The Effect of Prolonged Administration of Ethanol on Cardiac Metabolism and Performance in the Dog

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ABSTRACT The effect of prolonged administration of alcohol on mitochondrial function and high-energy phosphate (ATP) of heart muscle was investigated in dogs. Animals were divided into two groups, a control group and a group that received alcohol. In the experimental series, dogs received 400 ml of a 25% solution of alcohol added to the food and drinking water. Measurements were carried out after ethanol had been withheld for 2 days. Total myocardial blood flow, cardiac output, and myocardial O_2 consumption remained at control levels. Measurement of cardiac contractility using the maximal rate of left ventricular pressure rise (dP/dt_{max}) showed no change in animals exposed to alcohol. When the afterload of the heart was increased with angiotensin, a slight but not significant decline in cardiac contractility was observed. Activities of various intramitochondrial and extramitochondrial enzymes were measured in both groups. After alcohol administration, the primarily intramitochondrial isocitrate dehydrogenase diminished. ATP in heart muscle of dogs exposed to alcohol declined, and mitochondrial oxygen consumption and respiratory control indices diminished. These observations suggest that the primary lesion leading to alteration of myocardial performance is a biochemical malfunction of the mitochondria, which at this early stage is not reflected in changes in myocardial contractility.

INTRODUCTION

The chronic effect of alcohol on the heart has been the subject of a series of investigations (1–4). In general, the conclusion was reached that alcohol has a direct toxic effect on the heart muscle. This conclusion is based both on electron-microscopic and metabolic changes (3, 5–8). It has been shown, for example, that in the heart of alcoholic patients, as well as in that of animals exposed to large doses of alcohol, electron-microscopic observations of mitochondria reveal defects which resulted in enzymatic changes within these structures, affecting the whole intracellular metabolic state.

Alcohol also exerts profound changes on the lipid profile of heart muscle, liver, and serum. Dogs exposed to a prolonged period of alcohol intake show a marked increase in triglycerides (1, 9, 10) and a diminution in free fatty acid content of heart muscle. Ethanol also increases plasma free fatty acids, triglycerides, and cholesterol.

In general, the hemodynamic effects of prolonged alcohol ingestion appear to depend on the degree of heart failure and the presence of liver disease. However, it has been demonstrated that left ventricular function of patients is depressed even in alcoholics without heart disease (4).

Because of the implication of mitochondria in the toxic effects of alcohol, this study was initiated to investigate mitochondrial function in heart muscle in dogs exposed to alcohol for a period of 14 wk. In addition, the effect of ethanol on coronary flow, myocardial oxygen consumption, and myocardial contractility was investigated.

METHODS

Experiments were carried out on 28 anesthetized mongrel dogs (30 mg i.v. Na pentobarbital/kg). The animals were divided into two groups, one serving as control (group C), the other receiving alcohol (group A). Whenever possible, each animal served as its own control. Consequently, data collected in 14 animals before alcohol administration (group A_1) were compared with those collected at the end of the experimental period of 14 wk (group A_2). The same procedure was followed for animals not exposed to alcohol (groups C_1 and C_2). However, some analyses, such as heart

Received for publication 26 February 1973 and in revised form 18 June 1973.

muscle enzymes, mitochondria, and high-energy phosphates, required large amounts of cardiac tissue which could be obtained only at the end of the period of observation when the whole heart was available. In these instances, groups C_2 and A_2 were compared.

Each dog receiving alcohol was paired with a control animal of similar body size and weight which was maintained for the same period under identical conditions, except that alcohol was withheld. All animals received the same diet, which consisted of about 2,000 cal of meat, meat by-products, and vitamin supplementation. Alcohol (400 ml of a 25% solution) was added to the food and drinking water, and the animals consumed this amount of alcohol within 24 h. Spot checks on blood alcohol levels were taken repeatedly. The blood alcohol levels in these animals averaged 180 mg/100 ml, with a peak of 240 mg/100 ml after ingestion of the food (Fig. 1). In animals exposed to ethanol, studies were carried out 2 days after alcohol had been withheld.

Heart muscle biopsies (performed with a Menghini needle) for determination of isocitrate dehydrogenase were obtained in both groups at the onset of experimental period. At the end of the experimental period, the animals were killed, and the heart was used for biochemical examination.

