Z Rechtsmed 84, 195–207 (1980)

Zeitschrift für
Rechtsmedizin

Changes in the Myocardium and Skeletal Muscle in Guinea Pigs in Cold Exposure with and Without Ethanol*

J. Hirvonen, J. Penttinen, P. Huttunen, and P. Saukko

Department of Forensic Medicine, University of Oulu, Kajaanintie 52 D, 90220 Oulu 22, Finland

Summary. The effect of hypothermia with and without ethanol on the myocardium and skeletal muscle was studied. Changes were observed in both muscle types. The mildest lesions were discoloration of the muscle cells with acid fuchsin and Heidenhain's iron haematoxylin staining, these being more marked in the skeletal muscle. Waving and contraction bands in the muscle were seen in hypothermia. The most severe lesion was a focus with oedema and haemorrhage, a reduced reaction of β-hydroxybutyrate dehydrogenase and fragmentation of the muscle cells, and this was more frequent in the myocardium. Occasionally discoloration, contraction bands and waving were also seen in the controls killed by a blow on the neck. The changes were more numerous in the guinea pigs given ethanol before cold exposure, and serum creatinine phosphokinase was elevated in the same group. Urinary excretion of adrenaline increased in cold exposure, but noradrenaline did not change significantly. Hypoxia, catecholamines, and sludging of the blood are discussed as possible aetiological factors for the lesions.

Key words: Hypothermia, myocardial degeneration – Myocardial degeneration, hypothermia – Ethanol and myocardium

Zusammenfassung. Die Einwirkung von Hypothermie auf das Myokard und den Skelettmuskel sowie die Mitwirkung von Äthylalkohol wurde an Meerschweinchen studiert. Veränderungen konnten in beiden Muskelgruppen festgestellt werden. Eine leichte Läsion der Muskelzellen zeichnete sich als Dyskolorierung mit der Acid-Fuchsin- und Heidenhains-Eisen-Haematoxylin-Färbung. Die Veränderungen waren ausgeprägter in der Skelettmuskulatur.

Die Hypothermie-Gruppe zeigte wellenförmige Deformation der Muskelfasern und sog. hyaline Querbänder. Die stärksten Veränderungen: ein Focus mit Oedem, Haemorrhagie, Fragmentierung der Muskelzellen sowie eine

* This paper was presented in part at the 8th Scandinavian Meeting of Forensic Sciences, June 1979, in Sandefjord, Norway

Offprint requests to: Jorma Hirvonen, M.D. (address see above)

0044-3433/80/0084/0195/$ 2.60

herabgesetzte Reaktion der β-Hydroxy-Buttersäure-Dehydrogenase wurden häufiger im Myokard beobachtet. Gelegentlich sah man auch bei den Kontrolltieren, die durch einen Schlag in den Nacken getötet wurden, Dyskolorierung und wellenförmige Deformierung der Muskelfasern sowie hyaline Querbänder.

Die Veränderungen wurden häufiger bei denjenigen Meerschweinchen gesehen, die Alkohol vor der Kälteeinwirkung bekommen hatten. In derselben Gruppe waren auch die CPK-Werte im Serum erhöht. In der Kälte war die Urinexkretion von Adrenalin erhöht, dagegen beim Noradrenalin gab es keine statistisch signifikante Veränderung. Als mögliche ätiologische Faktoren der Läsionen werden Hypoxie, Katecholamine und Erythrozytenaggregation diskutiert.

Schlüsselwörter: Hypothermie, Myocardveränderungen – Myocardveränderungen, bei Hypothermie und Alkoholeinfluß – Alkoholwirkungen, Myocard

Signs of focal myocardial degeneration have been noticed both in therapeutic hypothermia (Smith 1940) and in victims of accidental hypothermia. The lesions consist of myofibre fragmentation or microinfarctions (Duguid et al. 1961) and discoloration of segments of the fibres with phosphotungstic acid haematoxylin, acid fuchsin, and iron haematoxylin (Hirvonen 1976). Similar lesions have been seen to develop in dogs during prolonged hypothermia, especially if respiration is not assisted, and small patches of myofibrosis or calcifications are seen in those which survive hypothermia. Thus, heart muscle seems to be affected by hypothermia and, if the lesions are widespread, reanimation is unlikely. These results together with the earlier, sometimes contradictory, literature (Fisher et al. 1957; Sano and Smith 1940) are extensively reviewed by Sarajas (1961).

When investigating deaths due to hypothermia one is faced with an absence of reliable signs pinpointing hypothermia as the actual cause of death. Focal myocardial lesions were found in 70% of cases, and did in fact constitute the most frequent sign suggesting hypothermia (Hirvonen 1976). We therefore set out to make a closer experimental study of these lesions, comparing them with the changes in skeletal muscle. The severity and type of the lesion was studied using proven muscle stainings and examining the release of muscle enzymes into the serum. In addition, the effect of ethanol on the development of the lesions was tested, as many studies have shown that ethanol can keep the heart beating in deeper hypothermia than could be survived otherwise (McGregor et al. 1966; Webb et al. 1968; Duthie and White 1977).

