Both acute and chronic exercise enhance in vivo ethanol clearance in rats

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ARDIES, C. M., G. S. MORRIS, C. K. ERICKSON, AND R. P. FARRAR. *Both acute and chronic exercise enhance in vivo ethanol clearance in rats.* J. Appl. Physiol. 66(2): 555–560, 1989.—Rates of ethanol clearance were measured at rest and with acute exercise in four groups of female Sprague-Dawley rats. Two groups were trained to run on a motor-driven rodent treadmill at 27 m/min, 1 h/day, 5 days/wk and were given a nutritionally balanced liquid diet; one of these groups received 35% calories as ethanol whereas in the other, sucrose was isocalorically substituted for the ethanol. Appropriate sedentary and nonethanol controls were also used. Clearance of a 1.75-g/kg ethanol dose injected intraperitoneally was determined by measuring ethanol levels in the blood each hour and utilizing these values in the Widmark equation (R. Teschke, F. Moreno, and A. Petrides, *Biochem. Pharmacol.* 30: 1745–1751, 1981) for calculating whole-body ethanol clearance. Rates of ethanol clearance were determined for each rat at 4 and 7 wk of training. The clearance tests at 4 wk included a 60-min period of running exercise, whereas the tests 3 wk later were conducted at rest. The results indicate that both acute exercise and exercise training can increase rates of in vivo ethanol clearance. In addition, the chronic exercise appeared to increase in vitro ethanol metabolism by hepatic microsomes without altering in vitro hepatic alcohol dehydrogenase activity.

ethanol clearance rates; in vitro ethanol metabolism; in vitro hepatic alcohol dehydrogenase activity

IN A PREVIOUS STUDY from this laboratory (13) we observed that levels of ethanol in the blood of exercise-trained rats fed 36% of their calories as ethanol were generally lower than sedentary rats fed the same ethanol-containing diet. This observation led us to study the possible interactive effects of chronic ethanol consumption and acute and chronic exercise on ethanol clearance in vivo.

It is well known that ethanol consumption results in enhanced rates of ethanol metabolism (8, 21, 25, 34); however, the influence of acute exercise and chronic exercise on ethanol clearance rates has not yet been adequately determined. Carpenter et al. (5), Canzanelli et al. (4), and Pawan (28) have observed that muscular activity does not affect ethanol metabolism. More recent evidence, however, provided by Schurch et al. (31), has indicated that exercise may increase the rate at which ethanol is cleared from the blood. These results indicate that both intensity and duration of the activity used may be important factors in determining whether exercise might affect ethanol clearance. Schurch et al. (31) employed a 90-min bicycle ride at 50% of maximal O_2 consumption, whereas the work loads used in the previous studies were greater and of shorter duration. In a preliminary study (1) we tested this hypothesis in rats by determining ethanol clearance with a 1-h period of running at 75% of maximal O_2 consumption (7) starting 60 min after an ethanol injection. We then compared clearance rates with those obtained from a second group of rats tested without the exercise. We observed that clearance rates with exercise were significantly elevated compared with the resting animals. In the present study we confirm that rates of ethanol clearance are enhanced by acute running exercise and also demonstrate that 7 wk of endurance training can alter resting rates of ethanol clearance.

METHODS

Animal care. Sprague-Dawley rats were obtained from Timco Breeding Ranch (Houston, TX) and housed four per cage until used. When treated they were then housed separately in stainless steel mesh-bottomed cages in an animal care facility maintained at 22°C with a 12-h light-dark cycle. The lights remained on from midnight until noon each day.

Diet. Liquid diets were formulated to meet the recommendations of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies (29). The liquid diets for the control and ethanol formulations were mixed fresh daily as described by Miller et al. (26) with use of a kitchen wire whisk and bowl. All dietary components were purchased from US Biochemical with the exception of the oil and sucrose, which were purchased locally from a supermarket, and ethanol, which was obtained from campus chemical supply.

The diets were offered to the animals in glass bottles equipped with a version of the Richter tube at ~11 A.M. The glass bottles containing uneaten diet were removed the following day at ~7:30 A.M. During the following 3.5-h period the bottles were weighed, food intakes recorded, new diet mixed, and rats exercised.

