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Increased Urinary Concentration of Catecholamines in Hypothermia Deaths

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ABSTRACT: Observations are presented on 24 hypothermia deaths, either accidental or suicidal. Most cases occurred in dry, cold circumstances, the air temperature being below 0°C. More cases were seen in early winter, suggesting a lack of acclimatization to the cold. Purple skin and swelling of the ears and nose (mild frostbite) were the most frequent external signs of exposure. Frequent internal signs were stomach ulcerations or hemorrhagic gastritis and small degenerative foci in the myocardium. High blood alcohol (about 200 mg/dL) was the most common contributory factor, but psychotropic drugs were detected in a few cases. The total urinary catecholamine content was increased in the hypothermia deaths, with levels of 0.20 ± 0.16 μg/mL (mean ± standard deviation) versus 0.07 ± 0.07 μg/mL in sudden natural deaths and 0.02 ± 0.02 μg/mL in rapid violent deaths. Adrenaline was more abundant than noradrenaline. It is suggested that urine catecholamine measurements can give useful information for the diagnosis of acute hypothermia.

KEYWORDS: pathology and biology, hypothermia, death

Hypothermia deaths are difficult to diagnose, since so far no specific changes caused by cold have been observed in the organs, but only general indications of stress. Therefore the diagnosis must be made partly by exclusion and partly by relying on anamnestic information. The search for more reliable necropsy signs is thus important, although cold stress can be tolerated to some extent and cold itself is less harmful to tissues than is heat.

The most frequent macroscopic sign pointing to death from hypothermia seems to be small hemorrhages and ulcerations in the gastric mucosa (Wischnewski ulcers), which indicate a stress condition that lasted some hours. If the exposure occurs in severe frost, a swelling of the nose, ears, hands, and face is visible, indicating vital frostbite [*1*].

In cases of accidental hypothermia, the process of death usually lasts 4 to 10 h, at least under winter conditions in northern Scandinavia. It is therefore understandable that there should be few morphological changes, and biochemical analyses could perhaps give more valuable information. Catecholamines and other stress hormones are secreted in connection with death, and increased amounts of these can be detected in the urine and blood, depending on the type and rapidity of the death [*2*]. Cold is in any case a cause of severe stress, which activates the sympathetic nervous system, and this is followed by increased secretion of stress hormones, particularly catecholamines [*3,4*]. This opens up the possibility of a postmortem assay for catecholamines, which appear to be relatively stable after death.

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We have previously reported [*1*] that catecholamines may appear in larger amounts than usual in the urine of hypothermia victims, an observation that is consistent with findings in living persons. Since this provided a promising clue to a postmortem test for hypothermia, further material was collected; the urinary catecholamines, adrenaline and noradrenaline, were measured and the results were compared with those obtained in cases of rapid violent deaths and sudden natural deaths.

Materials and Methods

All the cases in which hypothermia had been determined as the primary or contributory cause of death were collected from the years 1974 to 1980. Most of the 24 cases were males, 18 having hypothermia as the primary cause of death and 3 as a secondary factor. Only 3 females were among the 24, but these were all "true" hypothermia cases. The frequency was thus three or four cases per year from a population of 420 000. The cases were divided into groups according to the type of exposure: dry exposures with air temperature above or below 0°C, or wet exposure (Table 1). Dry exposure was the most frequent, and more cases occurred in the early winter than in the later winter, but one case occurred even in June. The age range was 21 to 85 years for the males and 27 to 56 years for the females. The mean ages and other data are given in more detail in Table 1.

The reference material comprised eight cases of sudden natural death of cardiac origin and six cases of rapid violent death with causes such as hanging, skull fracture, shotgun wounds, and alcoholic intoxication.

A complete necropsy was performed within two or three days of death. Tissue samples were taken for histological investigation and a urine sample was taken for catecholamine determinations. Drug and alcohol analyses were also performed on blood, urine, and tissue samples.