Material and Methods

The experiments were performed using adult guinea pigs of both sexes (weight range 580—825 g). The animals had lived at a normal colony temperature of +20° C with a 12 h illumination period per day, and had been fed on vegetables and pellets (Hankkija LDT, Helsinki) with water ad libitum.

The 30 guinea pigs used were divided into three groups, each containing both sexes. The first group consisted of 11 animals who were given 5 ml of 0.9% NaCl solution i.p. 30 min before

exposure. The second group contained 13 animals which were given 2 g/kg of ethanol i.p. in a 10% solution, also 30 min before the exposure. The NaCl and ethanol animals were placed in separate metabolic cages and transferred to a cold chamber, temperature −20° C, where they were kept until dead. The control group consisted of six guinea pigs given neither injection and not subjected to cold exposure, but killed with a blow on the neck after having been in the metabolic cages for 6 h.

Parameters and Samples

Rectal temperature was measured once an hour during cold exposure and at death. The time of death was defined as the cessation of heart beats.

A myocardial sample was cut transversally through the middle part of the ventricles, and *a skeletal muscle sample* excised from the femoral muscle. Both samples were divided into two parts, the first being rapidly frozen in liquid nitrogen for histochemical examination, and the second fixed in 10% neutral formaline and processed in the normal way for paraffin sections.

A blood sample was taken from the heart and central vessels at death. One part was centrifuged and stored frozen for enzyme analysis, and another was used for blood alcohol determination.

Urine was collected during cold exposure for catecholamine assay.

Methods

Histological and Histochemical Methods. The formalin-fixed muscle samples were embedded in paraffin wax and sectioned at 7μ.

The following stainings were used for both the heart and skeletal muscle sections:

1. Haematoxylin-eosin
2. Heidenhain's iron haematoxylin (Romeis 1968)
3. Acid fuchsin (Poley et al. 1964)
4. Haematoxylin basic fuchsin picrin acid; HBFP (Lie et al. 1971)
5. Mallory's phosphotungstic acid haematoxylin; PTAH (Lillie 1965).

The frozen muscle samples were cut in a cryostate at 10μ and the sections processed for the β-hydroxybutyrate dehydrogenase (HBD) reaction (Barka and Anderson 1965).

Estimation of the Degree of Muscle Lesion. The degree of muscle lesion was assessed independently by two of the authors without knowing the group of the animal. Attention was paid to the following signs of muscle cell damage: hyperchromasia or discoloration, occurrence of contraction bands and waving, fragmentation of the muscle cells and small haemorrhages between the fibres, and reduced intensity of the HBD reaction.

A scale from 0 to 3 was used when viewing the paraffin sections

0 = no lesions
1 = hyperchromasia or discoloration in the fibres
2 = contraction bands and/or waving
3 = fragmentation of the fibres and small haemorrhages.

A similar scale was also adopted for the changes in the HBD reaction

0 = even, deep blue reaction throughout the section
1 = violet areas among the blue
2 = reaction focally very weak, but no complete loss of staining
3 = local, complete loss of the reaction.

The scores for each animal were summed to give a lesion index. A mean and standard deviation for the index was then calculated for each group.

Biochemical Assays. Serum creatinine phosphokinase (CPK) and glutamate oxalacetate transaminase (GOT) were determined using standard kits (Calbiochem.—Boehring Corp.).

The ethanol concentration in blood was determined by gas chromatography using propanol as the internal standard (Porapak Q, 120—125 mesh, column temperature 170° C). The proteins were removed from the blood with a solution of BaOH and $ZnSO_4$.

The catecholamines adrenaline and noradrenaline in the urine was assayed by a method proposed by Pekkarinen (1969) in which catecholamines are absorbed into Al_2O_3 at pH 8.5 and eluted with a solution of HCl and NaH_2PO_4. The catecholamines are oxidized to adrenochrome and noradrenochrome and then reduced to adrenolutine and noradrenolutine. The intensity of fluorescence of these compounds was determined using an Aminco-Bowman spectrophotofluorometer.

Statistical significances of the differences between the groups were calculated using Student's *t*-test.

Results

The *survival time* of the guinea pigs receiving NaCl at the ambient temperature of −20° C was 354 ± 48 min (mean ± SD), but this was significantly reduced ($P < 0.01$) to 275 ± 76 min in those which received ethanol. The drop in *rectal temperature* started earlier and was more rapid in the ethanol than in the NaCl group, where it remained above 35° C for about 4 h (Fig. 1). The average dying temperature did not significantly differ between the two groups (Fig. 1).

There were no special macroscopic findings, only congestion in the organs and dilation of the right heart ventricle.

Histological Results

Myocardial Changes. Changes were observed in both the right and left heart ventricle. The frequencies for the degrees of lesion and staining methods are given in Table 1.

