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Beta-Hydroxybutyric Acid—An Indicator for an Alcoholic Ketoacidosis as Cause of Death in Deceased Alcohol Abusers*

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ABSTRACT: We analyzed the postmortem blood of a total of 100 fatal cases for beta-hydroxybutyric acid (BHBA). In 25 cases of sudden and unexpected death of alcoholics we found pathologically increased levels of BHBA of 1260 to 47 200 (median 8000) μmol/L. This led us to the diagnosis of an alcoholic ketoacidosis (AKA) as cause of death in these cases. The control group of 69 postmortem cases revealed that BHBA concentrations below 500 can be regarded as normal, and values up to 2500 μmol/L as elevated. Our study shows that BHBA values over 2500 μmol/L could lead to death, if no medical attention is sought. During storage we did not find any indication of postmortem formation or decomposition of BHBA in blood in vitro or in the corpses. In our opinion, BHBA should be considered the diagnostic marker of choice for the postmortem determination of alcoholic ketoacidosis (AKA) as the cause of death. The classical indications of such deaths are: unexpected death of a chronic alcoholic; none or only traces of ethanol in the blood; increased acetone blood concentration; and neither autopsy, histology, microbiology, nor toxicology reveal the cause of death. In six further cases a diabetic ketoacidosis (DKA) was diagnosed as the cause of death.

KEYWORDS: forensic science, alcoholic ketoacidosis, diabetic ketoacidosis, beta-hydroxybutyric acid, beta-hydroxybutyrate, chronic alcoholics, cause of death, postmortem, degradation, stability, whole blood

Cases of sudden and unexpected deaths of chronic alcoholics have been noted in which no or only little ethanol could be determined in the postmortem blood samples. An acute alcohol intoxication as the cause of death can therefore be ruled out. Furthermore the autopsy, histology, microbiology, and toxicology could not determine the cause of death. Proportionally such deaths in chronic alcoholics make up 3 to 10% (2,3). In comparison, all the deaths investigated, where no cause of death could be determined through forensic science, are estimated at 1 to 3% (4).

We closely investigated those cases of unexpectedly deceased chronic alcoholics with no determined cause of death that were examined in the Institute of Forensic Medicine of Zurich between 1990 and 1998. In 1993 we published a report of three clinical survivors and six cases which ended in death (5). In the six examined corpses blood alcohol concentrations below 0.01 g/100 mL and BHBA concentrations between 4100 and 47 200 μmol/L (1 μmol/L = 0.104 mg/L) were determined. During that investigation we found the diagnostic marker of choice to be beta-hydroxybutyric acid (BHBA) for the postmortem investigation of alcoholic ketoacidosis (AKA). Our present investigation reconfirms the results of this study.

The Role of Beta-Hydroxybutyric Acid in the Metabolism

In order to understand how the BHBA concentration can increase, we need a closer look at the different steps of the metabolic pathway. Ethanol is mainly degraded via acetaldehyde and acetate to acetyl-CoA. Drinking copious amounts of ethanol, as is the case in chronic alcoholics, a large amount of NADH is produced in the first and second steps (Fig. 1). The $NADH/NAD^+$-equilibrium is shifted towards NADH (6–8). As shown in Fig. 2, the accumulation of NADH causes a rise in the lactate level, an increase in the ketogenesis, and a shift of the BHBA/acetoacetate ratio towards BHBA (8–14). This leads to a suppression of the citric acid cycle. There is an increased lactate concentration in the blood, but levels usually remain under those of a lactic acidosis (9,13–15).

Typical for chronic alcoholics and an important factor in the pathogenesis of AKA is the condition of primary nourishment with ethanol and minimal ingestion of food. Fasting causes the glycogen levels in the liver to be used up. The increase of the glucagon/insulin ratio causes increased lipolysis and ketogenesis. Due to the dropping insulin level, the lipolysis is increasingly less inhibited. The concentration of free fatty acids rises in the blood. The production rate of the ketone bodies is directly proportional to the amount of available free fatty acids. The result of this rapid increase of lipolysis seems to be responsible for an increased ketogenesis in alcoholic ketoacidosis (AKA) as well as in diabetic ketoacidosis (DKA). Additionally, low carbohydrate diets are ketogenic and lipolytic.

The production of the ketone bodies acetoacetate, acetone and BHBA is regulated, among others, through the available concentration of acetyl-CoA; see Fig. 3. The breakdown of ethanol, the suppression of the citric acid cycle, and the increase of fatty acids all increase the concentration of acetyl-CoA. Acetoacetate spontaneously decarboxylates to acetone, which is a neutral substance and does not contribute to an acidosis (16). BHBA is produced in a NADH-dependent reaction from acetoacetate. The ketone body ratio is two-thirds BHBA and one-third acetoacetate and acetone (17).

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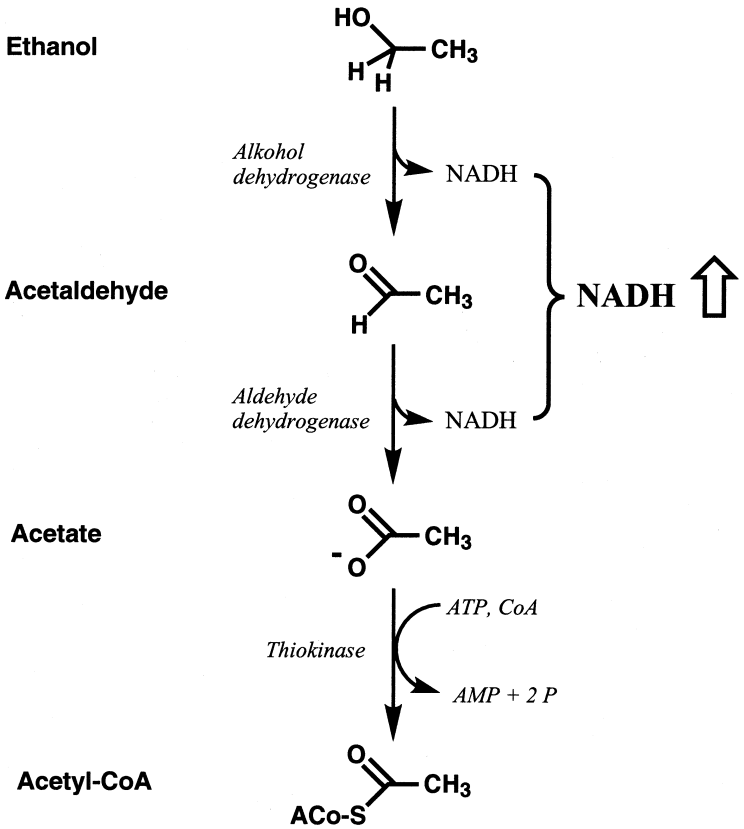

