The Effects of Chronic Alcohol Ingestion in Mice on Contractile Properties of Cardiac and Skeletal Muscle: a Comparison with Normal and Dehydrated-Malnourished Controls

The experiments described here were designed to measure contractile characteristics of mouse heart muscle following their isolation from animals restricted to a fluid intake of 20% alcohol for a prolonged period. The cardiac muscle performance of these mice was compared to that of normal controls, and to that of a group of mice whose volume of fluid intake was adjusted to that of the alcoholic treated group. Skeletal muscle contractility was studied in addition.

Methods. Swiss albino mice, obtained at 6 weeks of age, were maintained on one of the following intake regimens: Group 1 (normal), Purina Lab Chow and water ad libitum; Group 2 (alcohol), Purina Lab Chow and 20% ethanol by volume in water ad libitum; Group 3 (dehydrated-malnourished), Purina Lab Chow ad libitum and water intake limited to the fluid intake of the alcohol group on a day-to-day basis. The mice given alcohol were adapted to the 20% concentration by first giving them 5% alcohol for 3 days, then 10% for 3 days, 15% for 3 days, and finally the 20%. Food and fluid intake were measured for each group on a daily basis, as were body weights.

At the end of 27 weeks, the animals were sacrificed, and a strip of rectus abdominis muscle and the trabeculae carnae from the posterior wall of the left ventricle dissected from each mouse. Both muscles were clamped vertically in baths containing oxygenated Ringer's solution at 25 °C. The apparati permitted adjustments of resting length and tension as has been described previously[1,2]. Excitation thresholds were determined and the muscles stimulated with rectangular pulses supramaximally at a rate of 1/sec. Peak twitch tension, twitch time, and relaxation rate were recorded. For cardiac muscle, twitch tension is reported as mg/trabecular preparation, since all preparations were approximately the same size. For skeletal muscle, peak tension is reported as g/g dry weight of muscle. Relaxation rate in g/sec for both types of muscle was calculated as the slope of the line tangent to the relaxation curve at its steepest points. The period between stimulus and peak tension, referred to as time to peak tension, or TPT in Tables II and III, was also measured and is given in msec.

Results. The mice on 20% alcohol consumed about 25% of the fluid intake of the normal group, and hence Group 3 was appropriately fluid restricted. The fluid restriction resulted in a greatly reduced food intake similar to that of the alcoholic mice and in a body weight significantly different from the normal group but not from the alcohol group (see Table I).

Results of the twitch characteristics of the trabeculae and rectus muscles are seen in Tables II and III. Peak twitch tension of trabecula muscle is significantly reduced in both alcohol and dehydrated-malnourished groups when compared to normal controls. A significantly different rate of relaxation between normal and the dehydrated-malnourished group was also shown, but not between these and the alcohol group.

None of the characteristics of skeletal muscle contraction were significantly different between the three groups.

Discussion. Chronic alcoholism has been associated clinically with both heart failure and skeletal muscle weakness[3-5], though the etiology of both remains con-

[1] W. C. ULLRICK, Physiol. Chem. Phys. *2*, 385 (1970).
[2] S. L. BERK and W. C. ULLRICK, Experientia *29*, 809 (1973).
[3] J. C. HUGHES, in *Major Problems in Pathology* (W. B. Saunders Company, Philadelphia 1974), vol 4, p. 144.
[4] G. T. PERKOFF, A. Rev. Med. *22*, 125 (1971).
[5] P. G. LYNCH, J. neurol. Sci. *9*, 449 (1969).

Table I. Summary of general data

Group	Average fluid intake (ml per mouse/day)	Average food intake (g per mouse/day)	Average body wt. (g per mouse Terminal)
1. Normal (10)[a]	15.0	6.3	37.9 ± 2.4[b]
2. Alcohol (15)[a]	3.5	4.3	34.6 ± 3.3
3. Dehydrated-malnourished (10)[a]	3.5	4.8	34.0 ± 2.8

Terminal body weight comparisons: Between Groups 1 and 2, $p < 0.01$; between Groups 1 and 3, $p < 0.01$; between Groups 2 and 3, $p > 0.05$. [a]Number of animals in group. [b]Mean ± SE.

Table II. Contractile characteristics for cardiac muscle

Group	Peak twitch tension (g/g)	Twitch time (msec)	Rate relaxation (g/sec)	TPT (msec)
1. Normal	344 ± 30[a]	600 ± 28	1.19 ± 0.13	243 ± 14
2. Alcohol	218 ± 29	490 ± 21	0.93 ± 0.15	225 ± 17
3. Dehydrated-malnourished	197 ± 14	550 ± 26	0.72 ± 0.08	253 ± 10
Standard analysis: group comparisons (p)				
Groups 1 and 2	< 0.01	> 0.05	> 0.05	> 0.05
Groups 1 and 3	< 0.01	> 0.05	< 0.01	> 0.05
Groups 2 and 3	> 0.05	> 0.05	> 0.05	> 0.05

[a]Mean ± SE.