The first-degree lesion was a change in the staining of the myofibres with simultaneous homogenous appearance in the discoloured part. The lesion was visible as eosinophilia in H-E, red colour in acid fuchsin, and dark colour in iron

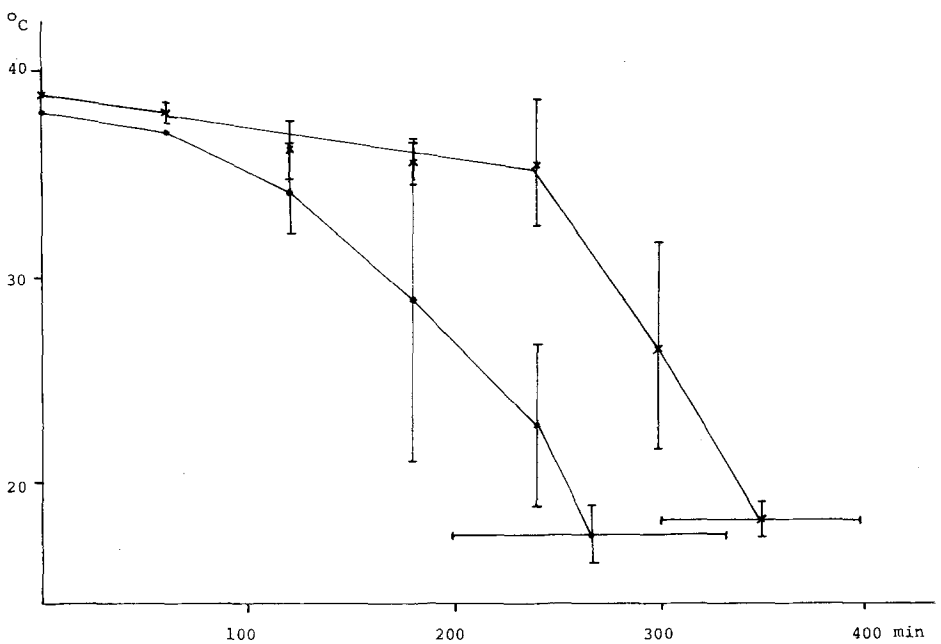

Fig. 1. Drop in body temperature and survival time of guinea pigs kept at −20° C

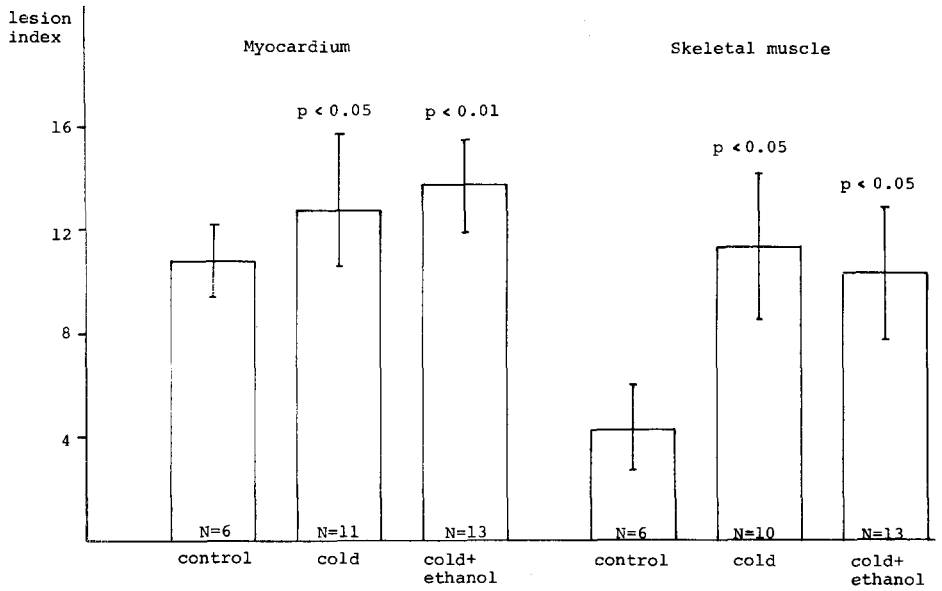

Fig. 2. Degree of morphological changes after exposure. The lesion index is the sum of the changes (graded 1—3) observed in the six histochemical stainings. Student's *t*-test

haematoxylin and PTAH (Fig. 3). Such changes could occasionally be seen in a few myocardial fibres in the control animals, but in cold exposure they were visible in a number of fibres evenly distributed in the section. In HBFP sections fuchsinophilia was rare and was confined to small areas. No difference was observable between the NaCl and ethanol groups.

Second-degree lesions, waving and/or contraction bands in the muscle fibres, were seen almost invariably in cold exposure (NaCl and ethanol groups), and occasionally in the controls. When appearing at the edge of the section those signs were discounted, as an artefact was probable. The most advanced lesion, a small focus consisting of fragmented myocytes which in addition were also discoloured and an occasional small haemorrhage, was found in both cold exposure groups in 27—64% of cases, depending on the method used. This was found only in one control animal. A slight focal decrease in the reaction intensity of HBD was seen in the non-exposed animals, while small weakly stained spots or single fibres, a second-degree lesion, were seen together with contraction bands in cold exposure.