FIG. 1—*Major pathway of ethanol degradation in the human body.*

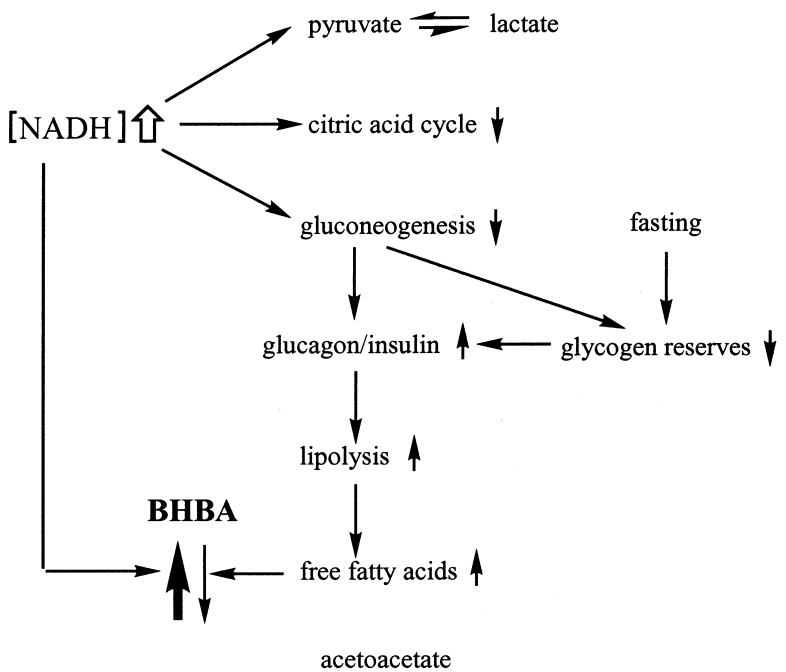

FIG. 2—*Accumulation of NADH may cause dramatic changes in different metabolic pathways and leads to an increase of BHBA.* ⌓ *massive increase,* ↑ *increase,* ↓ *decrease,* ⥋ *shift to right side.*