Ethanol clearance rates. Ethanol clearance rates at rest were determined by measuring the disappearance of ethanol from the blood after an intraperitoneal injection of 1.75 g ethanol/kg body wt. The ethanol was diluted to 20% (wt/vol) in saline. To follow the disappearance of ethanol from the blood, a 50-μl sample of blood was

obtained from the tail of each rat every 60 min for 5 h after the initial injection of ethanol. No anesthetic was administered while blood was collected to avoid the possibility that drug interactions with the ethanol might affect ethanol clearance. Each blood sample was placed into a 20-ml glass vial, sealed, and stored at −80°C until analyzed for ethanol content by headspace gas chromatography with a Perkin-Elmer model F40 Multifract instrument interfaced with a Sigma 2000 integrator as described by Erickson et al. (11). The resulting values were used to calculate ethanol clearance rates using the Widmark equation (35)

$$A = pr(C_t + B_t)$$

where A is ingested dose of ethanol in grams, p is body weight, r is apparent volume of distribution of alcohol expressed as a fraction of body weight, C_t is blood concentration of alcohol at *time t*, *B* is slope of the apparent linear decline of alcohol concentrations with time, and *t* is time after alcohol dosing. The Widmark equation is preferable for calculating ethanol clearance because it factors into the equation the initial dose of ethanol, the animal's body weight, and predicted water space in which the ethanol is distributed after absorption rather than simply using only the slope of the curve obtained when ethanol concentration in the blood is plotted over time. This analysis makes the comparison of clearance rates between groups that differ in body size more equitable (36).

Experimental procedures. In *experiment 1* the effect of fasting and acute exercise on ethanol clearance and subsequent food consumption were determined. Four groups of rats ($n = 10$) were fed liquid diet (without ethanol) ad libitum for 3 wk. After a 12-h fast, two groups were given an ethanol clearance test, one while remaining sedentary in their home cage for 5 h and the second group running on the treadmill for 60 min at 20 m/min on a 0% grade starting 60 min after the injection of ethanol. Ethanol clearance was determined in the same manner on two additional groups that were allowed access to their food until the time of the test. Food consumption was also determined on a daily basis for 10 days after the clearance test.

In *experiment 2* three groups of rats, run/ethanol (R/E, $n = 10$), run/pair fed (R/P, $n = 10$), and run/control (R/C, $n = 10$), were trained to run on a rodent treadmill (Quinton Instruments), on a daily basis utilizing a low-intensity endurance protocol. The rats ran at a speed of 27 m/min (0% grade) for 20 min the 1st day, and the running time was increased by 2 min each day until the rats ran continuously for 60 min. This work load was maintained for the duration of the study. The exercise periods were initiated at 8 A.M. each day 5 days/wk. Three other groups of rats, sedentary/ethanol (S/E, $n = 10$), sedentary/pair fed (S/P, $n = 10$), and sedentary/control (S/C, $n = 10$), did not receive any exercise training. All six groups were fed liquid diets with the R/P and S/P rats pair fed to the R/E and S/E rats, respectively, and the R/C and S/C rats given sufficient liquid diet to maintain weight gains similar to a group of 10 rats maintained on Purina rat chow and water ad libitum. The Purina rat chow group was not utilized in the study for ethanol clearance determinations.

Rates of ethanol clearance were measured in all rats on the 29th day of the study and again on the 50th day. In all cases, the clearance tests were performed 72 h after the last exercise period. On the 29th day of the study the ethanol clearance test included a 1-h run on the rodent treadmill, which started 60 min after the injection of ethanol. The R/E, R/P, and R/C animals ran at a speed of 27 m/min compared with 20 m/min for the S/E, S/P, and S/C groups in order that the relative intensity of the run for the trained rats be approximately equal to that of the untrained rats, 75% of maximal O_2 consumption for both groups (7). Five hours before the ethanol injection to initiate the clearance test at 7 A.M., feeding bottles containing ethanol were replaced with bottles containing diet without ethanol. Previous experience indicated that 5 h provided sufficient time for the animals to eliminate any ethanol from the blood so that the only ethanol present was the amount injected. On the 50th day of the study a second ethanol clearance test without the 60-min exercise period was administered to all the animals.

Enzyme analysis. Seven days after the second clearance test, the unanesthetized rats were killed by decapitation, and samples of liver tissue were frozen in liquid N_2 and stored at −80°C. The assays for ethanol oxidation by liver microsomes and for alcohol dehydrogenase activity were linear with respect to both time and protein added.