Table III. Contractile characteristics for skeletal muscle

Group	Peak twitch tension (g/g)	Twitch time (msec)	Rate relaxation (g/sec)	TPT (msec)
1. Normal	451 ± 51[a]	244 ± 28	23.6 ± 3.2	29.4 ± 2.9
2. Alcohol	393 ± 48	313 ± 22	19.6 ± 3.8	33.0 ± 1.6
3. Dehydrated-malnourished	357 ± 40	251 ± 31	18.8 ± 3.5	32.2 ± 2.7

[a]Mean ± SE. No significant difference occurred between any groups in any category.

troversial. BURCH et al.[6] have reported an experimental alcoholic cardiomyopathy in mice based on electron microscopic evidence, and MAINES and ALDINGER[7] showed a 70% decrease in ventricular systolic tension in rats on long term consumption of 25% ethanol. In both studies, as in our study, alcoholic animals consumed only a fraction of the fluid intake of normal controls. Clinically, most patients who carry a diagnosis of alcoholic cardiomyopathy have an accompanying decreased total fluid intake as an integral part of their alcoholism.

The decreased twitch tension in the cardiac muscles of both the alcoholic and chronically dehydrated groups must again raise the question of the role of malnutrition, specifically dehydration, and perhaps concomitant electrolyte abnormalities in alcoholic cardiomyopathy. As pointed out by BURCH and GILES[8], in published experimental animal studies, alcohol containing fluids served as the entire source of liquid intake. Control groups of these studies, however, were not designed to provide for a reduced fluid intake commensurate with that found in the experimental animals. Our studies did, however, attend to this matter, and our results force us to consider that dehydration per se may play an important role in the etiology of alcoholic cardiomyopathy.

It is of interest that while significant differences in peak twitch tension of cardiac muscle was demonstrated, no significant skeletal changes were induced. This fits well with the clinical observation that alcoholic cardiomyopathy is seldom accompanied by clinically detectable skeletal myopathy[4].

Summary. In vitro isometric contractile tension was measured in heart and skeletal muscle in 3 groups of mice: 1. a control group, 2. a group maintained for 27 weeks on 20% alcohol, and 3. a group whose fluid intake was restricted to the extent equaling that which occurred in the alcohol treated animals. Results showed a reduction in cardiac twitch tension in both the alcohol and fluid restricted group, as compared to normal controls. We therefore consider that dehydration per se may play an important role in the etiology of alcoholic cardiomyopathy.

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[6] G. BURCH, H. COLCOLOUGH, J. HARB and C. TSUI, Am. J. Cardiol. *27*, 522 (1971).
[7] J. E. MAINES and E. E. ALDINGER, Am. Heart J. *73*, 55 (1967).
[8] G. BURCH and T. GILES, in *The Biology of Alcoholism* (Plenum Press, New York 1974), vol. 3, p. 435.
[9] This research was supported in part by Public Health Service Grant No. HL 15462.

Hemodynamic and Ventilatory Effects of Skin-Cooling in Cattle

Cattle are often used to study cardiopulmonary responses to alveolar hypoxia[1–4]. Such studies are generally concerned with interactions among pulmonary and systemic hemodynamics, alveolar or arterial blood gases, and ventilatory minute volume. Although it is usually assumed that inspired oxygen tension is the only environmental variable of physiological importance, cattle use ventilation to help regulate body heat and the ambient temperature at which the animals are studied might have significant cardiopulmonary effects[5]. It is important, therefore, to determine if thermoregulatory ventilation at the usual laboratory temperature of 25 °C influences the inter-relationships among cardiopulmonary processes. Thus, we studied the effects of skin-cooling on pulmonary and systemic hemodynamics, arterial blood gases, and minute ventilation in cattle at an ambient temperature of 25 °C.

Materials and methods. Systemic and pulmonary blood pressures, cardiac output, and arterial blood gases were measured in 12, 3- to 4-month-old, unanesthetized, Hereford calves following catheterization of the thoracic aorta, pulmonary artery, and right atrium. The techniques used in this study have been described elsewhere[4]. Minute ventilation was measured with a muzzle mask and a dry gas meter. Skin and rectal temperatures were monitored with thermocouples. Measurements were made at an ambient temperature of 25 °C before and after the skin on the calf's back was cooled for 30 min with cold water and a stream of air.

[1] A. F. ALEXANDER, C. S. CARD, R. S. JAENKE, J. L. HICKS and D. H. WILL, in *Research Animals in Medicine* (Ed. L. T. HARMISON; NIH, Washington 1974), p. 193.
[2] R. F. GROVER, in *Cardiovascular and Respiratory Effects of Hypoxia* (Eds. J. D. HATCHER and D. B. JENNINGS; Karger, Basel/New York 1966), p. 307.
[3] J. A. WILL, G. E. BISGARD, A. V. RUIZ and R. F. GROVER, in *Research Animals in Medicine* (Ed. L. T. HARMISON; NIH, Washington 1974), p. 267.
[4] E. K. WEIR, A. TUCKER, J. T. REEVES, D. H. WILL and R. F. GROVER, Cardiovasc. Res. *8*, 745 (1974).
[5] J. R. S. HALES and J. D. FINDLAY, Resp. Physiol. *4*, 333 (1968).