Histological Methods

The tissue samples were normally fixed in buffered neutral formalin, except for the myocardium (left ventricle), from which an extra sample was also fresh-frozen for enzyme histochemistry. The formaline-fixed samples were processed into paraffin blocks as usual and stained with hematoxylin and eosin. In addition, acid fuchsin [*5*], Heidenhain's iron hematoxylin, and phosphotungstic acid hemotoxylin (PTAH) were applied to heart sections [*6*]. Reactions of cytochrome oxidase, succinate, and hydroxybutyrate dehydrogenase were determined in the frozen heart sample in order to study very recent hypoxemic changes [*7*].

Chemical Methods

The alcohol concentration in the blood and urine was determined using gas chromatography and the alcohol dehydrogenase (ADH) method. Drugs were measured by routine methods in the Laboratory of Forensic Chemistry at the University of Helsinki.

The catecholamines adrenaline and noradrenaline were assayed in the urine by the method proposed by Pekkarinen [*8*], in which catecholamines are absorbed into alumina at pH 8.5 and eluted with a solution of hydrochloric acid and monobasic sodium phosphate. They are then oxidized to adrenochrome and noradrenochrome and reduced to adrenolutine and noradrenolutine, whose intensity of fluorescence was determined by using an Aminco-Bowman spectrophotofluorometer. The modified fluorometric method of Anton and Sayre [*9*] was also used in determining total catecholamine concentration from some earlier samples.

TABLE 1—*Circumstances and contributory factors in cases of hypothermia, by type of exposure.*

Sex and Age	Exposure Type	Cases, *n*	Month	Ethanol, mg/dL		Drugs	Illnesses
				Blood	Urine		
Males[a]							
53 ± 12[b]; 40-63[c]	dry, 0°C	3	June, Oct., Dec.	200 ± 110; 70-280	250; *n* = 1	...	emphysema, cardiac hypertrophy
49 ± 18; 21-74	dry, < 0°C	12	Jan.-April, Oct.-Dec.	170 ± 110; 0-340	270 ± 180; 0-460; *n* = 8	thioridazine (2), diazepam, pericyazine	emphysema, cardiac hypertrophy, coronary sclerosis
52 ± 5; 48-55	dry, temperature unknown	2	April, Nov.	40 ± 60; 0-80	130 ± 180; 0-260	cyclobarbital, hexobarbital, chlordiazepoxide, oxazepam	diabetes
39	wet	1	Oct.	190	260	...	...
Females[a]							
34 ± 9; 27 and 41	dry, 0°C	2	March, Dec.	0	0	pericyazine, methylperone, chlorprothixene	depression
56	wet	1	Sept.	0	0	dibenzepin	...
Males[d]							
62 ± 20; 49-85	dry, 0°C	3	Jan., Oct., Dec.	250 ± 160; 100-410	310 ± 250; 130-490	thioridazine	cardiac hypertrophy, alcoholism

[a]Hypothermia as the primary cause of death.
[b]Mean ± standard deviation.
[c]Range.
[d]Hypothermia as a contributory cause of death.

Statistical Analyses

The results were tested with one-way analysis of variance where the hypothermia group was compared to both control groups and to the combined controls. Student's t test was used when the differences between the two control groups were tested.

Observations

The average age of the male victims in each group was about the same, around 50 years, but the females were younger (Table 1).

Manner of Death

There were three suicides, of which two were women and one a man, and also one man for whom the manner of death could not be determined (Table 2). Five males and one female, or one victim in four, had undressed.

Contributing Factors

The average blood ethanol concentration in the male victims was 200 mg/dL or a little less and that in the urine around 250 mg/dL (Table 2). There were three males who had not been consuming alcohol, but one of them had thioridazine, one cyclobarbital and chlordiazepoxide, and one severe arteriosclerosis and emphysema as contributing factors. Four males had ingested both alcohol and some psychotropic drug (neuperil [pericyazine], diazepam, oxazepam, or thioridazine). The female victims had no alcohol in the blood, but the one who died accidentally had pericyazine, methylperone (flubuperone), and chlorprothixene, a combination that had probably caused such a disturbance in thermoregulation that she had succumbed to mild cold at 0°C or to acute intoxication. The other two were suicidal exposures; one had taken dibenzepin and the other no drugs. Exposure had occurred in these cases under wet conditions at 0°C and in dry frost, respectively.