Fig. 3. **A** Myocardium of a guinea pig subjected to cold exposure after ethanol administration. Eosinophilia, waving, and lysis of the myofibres point to degeneration of the myocardium. Haematoxylin-eosin. 160×. **B** Myocardium of a guinea pig subjected to cold exposure without ethanol. Fuchsinophilia, contraction bands and lysis of the myofibres. Acid fuchsin. 160×. **C** Myocardium of a guinea pig subjected to cold exposure after ethanol administration. Loss of enzyme reaction focally and contraction bands. β-hydroxybutyrate dehydrogenase. 400×. **D** Myocardium of a guinea pig killed with a blow on the neck. No discolouration or other signs of acute degeneration. Iron haematoxylin. 160×.

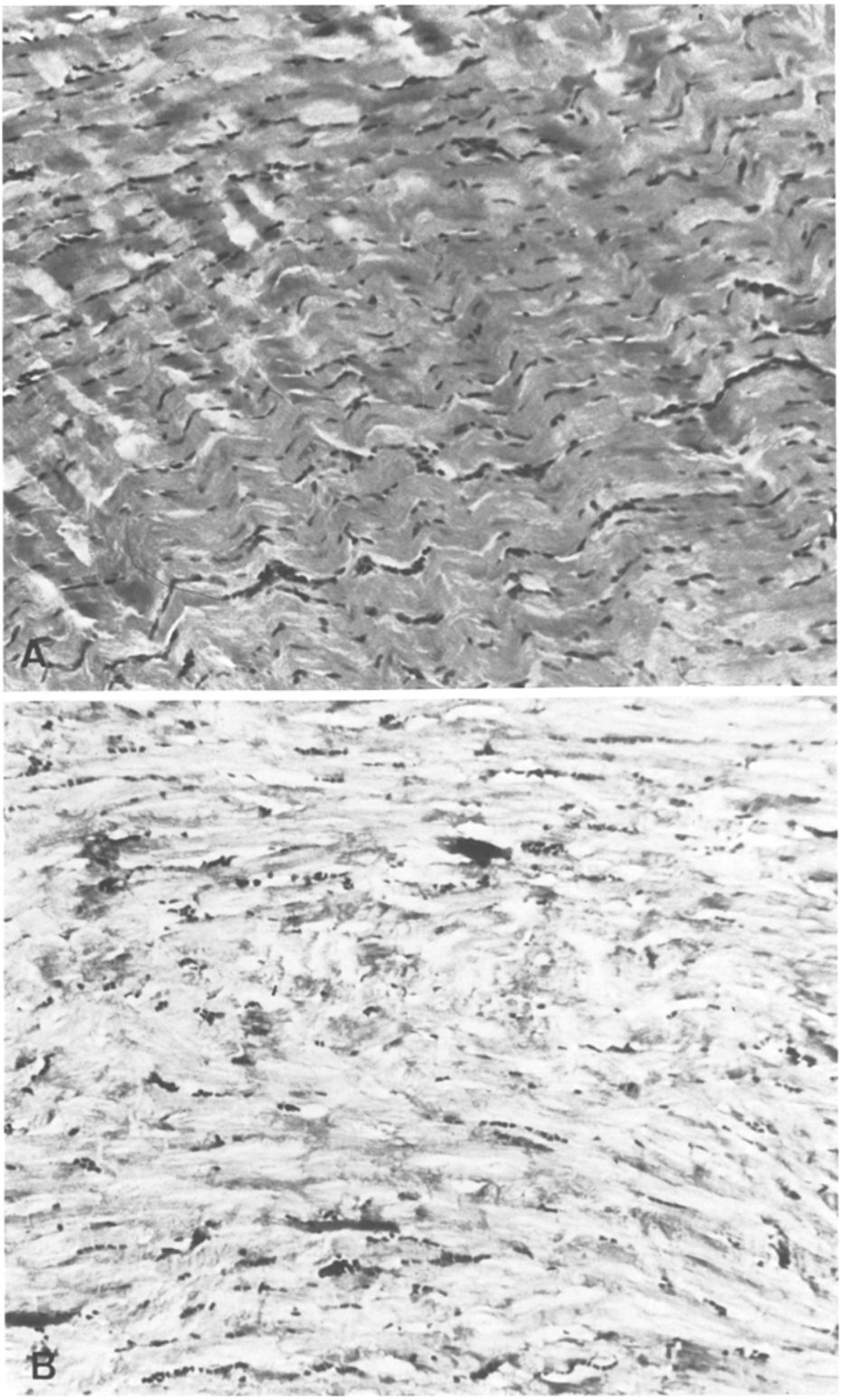

Fig. 3A, B (Legend see page 199)

