Corporation, Boston, Mass.,) was used as the substrate at a final concentration of 2 mM (50 nc/μmol). The released ^{32}P was extracted in isobutanol as phosphomolybdate and was measured in a liquid scintillation counter. Proteins were determined by the biuret method (Gornall *et al.*, 1949).

Ethanol metabolism and oxygen consumption by rat liver slices in the presence and absence of dinitrophenol and ouabain, ethanol metabolism *in vivo*, alcohol dehydrogenase activity in the cytosol fraction of liver homogenates and mitochondrial α-glycerophosphate oxidase activity were determined as described previously (Israel *et al.*, 1975).

Results are presented as mean ± S.E.M. and the significance of differences between means was determined according to Student's *t* test.

Results

Table 1 shows the rate of oxygen consumption by liver slices of cold-adapted and control animals. As can be observed, the rate of oxygen consumption was markedly increased by 2,4-dinitrophenol (DNP) in age-control and weight-control animals in both experimental series. In the cold-exposed group, the rate of oxygen consumption of the liver was found to be increased and was no longer sensitive to DNP.

TABLE 1

Effects of dinitrophenol and ouabain on the rate of oxygen consumption by rat liver slices after exposure to cold

Liver slices were prepared as described by Videla and Israel (1970) and incubated in Krebs-Ringer-bicarbonate buffer at 37°C (Israel *et al.*, 1975).

	Additions		Oxygen Consumption	
	DNP [a]	Ouabain [b]	Expt. A [c]	Expt. B [d]
			μmol/g liver/min	
Age-control	—	—		0.90 ± 0.03 (4)
	+	—		1.26 ± 0.10[e] (4)
	—	+		0.85 ± 0.02 (4)
Weight-control	—	—	0.95 ± 0.03 (7)[f]	1.22 ± 0.05 (4)
	+	—	1.80 ± 0.07[e] (7)	2.08 ± 0.13[e] (4)
	—	+	0.96 ± 0.04 (7)	1.30 ± 0.05 (4)
Cold-exposed	—	—	1.22 ± 0.04[g] (8)	1.62 ± 0.06[f] (4)
	+	—	1.33 ± 0.06 (8)	1.50 ± 0.10 (4)
	—	+	0.88 ± 0.06[e] (8)	1.30 ± 0.05[e] (4)

[a] [DNP] = 100 μM.

[b] [Ouabain] = 1 mM.

[c] Animals killed under ether anesthesia in order to obtain blood for simultaneous studies of another problem (K. V. Flattery, unpublished data).

[d] Animals killed by decapitation.

[e] Significantly different with respect to slices incubated with no additions for the same animal ($P < .02$).

[f] Values shown are mean ± S.E.M.; number of animals is shown in parentheses.

[g] Significantly different with respect to either age- or weight-controls without additions ($P < .01$).

The increase in the rate of oxygen consumption of the liver of the cold-exposed animals was blocked by ouabain, an inhibitor of the Na + K)-ATPase system. Oxygen consumption in the liver of the younger weight-control rats was found to be significantly higher than that of the older age-control animals (table 1, experiment B; $P < .01$). This is in agreement with data by other investigators (Terroine and Roche, 1925; Kleiber, 1941; Krebs, 1950; Weiss, 1954).

Table 2 shows that the activity of the hepatic (Na + K)-ATPase was increased by 100% in the animals exposed to cold, when expressed as either units per milligram of protein or units per milligram of tissue. The Mg^{++}-activated ATPase activity was increased by about 10% but this increment was significantly different only when expressed as units per milligram of tissue.

Table 3 shows the effect of cold exposure on the rate of ethanol metabolism by liver slices. As shown previously, (Videla and Israel, 1970; Israel *et al.*, 1975) ethanol metabolism was markedly increased by DNP in the livers of control rats. Cold exposure led to a 50% increase in the rate of ethanol metabolism; in this situation, DNP was not able to increase

TABLE 2

ATPase activity in liver homogenates after exposure to cold

Enzymatic assay was performed at 37°C as described by Bernstein *et al.* (1973).