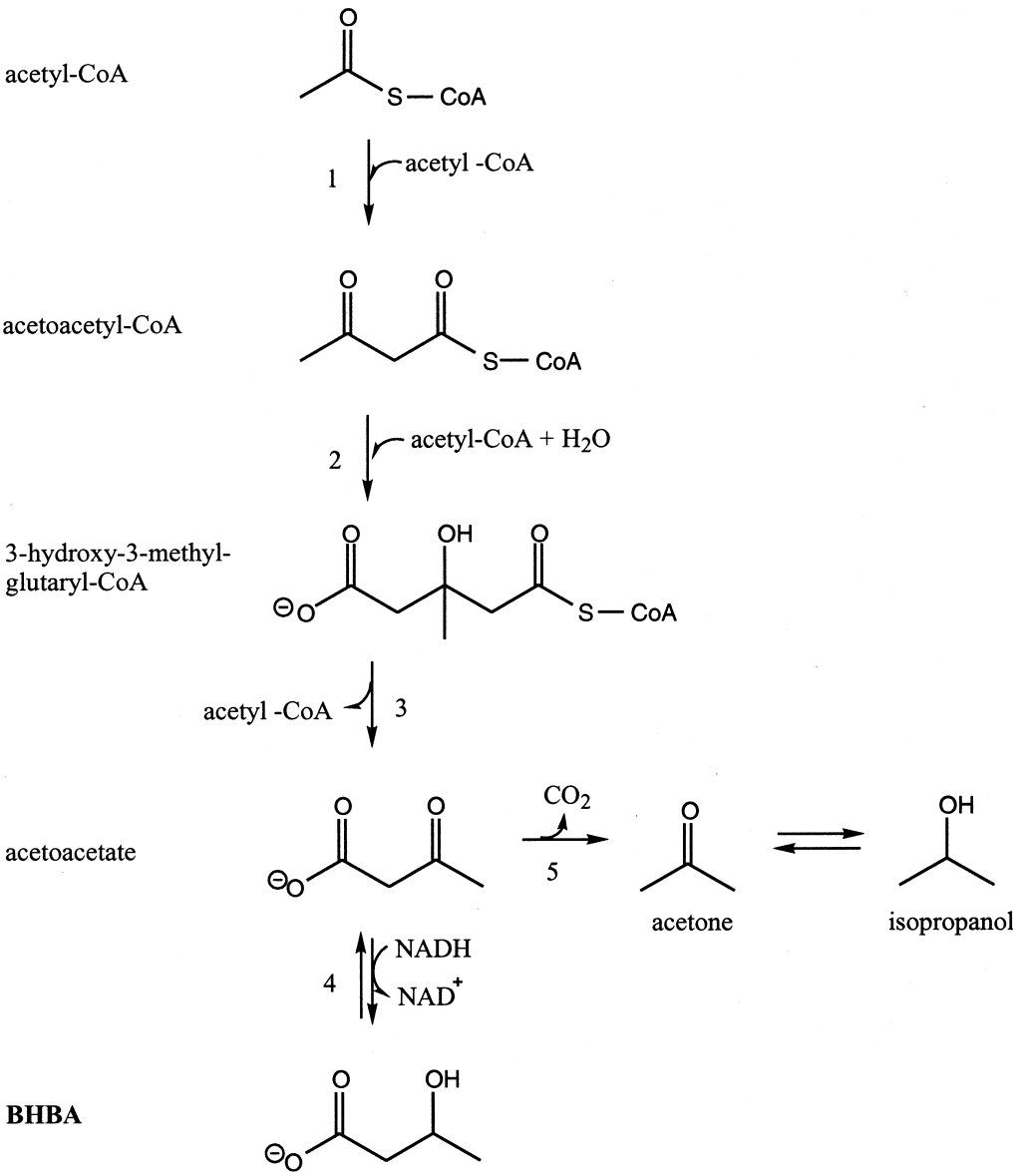

FIG. 3—*Metabolic pathway of acetyl-CoA to acetoacetate, acetone, and beta-hydroxybutyric acid (BHBA). 1 = beta-ketothiolase; 2 = HMG-CoA synthase; 3 = HMG-CoA lyase; 4 = D-3-hydroxybutyrate dehydrogenase; 5 = spontaneous decarboxylation.*

Review of the Literature

To date there have been few publications dealing with postmortem examinations of alcohol-induced metabolic disturbances as the cause of death in chronic alcoholics. The relevant laboratory results of these studies are summarized in Table 1. In 1993 Denmark (18), Thomsen et al. (19), and Caspar et al. (5) published the first studies.

Of the 13 chronic alcoholic deaths investigated by Denmark (18) the cause of death could not be determined through autopsy in six cases. He used BHBA as a diagnostic marker for the determination of AKA. Because of the advanced stage of haemolysis in many of the postmortem whole blood samples and the associated analytical problems, he investigated the vitreous humour and urine.

Thomsen et al. determined the ketone body concentration in the postmortem blood of 122 deceased persons (19) using gas chromatography with head-space injection (20). Sixteen of them were chronic alcoholics who suffered sudden and unexpected death with little or no ethanol in their blood. In those authors' opinion the cause of death in this group was in whole or partially due to ketoacidosis. In 1995, Thomsen et al. (21) published a second study where a total of 131 deceased persons were investigated. However, the group of 17 chronic alcoholics without ascertainable cause of death showed low BHBA blood concentrations of 147 to 833 μmol/L.

Pounder et al. (22) determined the total ketone body concentration (acetone + acetoacetate + BHBA) in 105 autopsy cases using gas chromatography with head-space injection in vitreous humour, pericardial fluid and blood from the Vena femoralis, superior/inferior Vena cava, and the aorta. The group of 22 chronic alcoholics contained only four cases with significantly elevated levels of total ketone bodies. The authors themselves mentioned that more data were needed to make a precise AKA diagnosis as cause of death. Furthermore, it was their assessment that a total ketone body concentration as an indicator for AKA be >10 000 μmol/L in the blood of the femoral vein and >5000 μmol/L in the vitreous humour.

TABLE 1—*Analytical data from the reviewed literature.*

Author(s)	Group	Number of Cases	BHBA			Acetone [Peripheral Blood (μmol/L)]	Acetone + Acetoacetate [Peripheral Blood (μmol/L)]	Acetone + Acetoacetate + BHBA [Peripheral Blood (μmol/L)]
			Peripheral Blood (μmol/L)	Vitreous Humour (μmol/L)	Urine (μmol/L)			
Caspar (5)	A	6	4100–47 200	…	…	…	…	…
Denmark (18)	A	6	…	1830–8170	2570–47 400	…	…	…
	B	7	…	0–8576	192–19 230	…	…	…
	C	36	…	<960	<960	…	…	…
Thomsen (19)	A	16	33–3585	…	…	…	22–3230	…
	B	27	0–1876	…	…	…	18–680	…
	C	79	0–702	…	…	…	0–490	…
Thomsen (21)	A	17	147–833	…	…	…	91–312	…
	B	35	39–133	…	…	…	34–80	…
	C	79	36–81	…	…	…	28–49	…
Pounder (22)	A	4	15 900–26 800	…	…	…	…	18 600–130 000
	C	71	…	…	…	…	…	230–8080
Brinkmann (23)	A	6	…	…	…	1700–6900	…	…
	C	218	…	…	…	<170	…	…
Iten (present publication)	A	25	1260–47 200	…	…	…	…	…
	C	69	52–2440	…	…	…	…	…

NOTE—A = deceased alcoholics with unknown cause of death; B = deceased alcoholics with known cause of death; C = control group.

In a first series, Brinkmann et al. (23) quantitated acetone in the postmortem blood of 24 sudden and unexpected deaths of chronic alcoholics. In six cases the autopsy revealed no cause of death, but acetone was found in a pathological range of 1700 to 6900 μmol/L (normal range 40 to 60 μmol/L in serum; 1 μmol/L = 5.8 μg/100 mL). BHBA was not examined. Brinkmann recommends an acetone analysis if no pathomorphological or toxicological cause of death can be determined in chronic alcoholics. Blood acetone levels of >1500 μmol/L are indicative of a severe ketoacidotic coma. In a second series of 45 questionable deaths of chronic alcoholics the sum values according to Traub (glucose + lactate) were determined in the cerebrospinal fluid.

In 1940, Dillon et al. were the first to describe severe metabolic acidosis in chronic alcoholics in clinical cases (24). They observed that an increased concentration of BHBA could be used as a good indicator for an alcoholic ketoacidosis (AKA). The patients suffered from severe vomiting, nausea, dehydration, abdominal pain and Kussmaul breathing, transpiration of acetone, and absentmindedness. The symptoms and the laboratory results they described were similar to those published in later studies (5,10,12,13,25–33). Since the clinical symptoms are very similar to those of a diabetic coma, a differential diagnosis is necessary. The laboratory findings of AKA patients reveal an increased anion gap, a metabolic acidosis and a positive reaction to serum ketone bodies. In contrast to DKA, AKA produces usually a hypoglycemia, but a slight hyperglycemia or glucosuria can also occur.