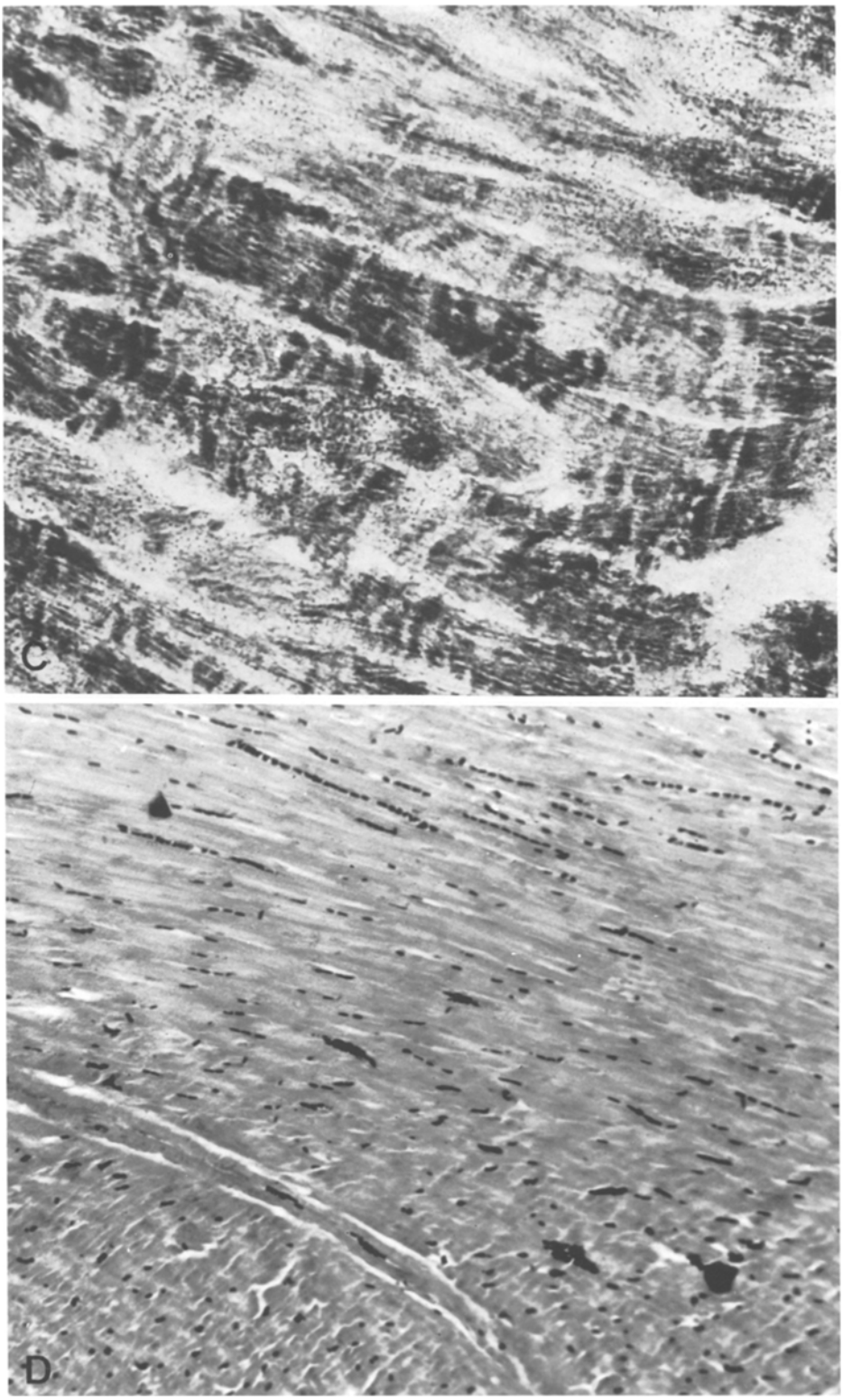

Fig. 3C, D (Legend see page 199)

Table 1. Frequency of myocardial changes in two exposure groups and controls as obtained using six stainings. The degrees of the lesions are explained in the text

Groups	HE	%	AF	%	HBFP	%	HIH	%	PTAH	%	HBD	%
Cold alone												
1st degree	—	—	2	18	5	45	—	—	—	—	2	18
2nd degree	8	73	5	45	6	55	6	55	4	36	8	73
3rd degree	3	27	4	36	—	—	5	45	7	64	1	9
($n=11$)												
Cold and ethanol												
1st degree	2	15	—	—	1	8	—	—	—	—	2	15
2nd degree	7	54	9	69	7	54	6	46	8	62	8	62
3rd degree	4	31	4	31	5	38	7	54	5	38	3	23
($n=13$)												
Control												
1st degree	—	—	3	50	3	50	—	—	—	—	1	17
2nd degree	5	83	3	50	3	50	5	83	6	100	5	83
3rd degree	1	17	—	—	—	—	1	17	—	—	0	—
($n=6$)												