	Weight-control	Cold-exposed	*P*
	(*n* = 8) [a]	(*n* = 8)	
$(Na^+ + K^+)$-activated ATPase			
(nmol P_i/mg protein/hr)	101.2 ± 26.1	207.1 ± 31.9	<.05
(μmol P_i/g tissue/hr)	19.4 ± 5.0	40.3 ± 6.4	<.05
Mg^{2+}-activated ATPase			
(μmol P_i/mg protein/hr)	2.92 ± 0.12	3.22 ± 0.17	N.S.
(mmol P_i/g tissue/hr)	0.56 ± 0.02	0.62 ± 0.02	<.05

[a] *n*, number of animals.

the rate of ethanol metabolism further, whereas the extra ethanol metabolism was completely abolished by ouabain. The effect of cold acclimation on the rate of ethanol metabolism was also studied *in vivo* and the results are presented in table 4. Ethanol metabolism, expressed in milligrams per kilogram of rat per hour, was found to be increased by about 50% after exposure of the animals to cold, when compared with age-control animals. However, this effect was not observed when results obtained with cold-adapted rats were compared with weight-controls. This lack of effect is due to the fact that weight-control rats were younger than the cold-exposed ones and had a significantly higher liver/body weight ratio. When the values given in milligrams per kilogram of rat per hour are corrected and expressed in terms of liver weight, it can be observed that in the cold-exposed animals, ethanol metabolism increases significantly by 21 and 37% when compared to weight and age-control animals, respectively. Under these conditions, alcohol dehydrogenase (ADH) activity was found to be decreased in the liver of the animals exposed to cold when compared to both groups of control animals at 23°C (table 5).

Mitochondrial α-glycerophosphate oxidase activity has been shown to be increased by both chronic ethanol treatment and thyroxine administration (Israel *et al.*, 1973). The same results were obtained after prolonged exposure of the animals to cold: cold-exposed: 4.2 ± 0.4 (8) nmol/mg of protein per min; weight-control: 2.8 ± 0.3 (8); $P < .05$. This is in agreement with data of Ruegamer *et al.* (1964).

TABLE 3

Effect of dinitrophenol and ouabain on the rate of ethanol metabolism by rat liver slices after exposure to cold

Slices were incubated at 37°C (see legend for table 1) and ethanol metabolism was measured as described by Israel *et al.*, (1975).

	Additions		Ethanol Metabolism (n = 5) [c]
	DNP [a]	Ouabain [b]	
			μmol/g liver/hr
Age-control	−	−	23.5 ± 3.9
	+	−	45.7 ± 5.3[d]
	−	+	23.5 ± 3.3
Weight-control	−	−	23.8 ± 3.2
	+	−	43.3 ± 7.9[d]
	−	+	25.4 ± 5.9
Cold-exposed	−	−	35.6 ± 2.9[e]
	+	−	30.7 ± 3.7
	−	+	17.6 ± 2.1[d]

[a] [DNP] = 100 μM.

[b] [Ouabain] = 1 mM.

[c] n, number of animals.

[d] Significantly different with respect to slices incubated with no additions in the same group of animals ($P < .05$).

[e] Significantly different with respect to either age or weight controls without additions ($P < .05$).

Discussion

The rate of oxygen consumption by liver slices of cold-acclimated rats was found to be

TABLE 4

Effect of cold exposure on the rate of ethanol metabolism in vivo

	Ethanol Metabolism	Liver/Body Weight Ratio	Ethanol Metabolism
	mg/kg rat/hr	*g liver/100 g rat*	*mg/g liver/hr*
Age-control (6)[a]	203 ± 19	3.28 ± 0.20	6.16 ± 0.39
Weight-control (6)	310 ± 21	4.45 ± 0.11	6.95 ± 0.40
Cold-exposed (6)	302 ± 24[b,c]	3.59 ± 0.07[d]	8.42 ± 0.80[b,e]

[a] n, number of animals.

[b] Significantly different with respect to age-control ($P < .05$).

[c] Ethanol metabolism was measured while the animals were still at 5°C; that for the control animals was done at 23°C.

[d] Significantly different with respect to weight-control ($P < .001$).

[e] Significantly different with respect to weight-control ($P < .05$).

TABLE 5

Alcohol dehydrogenase activity in the liver of rats exposed to cold

Assays were performed at room temperature as described previously (Israel *et al.*, 1975).

	Alcohol Dehydrogenase Activity
	nmol/mg protein/min
Age-control	7.2 ± 0.5[a] (4)[b]
Weight-control	8.7 ± 0.4 (8)
Cold-exposed	6.2 ± 0.3[c] (8)

[a] Age-control *vs.* cold-exposed ($.1 > P > .05$).

[b] Number in parentheses is number of animals.