Materials and Methods

Beta-Hydroxybutyrate-Analyses—The beta-hydroxybutyrate analysis (BHBA) was carried out at the Clinical Chemistry Laboratory of the Children's Hospital in Zurich (director Dr. N. Blau).[2] The method used was first described by Olsen (34) and modified by Dr. Blau. One mL of whole blood is deproteinized with 1 mL 0.6 N perchloric acid (5.2 mL 70% $HClO_4$ diluted with distilled water to a total volume of 100 mL). After centrifugation the supernatant is kept for analysis. The analysis is based on an enzymatic reaction using 3-hydroxybutyrate dehydrogenase (3-HBDH suspension, Boehringer Mannheim, Nr. 127833) in a carbonate buffer pH 9.5 (6.5 g $KHCO_3$ plus 4.84 g K_2CO_3 and 0.2 g Titriplex III (Merck 8418) diluted with distilled water to a total volume of 100 mL) with a surplus of NAD^+ (NAD, Boehringer Mannheim, No. 127302). The calibration points are set at 50, 100, 150, 200, 250, and 300 μmol BHBA per liter (sodium beta-hydroxybutyrate, Boehringer Mannheim, 106569). The calibration is linear within this range. Procedure: 50 μL deproteinized blood or 50 μL BHBA standard solution (in distilled water/0.6 N perchloric acid 1:1) plus 1 mL enzyme solution (1 mL buffer pH 9.5 plus 0.25 mg NAD plus 20 μL 3-HBDH suspension) are incubated at room temperature for 90 min. BHBA reacts enzymatically to acetoacetate and the NADH produced by this reaction is quantitated using a fluorometer (excitation at 340 nm, emission at 460 nm). According to the product information sheet of Boehringer, 3-HBDH has a high specificity: the enzyme oxidizes D-3-hydroxybutyrate (relative rate = 1.0), 3-hydroxypentanoate (rate = 0.05) and 3-hydroxyhexanoate (rate = 0.04). It does not oxidize 3-hydroxypropionate or L-3-hydroxybutyrate. To date no rapid diagnostic test has been developed for BHBA. Available tests for the detection of ketone bodies in the blood or urine are often quite sensitive to acetoacetate, less to acetone and not at all to BHBA.

Ethyl Alcohol, Acetone, and Isopropanol Analyses—Using gas chromatography/FID, blood was independently tested twice using two different methods for a total of four results. Method 1: 200 mg whole blood plus 2000 mg internal standard 1 (150 mg propionitrile in 1 L of distilled water), direct injection of 1 μL into a packed column (2 m × 3 mm, Porapak Q, 80/100 mesh, Art. No. 2-0331, Supelco), nitrogen with a flow rate of 25 mL/min was used as carrier gas and the oven temperature was set isothermal at 160°C. Method 2: 300 mg whole blood plus 800 mg internal standard 2 (200 mg acetonitrile plus 50 g NaF in 1 L of distilled water) head-space injec-

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tion, capillary column (Restek Stabilwax-DB, 30 m, 0.53 mm ID, 1 μm film, Cat. No. 10855), nitrogen gas with a flow rate of 5 mL/min was used as carrier gas and the oven temperature was set isothermal at 80°C. Aqueous ethanol standards were used for calibration (0.50, 1.00, 2.00 and 3.00 g/kg, Merck 8988.0001). RRT (Method 1/Method 2): methanol 0.196/0.642, ethanol 0.368/0.714, acetone 0.601/0.539, isopropanol 0.641/0.689, propionitrile 1.000/1.090, acetonitrile 0.481/1.000.

Glucose and Lactate Analyses—These compounds were analyzed in the clinical laboratory of the University Hospital Zurich using standard procedures. Glucose was quantitated in serum, cerebrospinal fluid, and vitreous humour with the hexokinase method (35) and lactate with the lactate oxidase/peroxidase method (36).

Results and Discussion

In our study, a total of 100 deaths were investigated between 1990 and 1998 for beta-hydroxybutyric acid (BHBA) using postmortem whole blood. In order to get as many BHBA positive cases as possible, all autopsy cases were selected which fulfilled the following criteria: unexpected death, suspicion of chronic alcohol abuse, none or only traces of ethanol in the blood, and where neither autopsy, toxicology, histology nor microbiology revealed the cause of death. Additionally we analyzed several cases with a possible history of diabetes or alcoholism. In order to reach a total number of 100 cases we randomly selected other cases including different manner and causes of death.

In 31 cases elevated BHBA blood concentrations were found. The differentiation between an alcoholic ketoacidosis (AKA) and a diabetic ketoacidosis (DKA) was done by carefully screening the case history and/or analyzing urine, serum, cerebrospinal fluid, or vitreous humour for elevated glucose concentration. In contrast to DKA, AKA usually produces a hypoglycemia, but a slight hyperglycemia or glucosuria can also occur. In this way 25 cases were assigned to the AKA group and six to the DKA group. The 69 cases with normal BHBA values were assigned to the negative group. The negative group should reveal information about the normal BHBA range in postmortem blood and possible postmortem BHBA changes in the concentration during the storage in vitro and in the corpses.

AKA Group

The 25 cases of the AKA group showed BHBA blood concentrations in a range of 1260 to 47 200 (median 8000) μmol/L; see Fig. 4. BHBA concentration in postmortem blood below 500 μmol/L were considered normal. Therefore the AKA group had between a 2.5- to 95-fold (median 16-fold) higher postmortem BHBA blood concentration than normal.

In 21 (84%) of the 25 AKA cases laboratory results confirmed the cause of death to be an alcoholic ketoacidosis. In two cases the diagnosed cause of death was primarily a cardiovascular failure due to massive damage of the heart. A metabolic disorder brought on through chronic alcoholism can also be taken into account as being the cause of death. The BHBA blood concentrations were 4000 and 4350 μmol/L, respectively. In the last two cases traumatic injuries (in one case combined with a sepsis) led to death. In both cases disorientation, a very poor state of health, and a highly increased BHBA value of 10 070 and 6000 μmol/L, respectively, led us to diagnose a severe AKA, which in turn could explain the unobserved way of getting injured.