HE, haematoxylin eosin; AF, acid fuchsin; HBFP, haematoxylin basic fuchsin picrin; HIH, Heidenhain's iron haematoxylin; PTAH, phosphotungstic acid haematoxylin; HBD, β-hydroxybutyrate dehydrogenase

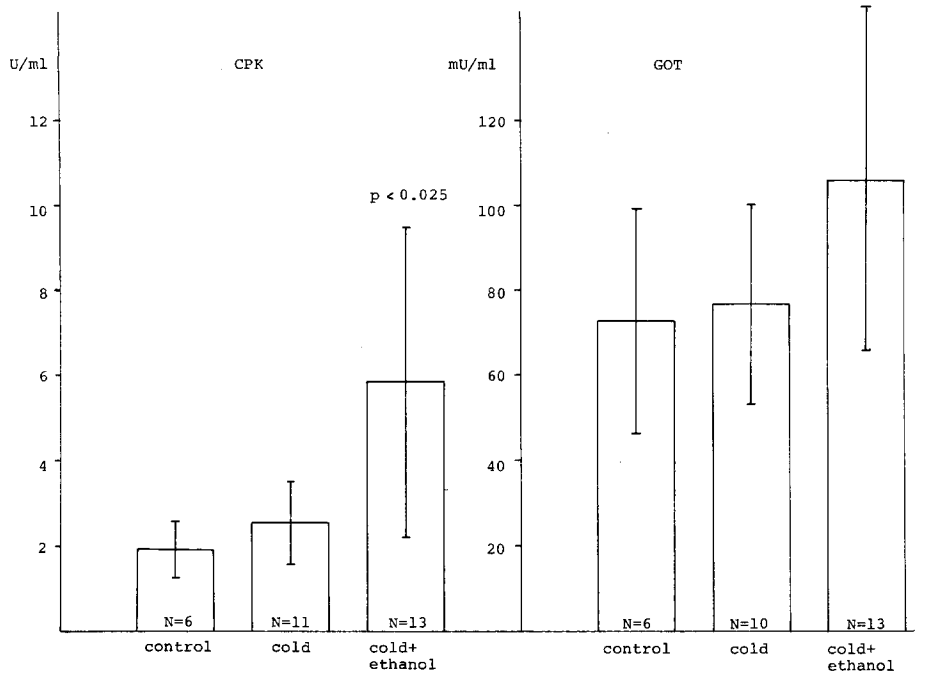

Fig. 4. Serum levels of creatinine phosphokinase (CPK) and glutamate oxalate transaminase (GOT) after exposure. Student's *t*-test

Complete focal loss of reaction was seen in the exposed animals, but never any more extensive negative areas.

The average lesion index for the NaCl group was 12.9 ± 1.7, which was significantly higher ($P < 0.05$) than in the control group, 10.8 ± 1.3. The index for the ethanol group was 13.8 ± 2.1, also significantly higher ($P < 0.01$) than in the control group.

The methods which demonstrated the mildest lesions best were those based on acid fuchsin and HBD staining.

Skeletal Muscle Changes. Changes were seen in all the exposed animals, involving deep red staining of extensive areas of the muscle fibres with acid fuchsin and single dark fibres with iron haematoxylin. Waving and contraction bands were seen in the fibres in every staining. Focal granulation of the cytoplasm and fragmentation of the fibre were seen in a few samples.

In the control group small parts of the muscle cells were orange in colour with acid fuchsin, red with basic fuchsin and dark with iron haematoxylin. Fuchsinophilia was never deep red in colour or widespread as in cold exposure. Mild waving was found with H-E in a few samples.

The lesion indices for the skeletal muscle were 11.4 ± 2.8 for the NaCl group, 10.9 ± 2.4 for the ethanol group and 6.0 ± 1.8 for the controls. The differences in relation to the control group were significant ($P < 0.01$).

Table 2. Urinary excretion of catecholamines (mean ± SD) in guinea pigs exposed to the cold (−20° C) with and without ethanol (2 g/kg). Controls were guinea pigs kept at room temperature

Groups	*N*	Adrenaline		Noradrenaline	
		μg/ml	μg/kg/h	μg/ml	μg/kg/h
Cold alone	6	0.09 ± 0.05	0.27 ± 0.04**	0.02 ± 0.05	0.12 ± 0.31
Cold and ethanol	6	0.10 ± 0.04	0.15 ± 0.12	0.01 ± 0.01	0.02 ± 0.03
Control	5	0.07 ± 0.02	0.17 ± 0.05	0.01 ± 0.01	0.01 ± 0.01

** $P < 0.01$ (Student's *t*-test)

Biochemical Results

The greatest *serum CPK* activity 5.8 ± 3.6 U/ml, was noted in the ethanol group. This was significantly higher ($P < 0.025$) than the control value of 1.9 ± 1.3 U/ml. The value for the NaCl group (2.6 ± 1.0 U/ml) did not differ significantly from the control value, but there was a significant difference ($P < 0.025$) with respect to the ethanol group (Fig. 4).