[c] Significantly different with respect to weight-control ($P < .001$).

increased by 30 to 80%. This is in agreement with previous data in which oxygen consumption was measured *in vitro* in liver slices (You and Sellers, 1951; Weiss, 1954, 1957; Hannon, 1958). Studies by Kawahata and Carlson (1959) have reported increases of 85% in liver oxygen consumption in cold-acclimated rats *in vivo*. Our studies show that all the increased metabolic capacity in the liver slices from cold-acclimated animals could be blocked by ouabain, an inhibitor of the (Na + K)-ATPase, thus suggested that an increased sodium pump activity is responsible for the increased oxygen utilization in these livers. Similar results have been recently obtained with preparations of diaphragm muscle from rats exposed to cold (Mokhova and Zorov, 1973). The idea of an involvement of the sodium pump is further supported by the fact that the (Na + K)-ATPase activity of the liver was increased by 100%. It is also interesting to note that DNP was not able to increase the rate of oxygen consumption further in the liver of the cold-acclimated animals. This would indicate that the mitochondrial oxidative capacity in the liver of these animals is no longer limited by the amount of phosphate acceptor. A reduction in the sensitivity to DNP is to be expected when a system which utilizes ATP and produces adenosine diphosphate, such as the (Na + K)-ATPase, is increased.

Previous studies indicate that the capacity of the liver tissue to metabolize ethanol depends on the rate of mitochondrial oxidation of reducing equivalents, since ethanol oxidation can be increased by mitochondrial uncouplers (Videla and Israel, 1970; Israel *et al.*, 1970, 1975; Seiden *et al.*, 1974). Table 6 shows that the rate of ethanol metabolism both *in vitro* and *in vivo* correlates in the liver of cold-acclimated animals and in controls. A good correlation also exists between *in vitro* and *in vivo* rates of ethanol metabolism when expressed per unit of liver weight. No correlation, however, exists between the rate of ethanol metabolism and the activity of ADH; this is consistent with our postulate (Videla and Israel, 1970) that the amount of ADH does not constitute the rate-limiting factor in ethanol metabolism.

Our data also suggest that the *in vivo* rate of ethanol metabolism should correlate with the rate of oxygen utilization by the liver in different species. Unfortunately, no data could be found on the relative rates of oxygen utilization by the liver of different species, using the same technique and experimental conditions. However, it is to be expected that liver metabolic

TABLE 6

Correlation between rates of oxygen consumption, ethanol metabolism and ADH activity in rat liver of age-control, weight-control and cold-exposed animals

	Correlation Coefficient (r)
QO_2[a] (*in vitro*) *vs.* Q_{ETOH}[b] (*in vivo*)	0.99
QO_2 (*in vitro*) *vs.* Q_{ETOH} (*in vitro*)	0.90
Q_{ETOH} (*in vitro*) *vs.* Q_{ETOH} (*in vivo*)	0.93
ADH activity[c] *vs.* Q_{ETOH} (*in vivo*)	−0.53
ADH activity *vs.* Q_{ETOH} (*in vitro*)	−0.80

[a] QO_2 = rate of oxygen consumption (micromoles per gram wet weight liver per minute): age-control: 0.90; weight-control: 1.22; cold-exposed: 1.62 (from table 1, experiment B).

[b] Q_{ETOH} = rate of ethanol metabolism. Experiments *in vitro* (micromoles per gram fresh weight liver per hour): age-control: 23.5; weight-control: 23.8; cold-exposed: 35.6 (from table 3). Experiments *in vivo* (milligrams per gram of liver per hour): age-control 6.16; weight-control: 6.95; cold-exposed: 8.42 (from table 4).

[c] ADH = alcohol dehydrogenase activity in liver supernatants (nanomoles per milligram of protein per minute): age-control: 7.2; weight-control: 8.7; cold-exposed: 6.2 (from table 5).

rate, in turn, is correlated with the basal metabolic rate for the whole animal. Figure 1 shows that the *in vivo* rate of ethanol metabolism does indeed correlate ($P < .001$) with the basal metabolic rate for a large number of species. This is in agreement with previous data by Lester and Keokosky (1967) who found that the rate of ethanol metabolism was proportional to the 0.75 power of the animal weight, which is known to correlate with the basal metabolic rate for different species.