In 64% of the AKA cases we detected increased acetone blood levels. Precise quantitation was made only in six cases, where we found 3400 to 7700 μmol/L (1 μmol/L = 5.8 μg/100 mL). Serum concentrations of 40 to 60 μmol/L are considered normal (37). Brinkmann et al. (23) also found pathological acetone values between 1700 and 6900 μmol/L in their AKA group. According to these authors, acetone concentrations in blood of more than 1500 μmol/L can be indicative of a serious ketoacidotic coma.

Among the 25 deaths in the AKA group, 9 were women (36%) and 16 men (64%), who were between 29 and 66 years of age (median 50 years). All of them had a history of chronic alcoholism. The autopsy revealed alcohol associated changes of the liver in 22 cases (88%) (clearly visible fatty degeneration of the liver 19 times, cir-

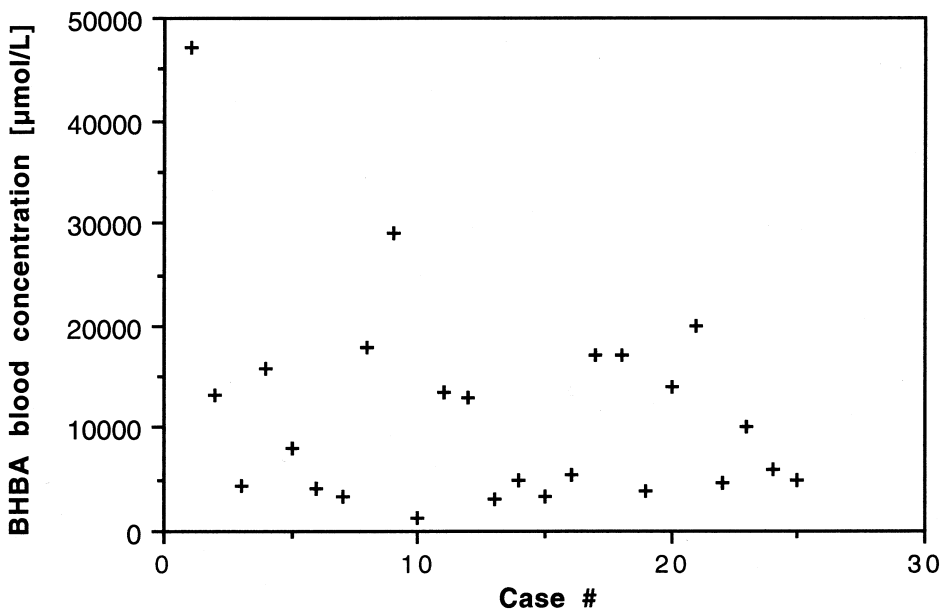

FIG. 4—*Beta-hydroxybutyric acid concentrations (BHBA concentrations) in postmortem whole blood of 25 cases with alcoholic ketoacidosis (AKA).*

rhosis 3 times, fatty liver hepatitis 2 times) and changes of the pancreas in 14 cases (56%) (lipomatosis 10 times, inflammation of the pancreas 2 times, pancreatic fibrosis 2 times).

All AKA cases showed similar findings. The following characteristics can be helpful for the recognition of such cases: (1) sudden and unexpected death of a chronic alcoholic; (2) no or only traces of ethanol in the postmortem blood (also indicating that the cause of death was not an acute alcohol intoxication); (3) increased acetone blood concentration; and (4) autopsy as well as histology, microbiology, and toxicology reveal no cause of death. Additionally, a number of characteristic features were recognized: neglected food intake or minimal eating habits, poor general condition, discovery of the body in living quarters, vomit in vicinity of the corpse, neglected appearance, messy living area, and empty bottles of alcohol present.

TABLE 2—*Beta-hydroxybutyric acid (BHBA), lactate and glucose concentrations of the DKA group. In comparison, the ranges of the AKA group and the normal ranges are shown.*

	BHBA (μmol/L)	Lactate (mmol/L)	Glucose (mmol/L)
DKA case #1	23 000*	37.2‡	43.8†, 31.9‡
DKA case #2	37 800*	‖	‖
DKA case #3	7260*	‖	45.0†, 59.4‡
DKA case #4	27 500*	38.8‡, 35.2§	61.6‡, 63.2§
DKA case #5	2290*	60.1‡, 51.8§	4.9‡, 11.6§
DKA case #6	21 000*	27.2‡, 26.8§	12.2‡, 16.9§
Range of the AKA group¶	1260–47 200*	23.8–46.5‡ 20.8§	1.1–3.3† 1.2–1.7‡
Normal range	clinical <340† postmortem <500*	0.6–2.4† 1.2–2.1‡ 0.6–2.4§	3.9–5.8† 2.4–4.2‡ 3.9–5.8§

* Postmortem whole blood.
† Blood serum.
‡ Cerebrospinal fluid.
§ Vitreous humour.
‖ Not analyzed.
¶ Lactate and glucose were analyzed only in a few cases.

Case Study of the AKA Group—A 65-year-old heavily alcohol dependent man was found dead in his apartment. He lived a secluded life for over 30 years. His apartment was in a state of disorder, dozens of whiskey and wine bottles were lying around. During his last months, he hardly ate and had a sip of whiskey about every 10 minutes. The man, who appeared listless and weak, complained about epigastric pain prior to his death. The autopsy and histology revealed a fatty degeneration of the liver and a lipomatous atrophy of the pancreas. No ethanol was detected in the peripheral blood, but a high BHBA concentration of 17 000 μmol/L, a highly elevated acetone concentration, and a low glucose level of 1.8 mmol/L were found.