Ethanol oxidation by hepatic microsomes (MEOS). Samples of liver from *experiment 2* were thawed within 2 wk from the time the animals were killed, and a washed microsomal fraction was prepared by the methods of Lieber and DeCarli (21). Rates of ethanol metabolism were then determined by incubating the microsomal samples with ethanol for 10 min in 30-ml rubber-stoppered glass tubes and measuring the amount of acetaldehyde produced. The reaction mixture contained, in a final volume of 3.0 ml, 100 mM phosphate buffer (pH 7.4), 100 μM sodium azide, 5.0 mM $MgCl_2$, 1.0 mM EDTA, 4 mM $NADP^+$, 8 mM sodium isocitrate, 0.34 U/ml isocitrate dehydrogenase, and 100 μl of microsomal suspension. The microsomes were preincubated in the reaction mixture without ethanol for 5 min at 37°C. The reaction was initiated by the injection of ethanol through the rubber stopper to yield a final concentration of 50 mM ethanol. After 10 min the reaction was stopped by injecting 1.0 ml 70% perchloric acid, and the reaction tubes were then placed in an ice-water bath. After 15 min the rubber stoppers were removed, and a 200-μl sample of the mixture was placed in a 20-ml glass vial, which was prechilled to 0°C and contained 300-μl ice-cold distilled water. The vials were immediately sealed and stored at −80°C until analyzed for acetaldehyde content by headspace gas chromatography with a Perkin-Elmer F40 Multifract instrument interfaced with a Sigma 2000 integrator as described by Eriksson et al. (12).

Alcohol dehydrogenase (ADH). A second sample of frozen liver was thawed, and alcohol dehydrogenase activity of the 100,000 *g* supernatant was measured by the

methods of Lumeng et al. (24).

Protein determination. Protein concentrations in the samples for the ADH and MEOS assays were estimated by the method of Bradford (2). Dilutions of 1:100 and 1:50, respectively, of the microsomal and ADH samples were prepared. These dilutions produced protein concentrations within the linear range of the standard curve (10–50 μg/100 μl).

Statistical analysis. To compare results between groups utilized in this experiment two-way analyses of variances (ANOVAs) were performed for each variable measured. The criterion for significance was the 0.05 level, and when significant differences were determined a Student Neuman-Keuls post hoc test was employed to locate the source of the differences (37). Ethanol clearance rates from the R/P and R/C groups were identical so these two groups were combined into one R/C group, as were the data from the S/P and S/C groups combined into one S/C group.

RESULTS

Effect of a 12-h fast and acute exercise on ethanol clearance. In comparing clearance rates with and without a prior 12-h fasting period the clearance rates at rest were 26% lower in the fasted group (Fig. 1). When a 60-min exercise period was included during a clearance test under these two conditions, we also observed a 27% lower clearance rate in the fasted group compared with the fed group (Fig. 1). Whether preceded by a 12-h fast or not, the exercise clearance rates were 8–10% greater than resting clearance rates.

Effect of fasting before the ethanol clearance test on subsequent food consumption. The effects of a fast on ethanol clearance rates were not limited to decreasing in vivo ethanol clearance. In *experiment 1* (Fig. 2) food consumption for 1 wk after the test was decreased to a much larger degree when the test was preceded by a fast compared with the nonfasted rats.

Effects of endurance training and ethanol consumption on ethanol clearance. Figure 3 illustrates the results of the clearance tests (from all groups) after 7 wk of endurance training. As expected, the resting clearance rates of the animals that received the ethanol treatment were

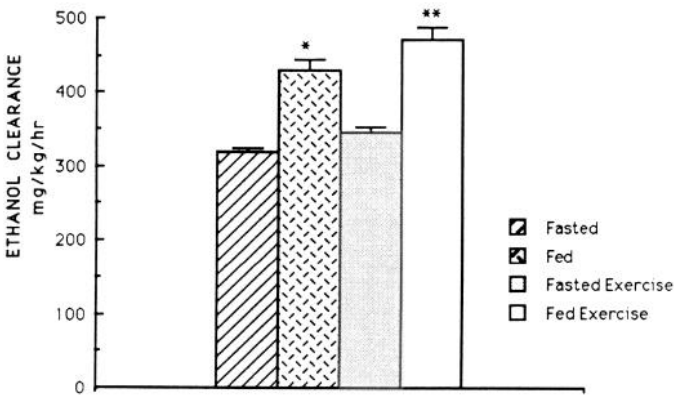

FIG. 1. Effect of a bout of exercise on ethanol clearance after a 12-h fast. Values are means ± SE; $n = 10$ for all groups. * Significantly greater than both fasted groups ($P < 0.05$); ** greater than all other groups ($P < 0.05$).