Signs of Exposure

A breakdown of the signs of exposure by the sex of the victim and environmental conditions is given in Table 2. Discoloration of the skin (purple color, marble-like pattern on the extremities) was seen in 22 cases out of 24 (92%), but this is a sign that can also develop postmortem.

Frostbite (swelling of the ears, nose, hands) was seen in 14 cases, a sign that can be taken as critical. Stomach mucosal lesions were found in 15 victims (62%), and small myocardial degenerative foci were seen in all cases. The latter change consisted of fragmentation and hyalinization of the myofibers, possible contraction bands and coloration of a part of myofiber red with acid fuchsin, black with iron hematoxylin and dark violet with PTAH, and perhaps a patchy disappearance of enzyme reactions.

Catecholamines

The mean urinary total catecholamine concentration (Table 3) was increased fourfold in the hypothermia deaths over the combined controls, sevenfold over the rapid violent deaths, and about threefold over the sudden natural deaths. These differences were statistically significant with the one-way analysis of variance. The total catecholamine content was significantly greater in the natural deaths than in the violent deaths within the control groups ($P < 0.05$, t test). When urinary adrenaline and noradrenaline were analyzed separately in five cases of hypothermia death, both were found to be significantly more elevated in the hypo-

TABLE 2—*Findings in hypothermia deaths, by type of exposure.*

Sex and Number of Cases	Type	Number of Cases					Manner of Death
		Undressing	Frostbite	Skin Discoloration	Stomach Lesions	Myocardial Degeneration	
Males[a]							
3	dry, < 0°C	...	1	3	1	3	all accidents
12	dry, < 0°C	5	11	12	9	12	10 accidents, 2 undetermined
2	dry, temperature unknown	...	...	1	2	2	1 suicide, 1 undetermined
1	wet	...	...	1	1(?)	1	accident
Females[a]							
2	dry, < 0°C	...	1	2	...	2	1 accident, 1 suicide
1	wet	1	...	1	...	1	suicide
Males[b]							
3	dry, < 0°C	...	1	1	2	3	2 accidents,[c] 1 natural

[a]Hypothermia as the primary cause of death.
[b]Hypothermia as a contributory cause of death.
[c]One involving alcoholic intoxication, one involving alcoholic and drug intoxication.

TABLE 3—*Urinary catecholamines in hypothermia deaths and controls.*[a]

Type of Death	Concentration of Catecholamines, μg/mL		
	A[b]	NA[b]	A + NA[b]
Hypothermia	0.176 ± 0.139 (5)	0.112 ± 0.097 (5)	0.206 ± 0.169 (24)
Natural	0.031 ± 0.025 (8)	0.047 ± 0.033 (8)	0.079 ± 0.048[c] (8)
Violent	0.013 ± 0.005 (6)	0.015 ± 0.023 (6)	0.028 ± 0.025 (6)
Natural and violent combined	0.024 ± 0.021 (14)	0.034 ± 0.033 (14)	0.057 ± 0.046 (14)

[a]In the one-way analysis of variance the differences in the catecholamine concentrations between the hypothermia and the two control groups (first figure) as well as the combined control group (second figure) were significant as follows. For A, $F = 8.49, 17.56$; $P = 0.003, 0.001$. For NA, the ratio of variances $F = 4.36, 7.43$; $P = 0.031, 0.014$. For A + NA, $F = 5.34, 10.39$; $P = 0.009, 0.003$.

[b]The number in parentheses is the number of cases.