Serum GOT was also highest in the ethanol group (105.0 ± 39.2 mU/ml), but the difference was not significant with respect to either the NaCl group (76.9 ± 23.5 mU/ml) or the control group (72.0 ± 26.3 mU/ml) (Fig. 4).

The average *blood alcohol* concentration in the ethanol-treated animals at death was 1.36 ± 0.37‰ (mean ± SD).

Catecholamines. Cold exposure increased the urinary excretion of adrenaline in the absence of ethanol from 0.17 ± 0.05 μg/kg/h to 0.27 ± 0.04 μg/kg/h ($P < 0.01$), but not in its presence (Table 2).

Discussion

The myocardial and skeletal muscle changes occurring in hypothermia in these experiments were small, and there were no macroscopic signs of any lesion. Investigation at the microscopic level revealed focal degeneration in the myocardium, which varied from discoloration of a short part of the muscle fibre to destruction of a number of fibres with oedema and small haemorrhages. Same kind of local necrosis has also been noted in dogs which had been hypothermic for several hours, leading to fibrosis and calcification (Sarajas 1961). The foci are also observable in the hearts of victims who have succumbed to hypothermia (Duguid et al. 1961; Hirvonen 1976). If there is no destruction of the muscle fibres, the change may be demonstrated with greater certainty using special methods such as acid fuchsin and HIH, which stain the altered muscle cell cytoplasm. The cause of the discoloration is not clear, but it is also associated with a change in composition, the muscle cell turning homogenous. This part of the fibre had then picked up a different colour. A rearrangement of the muscle cell parts, or a change in the cytoplasm pH, e.g., would be possible explanations for this discoloration.

The mildest lesions, i.e., the discoloration, can apparently develop very rapidly, as they were occasionally present, though in a very mild form in those animals which had been killed with a blow on the head, where the heart had been removed immediately and fixed. These lesions are probably attributable to either a brief state of hypoxia or the mobilization of catecholamines due to the brain trauma.

The skeletal muscle was injured less than was the myocardium, but the lesion indices were higher in both hypothermic groups than in the controls, excluding the possibility of an artefact. It is therefore probable that some of the increased serum CPK activity may be derived from the skeletal muscle. The nature of the change in the muscle cell which causes discoloration is unclear, but acidosis and a decrease in glycogen are possibly involved.

The CPK values were highest in the group in which histological changes in the myocardium were most numerous. This implies that the main source of CPK may be the myocardium. Contrary to the results of Blain and Hook (1961), the GOT values did not change. The results parallel those in human victims of accidental hypothermia, in which high levels of CPK were also found by Maclean et al. (1968), who were able to find one small lesion in the skeletal muscle, but none in the myocardium in their necropsied hypothermia cases. The association of a CPK rise with ethanol treatment and hypothermia but not with hypothermia alone is difficult to explain. Perhaps only the double effect of cold and ethanol was enough to cause the cellular lesion which resulted in the release of CPK.

The aetiology of focal lesions is not clear. One mechanism would be the small platelet thrombi seen in dogs (Sarajas 1961), or sludging of the blood in some capillaries. Neither thrombi nor red cell plugs were seen in our experiments, however, which renders these explanations less likely. A second possibility is that the lesions may be due to myocardial hypoxia when the blood pressure falls and thus coronary perfusion can become relatively insufficient, and diffusion of oxygen into the muscle cells can also be slowed down. Hypoxia can also follow from the cessation of respiration once the heart continues beating. Relative hypoxia explains the diffuse patchy decrease in the HBD reaction in our experiments and the similar change in the succinate dehydrogenase reaction in the hypothermia experiments of Löfgren (1961). The role of hypoxia is further supported by the observation in dogs that, when respiration was assisted, the myocardial lesions were milder or non-existent (Sarajas 1961). Lesions are more numerous in deep hypothermia (below 30° C) lasting more than 1 h, two further factors increasing hypoxia (Sarajas 1964). A third explanation for the lesions would be that they are caused by catecholamines mobilized in hypothermia. It has been shown that the administration of l-noradrenaline to rats or isoprenaline to dogs induces myocardial lesions with contraction bands and patchy loss of the succinate dehydrogenase reaction (Kreinsen and Büsing 1975; Gopinath et al. 1978), the same changes in fact, as were observed here. Urinary excretion of adrenaline was increased in the present experiments, which fits in with the involvement of catecholamines in the lesion formation. The observations of Gilmour and Mallow (1977) that CPK, GOT, LDH, and HBD are released from perfused rat heart after a single dose of epinephrine speaks in favour of the same aetiology.

It has been proposed that the small lesions in the myocardium could act as ectopic foci, i.e., serving as a site for the initiation of ventricular fibrillation, which is often the mechanism of death in hypothermia of bigger animals (Duguid et al. 1961; Sarajas 1964).

The present experiments confirm our earlier experience that the guinea pig heart does not fibrillate, but simply ceases to beat. Thus, it seems that factors other than the foci are involved in the initiation of the fibrillation occurring in larger animals, e.g., the mass of the myocardium or an inhibition of muscle cell metabolism followed by increased irritability.