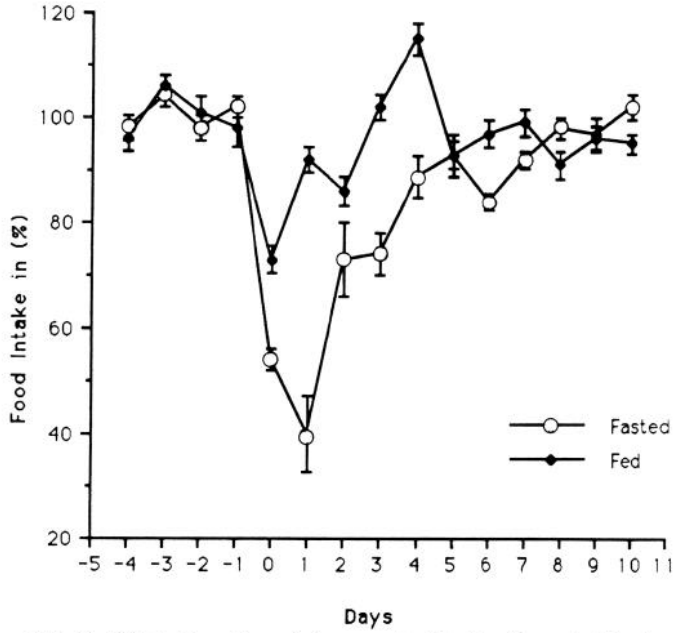

FIG. 2. Effect of an ethanol clearance test both with and without a prior fast on subsequent food consumption. Ethanol clearance test was performed on *day 0*, and food consumption, expressed as percent of average food intake for 4 days before clearance test, was measured for subsequent 10 days.

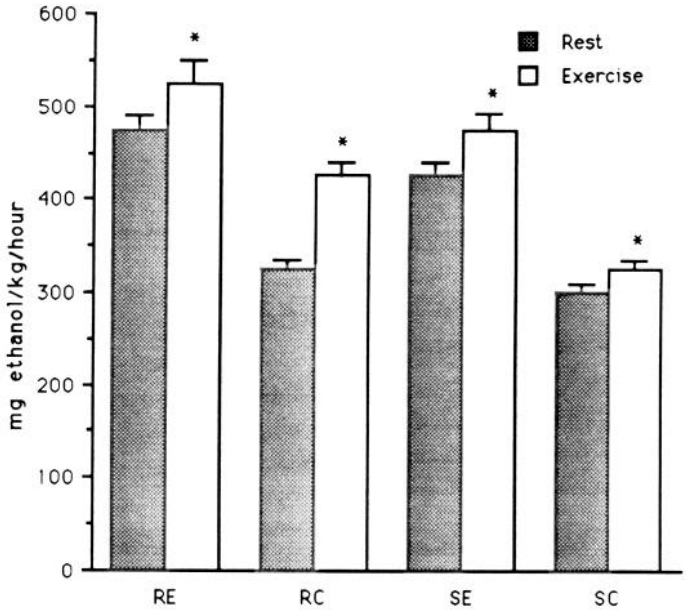

FIG. 3. Effect of training by running combined with chronic ethanol consumption (R/E, $n = 9$), training (R/C, $n = 16$), and ethanol consumption (S/E, $n = 10$) on ethanol clearance rates measured at rest and with 1 h of running compared with controls (S/C, $n = 17$). Values are means ± SE. * $P < 0.05$ vs. rest. Significant main effects due to both ethanol consumption and exercise training were observed.

36% greater than those that did not receive any ethanol in their diet. The effect of acute exercise on ethanol clearance was consistent with the previously observed clearance rates of *experiment 1*. Of major significance in this study is the observation that ethanol clearance rates at rest were significantly greater in the two exercise-trained groups compared with their sedentary controls. This increase in ethanol metabolism at rest resulting

from the exercise training appeared to be, in fact, additive to the effect of chronic group consumption on resting clearance rates.

Effect of exercise training and ethanol consumption on ADH activity. There were significant main effects due to chronic ethanol consumption on ADH activity of the liver (Table 1); however, post hoc analysis failed to discern that any individual group was significantly different from any other.

Effects of exercise training and ethanol consumption on MEOS. There were significant ethanol and training effects on MEOS, with activity of the R/C and R/E rats significantly greater than that of the S/C and S/E rats by 19 and 16%, respectively (Table 1).