Both the mitochondrial oxidative capacity of the intact liver cell and the rate of ethanol metabolism are increased in animals chronically treated with ethanol or with thyroxine (Videla *et al.*, 1973; Israel *et al.*, 1975). The increased metabolism induced after these two types of treatment appears to be mediated by an increased activity of the (Na + K)-ATPase system (Bernstein *et al.*, 1973; Israel *et al.*, 1973, 1975). We have now shown that the liver of cold-acclimated animals, in which the metabolic capacity is also increased, apparently by an increased sodium pump activity, also metabolizes ethanol at a higher rate. Furthermore, in these livers, the increased metabolism of ethanol cannot be further increased by DNP; this is also true in animals chronically treated with ethanol or given thyroxine. In all cases, however, this increased metabolic rate can be abolished by ouabain.

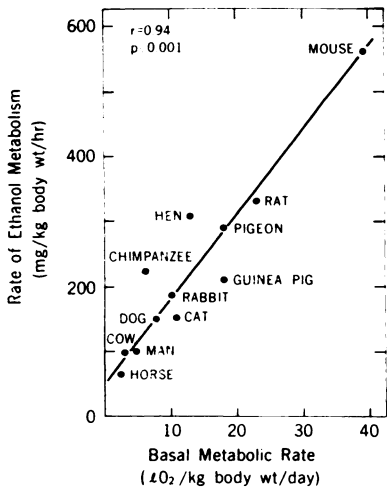

FIG. 1. Correlation between the *in vivo* rate of ethanol metabolism and the basal metabolic rate for several species. Data of ethanol metabolism for the different species were taken from Wallgren (1970) and Lundquist (1971), except for the chimpanzee (Pieper and Skeen, 1973). Data of basal metabolic rates in the same species were taken from Kleiber (1961) and Prosser and Brown (1962). Regression line: $y = 50.6 + 12.8x$; $P < .001$. A similar correlation is obtained when basal metabolic rate is plotted against the *in vivo* rate of ethanol metabolism expressed as milligrams of ethanol metabolized per gram of liver × hour ($y = 2.3 + 0.29x$; $r = .95$; $P<.001$).

Although it is known that thyroidectomized or hypothyroid animals are not able to acclimate to cold (Sellers and You, 1950; Johnson *et al.*, 1967), there has been much controversy as to whether thyroid hormones exert more than a permissive role in nonshivering thermogenesis in acclimation to cold (Jansky, 1973; Gale, 1973). The increases in (Na + K)-ATPase activity and in ouabain-sensitive oxygen consumption we have found in the liver of cold-acclimated animals parallel those obtained in euthyroid animals given thyroid hormones (Ismail-Beigi and Edelman, 1970, 1971; Israel *et al.*, 1973). However, it has been reported that plasma thyroxine levels in cold-acclimated animals are somewhat reduced rather than increased (Jobin *et al.*, 1971; Reichlin *et al.*, 1973). The rather recent recognition that clinical thyrotoxicosis can occur in the presence of normal levels of thyroxine but with higher levels of triiodothyronine (Hollander, 1968; Sterling *et al.*, 1970b), and the finding that thyroxine is peripherally converted into the more active triiodothyronine (Braverman *et al.*, 1970; Sterling *et al.*, 1970a, 1973; Pittman *et al.*, 1971; Schwartz *et al.*, 1971), suggest that plasma levels of triiodothyronine should also be considered. It is therefore most interesting that Reichlin and co-workers (1973) have reported that in cold-acclimated animals, plasma levels of triiodothyronine increase up to 80%. Recent studies by Balsam (1974) have also shown an increased metabolism of thyroid hormones, and an increased uptake of thyroxine by the liver in cold-acclimated rats. These studies and the observations of Weiss (1957) who found that only the tissues that respond to thyroid hormones with a calorigenic effect show an increased rate of oxygen consumption after cold acclimation would suggest a direct involvement of thyroid hormones in nonshivering thermogenesis. A hyperthyroid state in cold-acclimated rats is also suggested by the increased mitochondrial α-GP oxidase activity in the livers of

these animals. This enzyme has been classically considered a good indicator of thyroid hormone levels in the rat (Lee and Lardy, 1965; Ruegamer *et al.*, 1965).

It is well established that administration of thyroid hormones produces marked supersensitivity to many of the effects produced by norepinephrine and epinephrine (Harrison, 1964). Such a supersensitivity is also known to occur in cold-acclimated animals (Hsieh and Carlson, 1957; Bartunkova *et al.*, 1971; LeBlanc, 1971; Himms-Hagen *et al.*, 1972). A functional hyperthyroid state in cold-acclimated animals would explain this supersensitivity.

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