The lesions were more marked when ethanol and hypothermia were combined, as shown both microscopically and from the rise in serum CPK. This result runs contrary to the observation that ethanol protects the myocardium in hypothermia (Webb et al. 1968; MacGregor et al. 1966) nor does it fit in completely with the notion that myocardial lesions in hypothermia are caused by increased catecholamines, as Gilmour and Mallow (1977) found that ethanol prevents the myocardial damage caused by a single dose of adrenaline, the effect even being dose-dependent. These discrepancies cannot be explained, but species characteristics and some unknown combined effect of ethanol and hypothermia on muscle cell metabolism may perhaps be involved. Ethanol can have a deleterious effect of its own on the myocardium (Regan et al. 1966), but the degeneration seen in the present study cannot be ascribed to ethanol alone, as the NaCl group also showed histological signs of damage.

Acknowledgements. The authors are grateful to Ms. Marja-Liisa Hukkanen, Ms. Silja Leo, and Ms. Marjut Paitsola for the skillful technical assistance.

References

1. Barka T, Anderson PJ (1965) Oxidative enzymes. In: Histochemistry. Harper and Row, New York
2. Blair E, Hook R (1961) Serum glutamic oxalacetic transaminase content in hypothermia. Science 133:105–106
3. Duguid H, Simpson RG, Stowers JM (1961) Accidental hypothermia. Lancet 2:1213–1219
4. Duthie AM, White JF (1977) Cardiac arrest temperature: The effect of ethyl alcohol and carbon dioxide on rats, guinea pigs, and isolated rat hearts. Clin Exp Pharmacol Physiol 4:375–381
5. Fisher ER, Fedor EJ, Fisher B (1957) Pathologic and histologic chemical observations in experimental hypothermia. AMA Arch Surg 75:817–827
6. Gilmour RF, Mallow S (1977) Protective effect of ethanol against epinephrine-induced myocardial necrosis in rats. Res Comm Chém Pathol Pharmacol 18:303–327
7. Gopinath C, Thuring J, Zayed I (1978) Isoprenaline-induced myocardial necrosis in dogs. Br J Exp Pathol 59:148–157
8. Hirvonen J (1976) Necropsy findings in fatal hypothermia cases. Forensic Sci 8:155–164
9. Kreinsen U, Büsing CM (1975) Experimentelle Herzmuskelnekrosen bei der Ratte nach Gabe von l-Noradrenalin und Strophanthin. Virch Arch A, Pathol Anat and Histol 367:47–57
10. Lie JT, Holley KE, Kampa WR, Titus JL (1971) New histochemical method for morphologic diagnosis of early stages of myocardial ischemia. Mayo Clin Proc 46:319–327
11. Lillie RD (1965) Histopathologic technic and practical histochemistry, 3rd edn. McGraw Hill, New York

12. Löfgren L (1961) Histochemical investigation of succinic dehydrogenase in experimental hypothermia and parallel histo-pathological observations. Acta Pathol Scand 51:331–341
13. Maclean D, Griffiths PD, Emslie-Smith D (1968) Serum enzymes in relation to electrocardiographic changes in accidental hypothermia. Lancet Dec 14:1266–1270
14. MacGregor DC, Armour JA, Goldman BS, Bigelow WG (1966) The effects of ether, ethanol, propanol, and butanol on tolerance to deep hypothermia. Dis Chest 50:523–529
15. Pekkarinen A (1969) Fluorometric determination of adrenaline and noradrenaline. In: Manninen K (ed) (doctoral thesis) Regulation of the increased adrenomedullary secretion by drugs. Jaakkoo-Taara, Turku, pp 1–147
16. Poley RW, Fobes CD, Hall MJ (1964) Fuchsinophilia in early myocardial infarction. Arch Pathol 77:325–329
17. Regan TJ, Koroxenidis G, Moschos CB, Oldewurtel HA, Lehan PH, Hellems HK (1966) The acute metabolic and hemodynamic responses of the left ventricle to ethanol. J Clin Invest 45:270–280
18. Romeis B (1968) Mikroskopische Technik. Oldenburg, München
19. Sano ME, Smith LW (1940) A critical histopathologic study. Fifty post-mortem patients with cancer subjected to local or generalized refrigeration compared to a similar control group of 37 nonrefrigerated patients. J Lab Clin Med 26:443–456
20. Sarajas HSS (1964) Myocardial damage induced by immersion hypothermia. Am J Cardiol 13:355–366
21. Sarajas HSS (1961) Heart damage in dogs subjected to hypothermia, with or without complicating cardiac operations. Ann Acad Sci, Fenn A, Helsinki
22. Smith LW (1940) Pathologic changes observed in human tissues subjected to subcritical temperatures. Arch Pathol 30:425–439
23. Webb WR, Harrison N, Dodds R, Wax SD, Sugg WL (1968) Protective effect of ethyl alcohol in profound hypothermia. Cryobiology 4:290–294

Received October 18, 1979