The following enzymes were determined: NAD-specific isocitrate dehydrogenase (NAD-ICDH), malate dehydrogenase (MDH), glutamate oxalacetate transaminase (GOT), glyceraldehydephosphate dehydrogenase (GAPDH, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), α-glycerophosphate dehydrogenase (α-GPDH), 6-phosphogluconate dehydrogenase (6-PGDH), and fructose-1,6-diphosphate aldolase (ALD). The enzymes were extracted and analyzed in a Beckman Kintrac spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) as previously described (11). NAD-ICDH was extracted as follows: A small piece of left ventricular myocardium (8–10 mg) was placed on a small piece of aluminum foil and wrapped with a large piece of foil. This wrapped tissue was quick frozen in liquid nitrogen. The frozen tissue was weighed and then washed in 1 ml of 5 mM potassium buffer (pH 6.5). The washed tissue (blood-free) was homogenized by a Tri-R tissue homogenizer (Tri-R Instruments, Inc., Rockville Centre, N. Y.) for 1 min at 0°C in 0.4 ml of extraction medium containing 0.5% (wt/vol) Triton X-100, 1 mM ATP, 80 mM NaCl, and 5 mM potassium phosphate buffer (pH 6.2). The homogenate was centrifuged at 1,000 *g* for 10 min. The supernate was removed and the precipitate was resuspended in 0.4 ml of the extraction medium and centrifuged at 1,000 *g* for 10 min. The resulting supernates were combined and stored at 0°C for the enzyme assay.

Mitochondria were isolated with a modification of the procedure used by Lindenmayer, Sordahl, and Schwartz (12), and Jennings, Herdson, and Hill (13). 2 g of the left ventricular myocardium was freed from connective tissue and minced and homogenized with a Teflon pestle and glass homogenizer in 12 ml of ice-cold isolation medium containing 180 mM KCl, 10 mM EDTA, and 0.5% bovine serum albumin adjusted to pH 7.4 with Tris base. Only two strokes (20 s of each stroke) were made with a loosely fitting Teflon pestle driven at 500 rpm to minimize mitochondrial damage. The resulting homogenate was adjusted to pH 7.4 with 1 M Tris base and centrifuged at 500 *g* for 6 min. The supernate was passed through four layers of cheesecloth and centrifuged at 7,700 *g* for 10 min. The

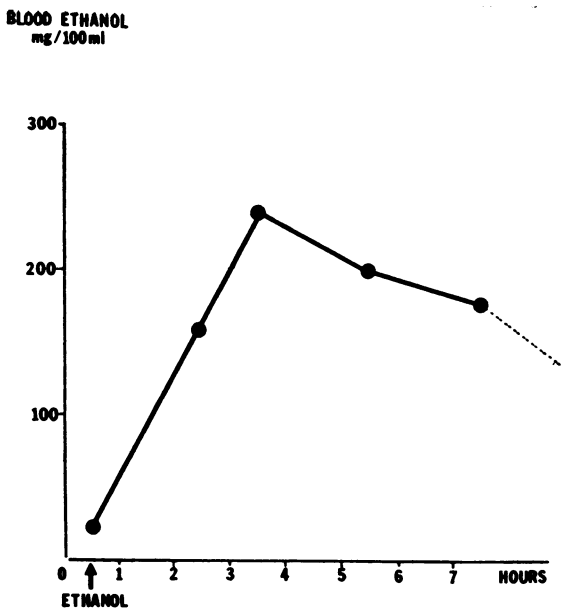

FIGURE 1 Blood ethanol concentrations after administration of the ethanol-containing diet to dogs. It may be seen that blood alcohol levels remain elevated during the period of observation, with a peak of 240 mg/100 ml.

resulting pellet was suspended in 10 ml of the isolation medium and centrifuged at 7,700 *g* for 5 min. The pellet was resuspended in 4 ml of the isolation medium and centrifuged at 160 *g* for 3 min. The supernatant fluid was removed and centrifuged at 7,700 *g* for 5 min. The purified mitochondrial pellet was suspended to a final concentration of 5–10 mg mitochondrial protein/ml (about 0.5 ml/g heart tissue) in a suspension medium containing 0.25 M sucrose, 1% bovine serum albumin, and 10 mM Tris-HCl at pH 7.4. In most instances, the mitochondrial preparation was also examined under the electron microscope.