DKA Group

The six cases of the DKA group showed highly elevated BHBA blood concentrations of 2290 to 37 800 (median 22 000) μmol/L. That is 5- to 76-fold (median 44-fold) higher than normal. In addition, the DKA group showed pathologically elevated glucose and lactate concentrations; see Table 2. All six cases of the DKA group could be diagnosed with a diabetic ketoacidosis (DKA) as probable cause of death. In contrast, the cases of the AKA group showed a hypoglycemia. Glucose was measured only in three cases. We found low concentrations of 1.1 to 3.3 in serum and 1.2 to 1.7 mmol/L in the cerebrospinal fluid. However, glucose concentrations have to been interpreted with care, because a postmortem decrease occurs, especially in whole blood.

The DKA group consisted of two women and four men, who died between the ages of 32 and 68 years (median 46). Two of them were known to have been chronic alcoholics. The autopsy revealed changes of the liver in five cases (clearly visible fatty liver 4 times, fibrosis 2 times) and changes of the pancreas in two cases (lipomatosis 1 time, pancreatitis 1 time).

Negative Group

The 69 cases of the negative group (control group) showed BHBA blood concentrations of 52 to 2440 (median 250) μmol/L; see Fig. 5. In this group neither an AKA nor a DKA, but rather an-

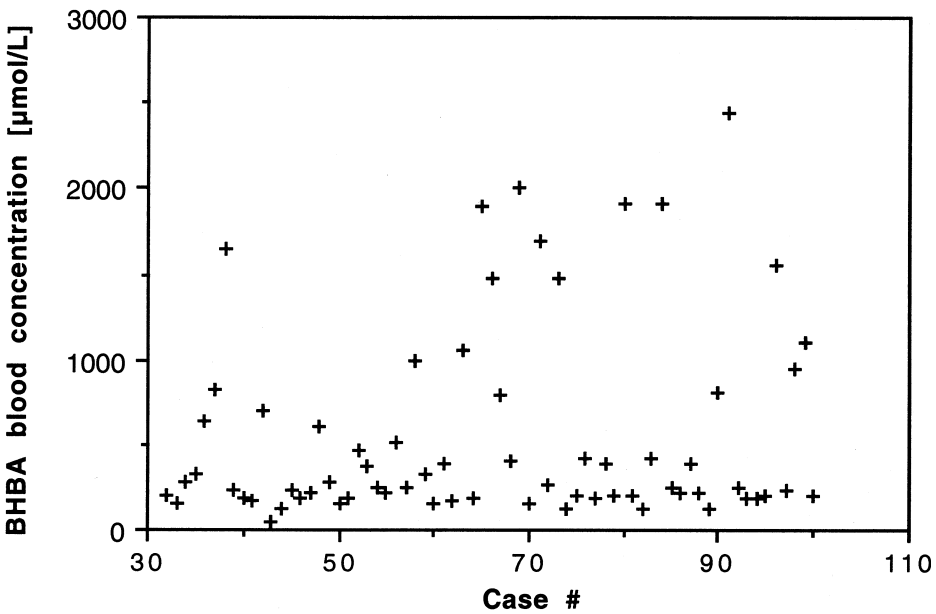

FIG. 5—*BHBA concentrations in postmortem blood in the negative group were within a range of 52 to 2440 (median 250) μmol/L.*

other cause of death, was diagnosed. As a result of the distribution of these values we propose the following limits for BHBA in postmortem whole blood: concentrations up to 500 μmol/L can be regarded as normal, 500 to 2500 μmol/L as elevated, and above 2500 μmol/L as pathological; see also Fig. 8. In living persons, values up to 340 μmol/L are considered normal, but prolonged fasting can produce elevated values of up to 2000 μmol/L (5).

Of the 69 deaths in the negative group 27 were female (39%) and 42 male (61%), who died between the ages of 23 and 83 years (median 45 years). The manner of death was as follows: death due to natural causes (41 cases), suicide (11), drug-induced accident (10), and unknown (7). As cause of death the following diagnoses were made: illicit drug poisoning, drug poisoning, cardiopulmonary arrest, pulmonary fat embolism, internal bleeding, and multi-organ failure.

Stability of BHBA in the Corpse and in Postmortem Blood Samples

In order to find out whether a formation or decomposition of BHBA occurs in the corpse during the time interval between death and drawing a blood sample, we plotted the BHBA blood concen-

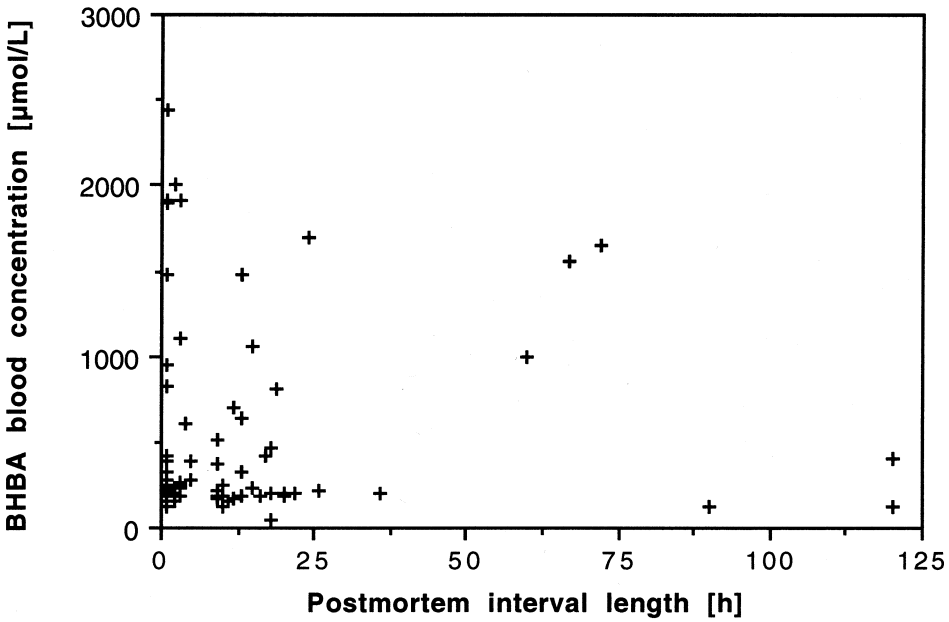

FIG. 6—*BHBA concentrations in the postmortem blood of the negative group, shown in relation to the postmortem interval length during the time of death and that of blood sample withdrawal.*