DISCUSSION

Effect of a 12-h fast on ethanol clearance. The results of this study demonstrate that the nutritional history of the rat before an ethanol clearance test must be carefully controlled. The advent of liquid diets in the field of ethanol research, coupled with the use of carefully controlled pair-feeding techniques, enabled the use of rat models that produced high ethanol ingestion in a nutritionally balanced diet. For the purposes of this project the diet employed by Miller et al. (26), which was designed to meet all the nutrient recommendations for rats as described by the American Institute of Nutrition Ad Hoc Committee, was used. In *experiment 1*, a 12-h fast significantly depressed the rate of ethanol clearance compared with the fed rats. Traditionally, ethanol clearance rates have been determined in fasted rats to eliminate any effects of food remaining in the gut. Lumeng et al. (24), however, have demonstrated that activity and content of ADH in liver are significantly reduced by either fasting or food restriction and that ethanol clearance rates are correspondingly reduced up to 33%. In addition, hepatic blood flow during acute intoxication is also affected by a prior fast. Iturriaga et al. (17) observed a 30% decrease in hepatic blood flow after an acute dose of ethanol in fasted ethanol-tolerant rats, which indicates that decrements in hepatic blood flow may be related to the withdrawal syndrome in a food-deprived ethanol-tolerant rat.

Because repeated measures of ethanol clearance were used in this study to establish the effect of a particular treatment on in vivo ethanol metabolism, it was important to use a model that was not compromised by artificially low ADH activity or impaired hepatic blood flow.

TABLE 1. *Interactive effects of exercise training and ethanol consumption on ADH and MEOS activity*

Group	ADH	MEOS
R/E	18±2.0	19.5±0.96*
R/C	12±1.0	16.4±1.16
S/E	15±2.0	16.8±1.12
S/C	13±1.0	13.8±0.42*

Values are means ± SE in nmol·min^{-1}·mg protein^{-1}. Significant main effects on alcohol dehydrogenase (ADH) activity were due to ethanol. MEOS, microsomal ethanol oxidizing system; R/E, run/ethanol rats; R/C, run/control rats; S/E, sedentary/ethanol rats; S/C, sedentary control rats. * $P < 0.05$ vs. all other groups.

For our purposes, fasting before assay procedures was contraindicated due to the effects of fasting on factors important to ethanol metabolism.

Effects of a fast before ethanol clearance test on subsequent food consumption. The effects of a fast on ethanol clearance rates were not limited to decreasing in vivo ethanol clearance. In *experiment 1* (Fig. 2) food consumption for 1 wk after the test was decreased to a much larger degree when the test was preceded by a fast compared with the nonfasted rats. Lumeng et al. (23) have also demonstrated that food restriction for 1 wk is almost as efficient as a 12-h fasting period in reducing ADH content and ethanol clearance. Because fasting before a clearance test has such effects on ethanol clearance and prolonged effects on subsequent food consumption, all clearance tests administered in *experiment 2* were conducted without a prior fast.

Effect of exercise on ethanol clearance. In *experiment 1* we also observed that the activity pattern of the rat during the clearance test influenced rates of ethanol clearance. When rats were run for 1 h during the clearance test, in this case 60 min after the ethanol injection, there was a 10% greater ethanol clearance rate. The fact that the net increase in clearance rates due to the exercise was proportionate with or without the 12-h fast raised the possibility that the enhancement in ethanol clearance by the acute exercise may have occurred through the ADH pathway.

Effect of endurance training and ethanol consumption on ethanol clearance. Figure 3 illustrates the results of clearance tests administered to the groups of animals used in the second experiment. As expected, the resting clearance rates of the animals that received the ethanol treatment were 36% greater than those that did not receive any ethanol in their diet. This degree of increase was similar to that seen by others (16, 17). The effect of acute exercise on ethanol clearance was consistent with that previously observed by us (Ref. 1 and *experiment 1*, this study). Similar to Schurch et al. (30), we used a work load that was of moderate intensity and long duration. Perhaps ≥60 min of aerobic exercise is necessary for the acute bout of exercise to have an effect on ethanol clearance, since previous studies, which did not observe any effects of exercise, used an exercise bout of higher intensity and shorter duration (4, 5, 26). Longer-term exercise would be expected to increase body temperatures for a long enough duration (3) to produce a significant "Q_{10}" effect on ADH. In addition, this type of exercise may also result in a sufficient metabolic demand for reducing equivalents (during glucose synthesis from lactate in the liver) to regenerate NAD^+ at a faster rate for ADH to oxidize ethanol more efficiently. Unfortunately the in vitro measurements of ADH activity in this study alone will fail to be predictive of in vivo activity when such factors of liver metabolism are taken into consideration.