Mitochondrial respiration was measured with a vibrating platinum oxygen electrode (oxygraph, Gilson Medical Electronics, Inc., Middletown, Wisc.). The apparatus contains an open-type electrode vessel (2.5 ml capacity cell) in a thermostatic water-jacketed cell, designed to minimize air diffusion into it. The vibrating electrode was preferred to the Clark electrode, since the response of the latter is slower and the slopes of the various states are less pronounced. Even though the ratios from the two electrodes are comparable, we have preferred the vibrating electrode because of its greater ease of operation and because of greater reproducibility.

The electrode measures the actual oxygen dissolved in the analysis solution, and since each analysis takes approximately 2 min to run, the back diffusion of oxygen is not a problem. The dissolved oxygen is adequate in the analysis solution to carry out three or four determinations on a sample of mitochondria. The oscillation frequency of the electrode is held constant at 120 cycles/s which permits a high sensitivity and decreased response time, both are critical for kinetic measurements. In addition, the vibration of the electrode, together with the small volume of the reaction medium (1.17 ml), keeps the suspension uniform during the period of measurements. The O_2 consumption of the electrode varies from 1.4 to 2.4 natoms O_2/min. This is

TABLE I

Enzyme Activities in Heart Muscle of Dogs Maintained with and without Alcohol

	NAD-ICDH*	MDH	GOT	LDH	ALD	GAPDH	α-GPDH	G-6-PDH*	6-PGDH*
					μmol/min/mg protein				
Group C_2 (n = 14)	21.3±1.3	11.4±1.0	2.0±0.1	0.7±0.1	0.2±0.02	0.6±0.1	0.1±0.01	0.1±0.04	0.1±0.05
Group A_2 (n = 14)	14.0±1.0	10.5±0.9	1.9±0.2	0.8±0.3	0.2±0.01	0.4±0.04	0.1±0.01	0.1±0.03	0.1±0.03
Significance of difference between C_2 and A_2	$P < 0.0005$	NS	NS	NS	NS	NS	NS	NS	NS

MDH, malate dehydrogenase; GOT, glutamate oxalacetate transaminase; LDH, lactate dehydrogenase; ALD, fructose-1,6-diphosphate aldolase; GAPDH, glyceraldehydephosphate dehydrogenase; α-GPDH, α-glycerophosphate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase.

* = nanomoles per minute per milligram protein.

C_2 = dogs after maintenance without alcohol for 14 wk.

A_2 = dogs after maintenance with alcohol for 14 wk.

less than 0.5% of the total O_2 content in the reaction medium. The O_2 consumption of the electrode is determined at the onset and at the termination of the experimental period. This background value is then subtracted from states 3 and 4. Consequently, the ratios between these two states are not changed.

Mitochondrial protein concentrations of samples obtained from control and ethanol-exposed groups were adjusted so that their protein concentrations varied by a maximum of 1.5%. Since the difference in respiratory control indices varies 30–40%, this slight difference in protein concentration is negligible. Table III illustrates the values obtained.

To measure respiratory function, mitochondria (1.5–2.0 mg/ml) were added to a reaction medium at 30°C containing 0.25 M sucrose, 8.5 mM K_2HPO_4, 10 mM Tris-HCl at pH 7.4, and substrates. The substrates used were glutamate (7.5 mM), oxalacetate (1.5 mM), and succinate (7.5 mM). Rotenone (4 μg/mg mitochondrial protein) was added to the reaction medium when succinate was used as substrate. The reaction was initiated by addition of ADP (251 nmol).

TABLE II

Values of Intramitochondrial NAD-ICDH before and after Administration of Alcohol

	nmol/min/mg protein		
Group A_1 (n = 6)	20.4±1.8	Group C_1 (n = 6)	17.8±1.6
Group A_2 (n = 6)	13.4±1.2	Group C_2 (n = 6)	21.7±2.5
Significance	$P < 0.005$		NS

A_1 = tissues obtained from heart by biopsy before administration of alcohol.

A_2 = dogs after administration of alcohol for 14 wk.

C_1 = tissues obtained from heart by biopsy at the onset of the experimental period.

C_2 = dogs after maintenance without alcohol for 14 wk.