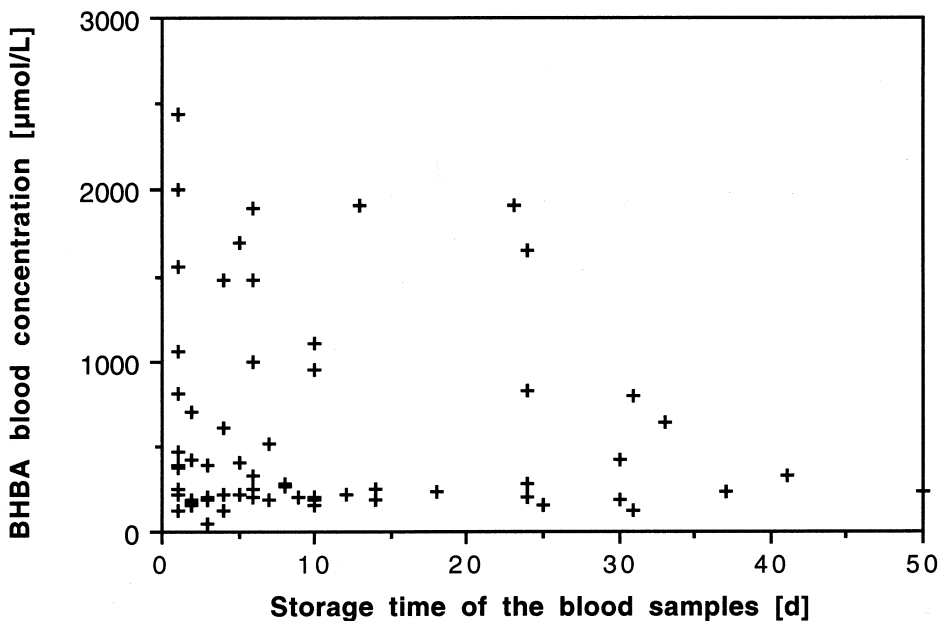

FIG. 7—*BHBA concentrations in the postmortem blood of the negative group, shown in relation to the storage time of the blood samples in a −20°C freezer.*

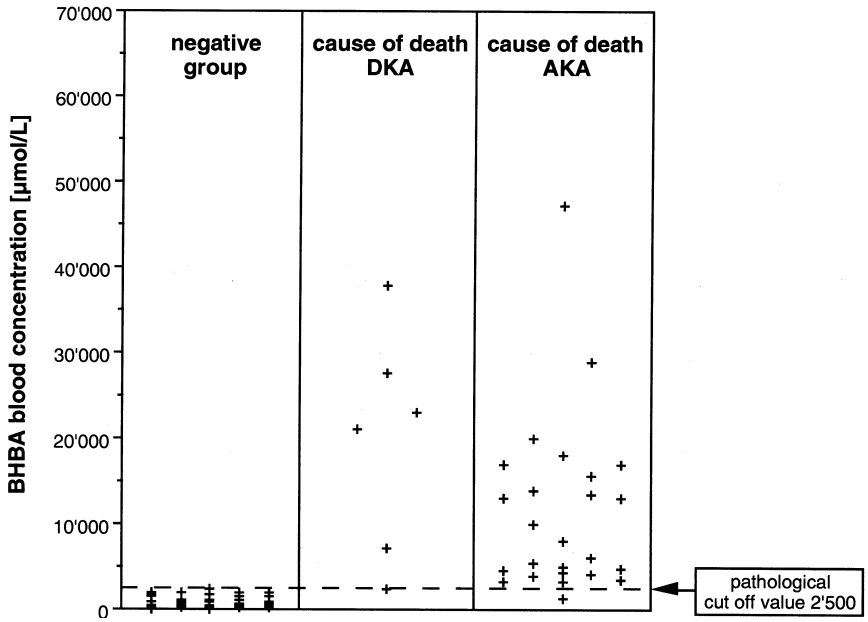

FIG. 8—*BHBA concentrations in postmortem blood of all 100 cases. The left column represents cases of the negative group, the middle column those with diabetic ketoacidosis as cause of death (DKA group), and the right column alcoholic ketoacidosis as cause of death (AKA group).*

trations of the negative group in relation to this postmortem interval length (Fig. 6). The Spearman rank correlation with a *p*-value of 0.63 demonstrates that there is no statistically significant change in the BHBA concentration along the time axis. The postmortem interval length ranges from 1 h to 14 days. The blood sample of the corpse with the longest postmortem interval length of 14 days and a BHBA concentration of 790 µmol/L is not shown in Fig. 6.

In order to study the stability of BHBA in the blood samples during storage at −20°C, we plotted the BHBA blood concentrations of the negative group in relation to the storage time (Fig. 7). The Spearman rank correlation with a *p*-value of 0.70 indicates no statistically significant correlation between BHBA concentration and storage time. This leads us to the conclusion that no significant changes in the BHBA concentrations occur even when stored in the freezer for a longer period of time. The storage times extended from 1 to 274 days. The two blood samples that were stored the longest are not shown in Fig. 7. They had BHBA concentrations of 250 µmol/L after 102 days and 175 µmol/L after 274 days of storage, respectively. The blood samples contained no preservatives.

Conclusions

Our study indicates that an alcoholic ketoacidosis (AKA) is more often than not the cause of death in alcohol abusers, and that an AKA can easily be diagnosed with a BHBA analysis in postmortem blood. A series of criteria was found to be helpful for the recognition of AKA cases: sudden and unexpected death of a chronic alcoholic no ethanol or only traces in the postmortem blood; increased acetone blood concentration; and where the autopsy, histology, microbiology, and toxicology reveal no cause of death. Figure 8 shows an overall view of the 100 postmortem BHBA blood concentrations collected in our study, each in its respective group. We propose the following BHBA limits in postmortem whole blood: concentrations up to 500 µmol/L are regarded as normal, 500 to 2500 µmol/L as elevated, and above 2500 µmol/L as pathological. Our study shows that values above 2500 µmol/L account for a serious AKA or DKA which can lead to death if no medical attention is sought. Values of nearly 50 000 µmol/L have also been recorded and were regarded as being extreme. We found no indication of a postmortem formation or decomposition of BHBA, either in the corpse or in vitro.