Effect of endurance training and ethanol consumption on ADH activity. As Table 1 illustrates, there were significant main effects due to the chronic ethanol consumption, which resulted in increased ADH activity. Post hoc analysis, however, failed to discern that any

individual group was significantly different from any other. An increase in ADH activity after chronic ethanol exposure has been observed in mice by others (10), although an increase in ADH activity after ethanol treatment has not been consistently observed in the rat (18). In the present study, although chronic ethanol consumption enhanced ADH activity, it is unknown whether this greater in vitro activity translates into greater in vivo clearance. There were no main effects of exercise training on ADH activity observed, and yet in vivo ethanol clearance was increased in the trained groups. This led us to believe that factors other than ADH may also be involved in the enhanced ethanol clearance observed in this study.

Effects of exercise training and ethanol consumption on MEOS. The ability of cytochrome P-450 to oxidize ethanol [first termed the microsomal ethanol oxidizing system (MEOS)] was first proposed by Lieber and DeCarli (19), and its role in the increased ethanol metabolism after ethanol treatment has been described (8, 32). Values reported in this study for MEOS activity in control rats were similar to those reported by Teschke et al. (33) and Lieber and DeCarli (21). The activity for the S/E rats was less than the 25.3 $nmol \cdot mg^{-1} \cdot min^{-1}$ reported by Lieber and DeCarli (20), although still significantly greater than the controls and consistent with an ethanol-induced increase described in the literature (8, 21, 33). The significant increases in MEOS activity reported here are probably of a lower magnitude compared with other studies (8, 21, 33) because we used older rats. Response to drug treatment was often much greater in immature rats than in the older rats (9).

Of major significance in this study is the observation that ethanol clearance rates at rest were significantly greater in the two exercise-trained groups compared with their sedentary controls. The training regime used results in enhanced activity of oxidative enzymes in skeletal muscle and in increased whole-body aerobic capacity (6). This is the first documentation that endurance training over a prolonged period (7 wk) can alter ethanol metabolism. Because there are insignificant differences in resting metabolic rates between trained and untrained rats, both ~25 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (27), the resting whole-body metabolic rate does not account for the greater clearance in the trained rats. A possible explanation for the increased resting ethanol clearance rates observed was provided by Frenkle et al. (15), who observed that endurance-trained rats had decreased hexobarbital-induced sleeping times compared with sedentary controls. This decrease in sleep time was related to an increase in metabolic tolerance to hexobarbital brought about through an increase in hepatic microsomal cytochrome *P*-450 and NADPH: Cytochrome *P*-450 reductase activity in the trained group. It is possible that in the present study, which also utilized endurance training for a similar duration, the hepatic cytochrome *P*-450-dependent mixed-function oxidase system was also increased by the exercise. The fact that MEOS activity of the R/C and R/E rats was significantly greater than the S/C and S/E rats (by 19 and 16%, respectively) provides evidence that exercise training alone is capable of inducing activity of the MEOS. The 16% greater activity of the R/E rats compared with the S/E rats also indicated that the effect of exercise training on MEOS activity may be additive with the effects of ethanol on MEOS.

From the results of this study we can conclude that acute running exercise for periods of at least 60 min will increase rates of ethanol clearance compared with rates measured at rest. More importantly, we conclude that endurance exercise training for 7 wk will also enhance clearance of the drug at rest. Although the exact mechanism responsible for this is unknown, it is possible that it is mediated in part through the cytochrome *P*-450-dependent monooxygenase system of hepatic microsomes as has been observed for other drugs (15). If metabolism of a drug can be increased by regularly performed endurance training through the hepatic mixed-function oxidase system, then the pharmacokinetics of commonly used drugs metabolized by this system must be reevaluated for application to participants of this type of activity. Further studies on the mechanism of induced ethanol clearance are being undertaken.

We gratefully acknowledge Kathe I. Byers for technical assistance in the ethanol and acetaldehyde analysis, and we thank Mina Rathbun for typing the manuscript.

This work was supported in part by Grant AA04114 to C. K. Erickson and by a Biomedical Research Support Grant from The University of Texas at Austin to R. P. Farrar.

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Received 28 March 1988; accepted in final form 25 August 1988.

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