Respiratory indices are defined as follows: Respiratory control index is the ratio of oxygen consumed in the presence of added ADP to that after the ADP had been phosphorylated. ADP/O represents the ratio of nanomoles of phosphorylated ADP to nanoatoms of consumed oxygen. QO_2 represents nanoatoms of oxygen consumed per milligram of mitochondrial protein per minute during state 3 respiration (rapid oxygen consumption during the phosphorylation of ADP). The respiratory control index is a measure of the degree of "coupling" or of "tightness of coupling" of oxidation and phosphorylation. Statistical analyses were performed using Student's t test (14).

Hemodynamic studies were performed under pentobarbital anesthesia (30 mg/kg i.v.) and included the measurement of heart rate, phasic and mean aortic pressures, left ventricular pressure, left ventricular end-diastolic pressure, the first derivative of the left ventricular pressure rise (dP/dt) (15), total myocardial blood flow, cardiac output, and myocardial oxygen consumption. Recordings were made on an Electronics for Medicine recorder (Electronics for Medicine, Inc., White Plains, N. Y.). Total myocardial blood flow was measured using ^{84}Rb and coincidence counting after catheterization of the coronary sinus using the Fick principle (16–18).

RESULTS

Enzymes in heart muscle. The enzymatic changes occurring in heart muscle of animals exposed to alcohol (group A_2) as compared with group C_2 (animals of the control series maintained without alcohol) are illustrated in Table I. Significant diminution in the NAD-ICDH occurred in group A_2 as compared with group C_2. Activities of other heart muscle enzymes did not change (Table I).

To obtain information on the change in the activity of intramitochondrial isocitrate dehydrogenase in the same animal, biopsy specimens were taken from heart muscle in groups C_1 (animals of the control group before maintenance for 14 wk) and C_2, and A_1 (animals

TABLE III

Mitochondrial Respiratory Function in Heart Muscle of Dogs Maintained with and without Alcohol

	Group C_2 (n = 6)			Group A_2 (n = 6)		
	RCI	ADP/O	QO_2	RCI	ADP/O	QO_2
Succinate	3.6±0.4	2.2±0.1	97.1±3.9	2.4±0.1*	2.1±0.1	62.0±4.5*
Glutamate/oxalacetate	10.1±0.8	3.7±0.1	105.2±8.2	6.6±0.5*	3.8±0.1	61.0±8.2*

RCI, respiratory control index; ADP/O, ratio of nanomoles of phosphorylated ADP to nanoatoms of oxygen; QO_2, oxygen consumption in nanoatoms O_2 per minute per milligram mitochondrial protein; C_2, dogs after maintenance without alcohol for 14 wk; A_2, dogs after maintenance with alcohol for 14 wk.

* Value compared with C_2 is statistically significant ($P < 0.0025$).

of the experimental group before exposure to alcohol) and A_2 (Table II). The first set of determinations was carried out on biopsy specimens obtained at the onset of the experimental period. The same determination was performed immediately before death. when it was possible to obtain an open biopsy. There was a significant diminution in intramitochondrial NAD-ICDH in group A. These changes were not present in group C (Table II).

High-energy phosphates and respiratory function of mitochondria. In 10 experiments, ATP values in the heart muscle of dogs exposed to alcohol diminished. The ATP content (micromoles/gram tissue) in the control series (C_2) was 5.2±0.2; in dogs exposed to alcohol (A_2) it was 4.7±0.1. Although this difference is statistically significant ($P < 0.025$), it may only indicate directional rather than quantitative changes. Table III shows that, using the vibrating platinum oxygen electrode, respiratory control indices were markedly diminished in the group exposed to alcohol (group A_2 compared with C_2) regardless of the substrate used. QO_2 was markedly reduced (Fig. 2). There was no significant difference in ADP/O ratio of both groups (A_2 and C_2).

Hemodynamic studies. In groups A and C, no significant changes occurred in myocardial contractility (left ventricular end-diastolic pressure, dP/dt_{max}, and tension/time index) at rest (Table IV). Coronary blood flow, as well as myocardial oxygen consumption and cardiac output, remained unchanged. This is in contrast to the acute effect of alcohol which has been shown to diminish myocardial contractility and increase coronary