[c]$P < 0.05$.

thermia group than in the violent and natural deaths or in the combined controls (analysis of variance).

Discussion

The observations on the presented cases of fatal hypothermia partly confirm those made earlier by others and by ourselves [*1, 10*] and partly point to some new approaches for diagnosing death from hypothermia.

Swelling of the ears, nose, and hands emerged as a relatively reliable sign of vital frostbite, and the same applies perhaps to the purple coloration of the skin, although the marble pattern of the skin on the hands and feet and the bright red color of the skin can also develop postmortem. Hemorrhagic gastritis or small 2- to 3-mm ulcerations in the gastric mucosa are also a good sign of antemortem cooling stress. The myocardial degenerative foci are also a change that points to difficulties in cardiac function known to occur in hypothermia [*11*]. Unfortunately, these signs are not specific but are merely changes that can result from any prolonged stress or shock with catecholamines as one etiologic factor [*12, 13*].

The earlier preliminary observation of increased urinary concentrations of catecholamines (CA) in hypothermia death [*5*] was now confirmed in a larger number of cases. The analysis also revealed that adrenaline (A) increased proportionally more than noradrenaline (NA). The possibility was ruled out that alcohol intake alone could have considerably increased the CA content in the urine, and thus cold stress can with more certainty be regarded as having caused the enhanced secretion of adrenaline and noradrenaline. The lowest urinary CA values were in the group of sudden violent deaths, mostly suicides, but the noradrenaline content was a little higher in the sudden natural deaths, perhaps reflecting some antemortem stress effect of the fatal illness, or of an intake of drugs. Total serum CAs are higher in natural deaths than in rapid traumatic deaths, but low in hospital deaths with slowly advancing agony [*14*]. According to Laves and Berg [*2*], serum CA content is increased in heart infarction deaths, and both A and NA are high, but A increases proportionally more. The same also occurs in suffocation, where A/NA relation is inverted from the normal. Similarly, A appeared in great concentrations in the urine in the present hypothermia cases, pointing to a greater activation of its secretion under cold conditions. This is consistent with the different roles of the catecholamines: A is more effective in mobilizing glucose, which is an important fuel for thermogenesis [*15*]. Similarly, A increases proportionally more than does NA in the serum during hypothermic operations in humans [*4*].

The concentration of CAs in the urine probably depends on the duration of hypothermia.

It was not possible to determine the exact duration in our cases, but some weight can perhaps be put on the trend for low values for total CA (below 0.1 μg/mL) to be detected mostly in cases with high blood alcohol (over 200 mg/dL). These latter persons are likely to succumb faster than more sober ones. Multiple diuresis caused by cold and alcohol [*16*] can affect the results, but the increase in CA excretion was still demonstrable in spite of the dilution of the urine. Of course, more accurate information could be obtained if serum values were available, but CA measurements from serum are difficult to obtain in frozen cadavers, because of hemolysis, for instance, whereas no such obstacles exist in the case of the urine.

What kind of evidence is needed for hypothermia to be regarded as the primary cause of death? First, the circumstances must point to cold stress: low temperature, wetness, wind, and physical exhaustion must be present. Second, there should be some signs of cold stress or frostbite in the body, for example swelling of the ears and stomach ulcerations or hemorrhagic gastritis. Third, ethanol or some other drug harmful to thermoregulation may well be found in the blood or urine. The ethanol concentration should be higher than 150 mg/dL, since it seems not to have the same dilating effect on the cutaneous vessels at lower concentrations [*17*]. Fourth, a high concentration of catecholamines (more than 0.1 μg/mL) should appear in the urine, with more adrenaline than noradrenaline. When these criteria, or at least three of them, are met, a diagnosis of hypothermia death can be made with reasonable certainty. The manner of death, which closely depends on the cause of death and the circumstances, can then be determined, whether it be an accident, suicide, or negligence on the part of some other person. The last possibility is often raised in cases of hypothermia, since the law demands that a helpless person should not be left in a dangerous situation.

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