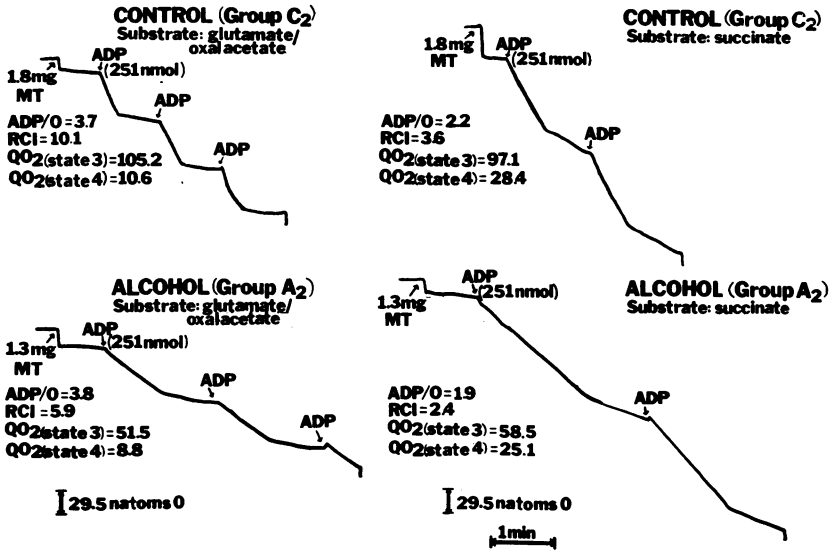

FIGURE 2 Representative oxygen electrode tracings (vibrating platinum electrode) of myocardial mitochondria from a control dog and from an animal exposed to alcohol for 14 wk. The mitochondria (MT) were isolated as described in the text. Succinate (7.5 mM), glutamate (7.5 mM), and oxalacetate (1.5 mM) were used as the substrates. Assays were carried out in 1 ml Tris-HCl (pH 7.4) at 30°C. ADP (251 nmol) was added where indicated. The differences in respiratory control indices (RCI) and mitochondrial oxygen consumption (QO_2) between group C_2 and A_2 were statistically significant ($P < 0.05$).

TABLE IV

Hemodynamic Studies before and after Alcohol Administration

	HR	MAP	LVP	LVEDP	dp/dt_{max}	TCF	CO	Vo_2
Group C_1 (n = 14)	116±13	126±4	143±5	6.3±0.9	2242±254	136±17	2557±260	13.5±1.8
Group C_2 (n = 14)	94±13	119±4	136±5	6.8±1.0	2215±183	137±15	2389±216	13.9±1.4
Significance between C_1 and C_2	NS	NS	NS	NS	NS	NS	NS	NS
Group A_1 (n = 14)	98±6	117±4	136±5	5.5±0.5	1964±59	131±9.8	2536±247	13.7±1.2
Group A_2 (n = 14)	103±16	121±5	135±6	5.1±0.7	2288±160	143±21	2601±223	13.2±1.3
Significance between A_1 and A_2	NS	NS	NS	NS	$P < 0.05$	NS	NS	NS
Response to isuprel								
Group A_1 (n = 14)	204±6	87±5	132±6	2.6±0.5	7372±554	381±40	6092±502	43.2±4.6
Group A_2 (n = 7)	216±8	92±5	140±8	4.0±1.0	7609±1076	351±81	7389±1078	37.3±9.9
Significance between A_1 and A_2	NS	NS	NS	NS	NS	NS	NS	NS

HR, heart rate; MAP, mean aortic pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; TCF, total coronary flow; CO, cardiac output; Vo_2, oxygen consumption, C_1, control data on dogs later not maintained on alcohol; C_2, dogs after maintenance without alcohol for 14 wk, A_1, control data on dogs later maintained on alcohol; A_2, dogs after maintenance with alcohol for 14 wk.

blood flow at blood levels exceeding 100 mg/100 ml (19).

To investigate whether or not increased cardiac load may reveal changes in cardiac contractility not otherwise noticeable, the heart was stressed with injection of isoproterenol or angiotensin (20) (Tables IV and V). Isoproterenol failed to cause changes in hemodynamics and contractility between groups C_1 and C_2, and A_1 and A_2. During angiotensin infusion, the maximal rate of rise of ventricular pressure (dP/dt_{max}) de-

TABLE V

The Effect of Angiotensin in Animals after Maintenance with and without Alcohol

	HR	MAP	LVP	LVEDP	dp/dt_{max}	TCF	CO
Group C_2, (n = 6)							
Before	118±10	123±2	140±3	4.9±0.6	2613±198	132±9	2230±151
After	117±22	167±5	186±10	8.8±1.5	2708±331	127±20	1614±114
Significance	NS	$P < 0.001$	$P < 0.005$	$P < 0.025$	NS	NS	$P < 0.01$
Group A_2, (n = 4)							
Before	73±5	127±8	141±8	4.9±0.5	2989±286	124±17	2599±335
After	84±10	177±6	198±8	11.2±1.3	2498±273	137±32	1850±287
Significance	NS	$P < 0.005$	$P < 0.002$	$P < 0.005$	NS	NS	$P < 0.05$

Refer to the legend of Table IV for definitions.

clined in animals exposed to alcohol. It increased slightly in the control animals. However, the changes were not statistically significant (Table V).

DISCUSSION

The study deals with the changes in mitochondrial and hemodynamic functions in dogs exposed to alcohol for 14 wk. Biochemical studies include the effect of alcohol on specific enzymes in heart muscle. In addition, the effect of alcohol on high-energy phosphate and respiratory functions of mitochondria was followed. The biochemical determinations were carried out in animals in which alcohol had been withheld for 2 days. Consequently, the results obtained could not have been due to elevated blood alcohol levels.

The principal biochemical findings obtained in dogs exposed to alcohol were as follows: (*a*) a diminution in intramitochondrial NAD-ICDH, (*b*) a fall in mitochondrial oxygen consumption and in the respiratory control index, and (*c*) a diminution in myocardial ATP content.

It is tempting to ascribe some of the biochemical alterations in heart muscle induced by alcohol to changes in the redox state in heart muscle. Since most investigators failed to demonstrate alcohol dehydrogenase activity in the heart, it is unlikely that heart muscle itself can metabolize ethanol (8, 21). Although some activity of this enzyme has been found in coronary sinus blood of chronic alcoholics (22), the overwhelming evidence now speaks against the presence of this enzyme in the heart (8, 21). At the present time, the question of the mechanism of action of ethanol on the heart and its metabolism is not fully understood.

However, in all likelihood, the diminution of intramitochondrial ISDH, the fall in mitochondrial oxygen consumption and respiratory control index, as well as the diminution in ATP content of heart muscle, are a direct toxic effect of ethanol itself. It is to be noted that only intramitochondrial ICDH was determined and that the decline in the activity of this enzyme from the control period to the termination of the experiments (A_1 to A_2) is 35% (Table II). This furnishes evidence of a disturbance in mitochondrial function.

No effects on hemodynamics or cardiac contractility were observed in unstressed animals that had been exposed to ethanol (group A_2). Regan also failed to discover changes in left ventricular function of dogs exposed to alcohol for several years, speculating that 10–15 yr of exposure to alcohol may be necessary to produce changes in contractility (1). Isoproterenol infusion failed to reveal any differences in hemodynamic response between groups A_1 and A_2 (Table IV). The response of total myocardial flow to the drug was also equal in both groups. This is not surprising, since this drug causes differences in myocardial flow only in the presence of restricted coronary circulation (23, 24).

In both groups (A_2 and C_2) end-diastolic pressures rose with angiotensin, and cardiac output decreased to the same degree. Consequently, no significance can be attached to these observations in the experimental group (group A_2). Afterload testing with angiotensin revealed a consistent reduction in dP/dt_{max} (Table V). Table V reveals that there was considerable scatter of the mean values. Consequently, the difference was not statistically significant.

The finding of normal hemodynamic function and cardiac contractility existing in conjunction with marked mitochondrial dysfunction deserves some comment. It could suggest that myocardial contractility is not dependent on the integrity of mitochondrial function. This opinion finds some support in our recent observations that both calcium binding and uptake by mitochondria and sarcoplasmic reticulum are severely reduced in dogs exposed to alcohol for a comparable period of time, even though cardiac contractility remains unimpaired. We would, however, agree with Sordahl, McCollum, Wood, and Schwartz that it is difficult to determine whether the changes in the activity in mitochondria or sarcoplasmic reticulum represent primary or secondary events in the development of cardiac failure (25).

ACKNOWLEDGMENTS

We wish to thank Esther Holden, Keith Seeler, Herbert Hansen, and Suzanne Cikowski, R. N., for their technical assistance and Barbara Biedebach and Susan Weisberg for their assistance in the preparation of the manuscript.

This work was supported by grants from the U. S. Public Health Service (RO1-MH21052-02), the Robert E. and May R. Wright Foundation, and the George and Miriam Gould Dock Fund.

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