Acknowledgments

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References

1. Meier M. Beta-Hydroxybutyrat (BHBA), ein Indikator zur Diagnostizierung einer alkoholischen Ketoazidose (AKA) als Todesursache [dissertation]. Zurich: University of Zurich, 1999.
2. Clark JC. Sudden death in the chronic alcoholic. Forensic Sci Int 1988;36:105–11.
3. Hansen AU, Simonsen J. The manner and cause of death in a forensic series of chronic alcoholics. Forensic Sci Int 1991;49:171–8.
4. Janssen W, Naeve W. Der plötzliche Tod aus natürlicher Ursache. In: Mueller B. Gerichtliche Medizin. Berlin: Springer-Verlag, 1975.
5. Caspar CB, Risti B, Iten PX, Jost R, Russi EW, Speich R. Alkoholische Ketoazidose. Schweiz. Med. Wochenschrift 1993;123:1929–34.
6. Israel Y, Videla L, Bernstein J. Liver hypermetabolic state after chronic ethanol consumption: Hormonal interrelations and pathogenetic implications. Federation Proceed 1975;34:2052–9.
7. Greiling H, Gressner AM. Pathobiochemie und klinisch-chemische Diagnostik der Organ- und Systemerkrankungen. In: Lehrbuch der Klinischen Chemie und Pathobiochemie. Schattauer, 1989;421–508.
8. Madison LL. Ethanol-induced hypoglycemia. Adv Metab Disord 1968;3:85–109.
9. Lundquist F, Tygstrup N, Winkler K, Mellemgaard K, Munck-Petersen S. Ethanol metabolism and production of free acetate in the human liver. J Clin Invest 1962;41:955–61.
10. Miller PD, Heinig RE, Waterhouse C. Treatment of alcoholic acidosis. Arch Intern Med 1978;138:67–72.

11. Randall B. Fatty liver and sudden death. Human Pathol 1980;11: 147–53.
12. Thompson CJ, Johnston DG, Baylis PH, Anderson J. Alcoholic ketoacidosis: an underdiagnosed condition. British Med J 1986;292:463–5.
13. Fulop M. Alcoholic ketoacidosis. Endocrin Metab Clin North Amer 1993;22:209–19.
14. Fulop M. Alcoholism, ketoacidosis, and lactic acidosis. Diabet Metab Rev 1989;5:365–78.
15. Lieber CS. Metabolism and metabolic effects of alcohol. Med Clinics North Amer 1984;68:3–31.
16. Oster JR, Epstein M. Acid-base aspects of ketoacidosis. Am J Nephrol 1984;4:137–51.
17. Wieland O. Ketogenesis and its regulation. Adv Metab Dis 1968;3: 1–47.
18. Denmark LN. The investigation of beta-hydroxybutyrate as a marker for sudden death due to hypoglycemia in alcoholics. Forensic Sci Int 1993;62:225–32.
19. Thomsen JL, Theilade P, Felby S, Nielsen E. Alcoholism and ketoacidosis. Forensic Sci Int 1993;60:3–4.
20. Felby S, Nielsen E. Determination of ketone bodies in postmortem blood by head-space gas chromatography. Forensic Sci Int 1994;64:83–8.
21. Thomsen JL, Felby S, Theilade P, Nielsen E. Alcoholic ketoacidosis as cause of death in forensic cases. Forensic Sci Int 1995;75:163–71.
22. Pounder DJ, Stevenson RJ, Taylor KK. Alcoholic ketoacidosis at autopsy. J Forensic Sci 1998;43:812–6.
23. Brinkmann B, Fechner G, Karger B, Du Chesne A. Ketoacidosis and lactic acidosis—frequent causes of death in chronic alcoholics. Int J Legal Med 1998;111:115–9.
24. Dillon ES, Dyer WW, Smelo LS. Ketone acidosis in nondiabetic adults. Med Clinics North Amer 1940;24:1813–22.
25. Jenkins DW, Eckel RE, Craig JW. Alcoholic ketoacidosis. JAMA 1971;217:177–83.
26. Levy LJ, Duga J, Girgis M, Gordon EE. Ketoacidosis associated with alcoholism in nondiabetic subjects. Annals Internal Med 1973;78: 213–9.
27. Cooperman MT, Davidoff F, Spark R, Pallotta J. Clinical studies of alcoholic ketoacidosis. Diabetes 1974;23:433–9.
28. Soffer A, Hamburger S. Alcoholic ketoacidosis: a review of 30 cases. J Amer Med Wom Assoc 1982;37:106–10.
29. Halperin ML, Hammeke M, Josse RG, Jungas RL. Metabolic acidosis in the alcoholic: a pathophysiologic approach. Metabolism 1983;32: 308–15.
30. Fulop M, Bock J, Ben-Ezra J, Antony M, Danzig J, Gage JS. Plasma lactate and 3-hydroxybutyrate levels in patients with acute ethanol intoxication. Amer J Med 1986;80:191–4.
31. Adams SL. Alcoholic ketoacidosis. Emergency Med Clin North Amer 1990;8:749–60.
32. Wrenn KD, Slovis CM, Minion GE, Rutkowski R. The syndrome of alcoholic ketoacidosis. Am J Med 1991;91:119–28.
33. Jobard JM. Acidocétose alcoolique. Annales de gastroentérologie et d'hépatologie 1993;29:233–5.
34. Olsen C. An enzymatic fluorometric micromethod for the determination of acetoacetate, beta-hydroxybutyrate, pyruvate and lactate. Clin Chim Acta 1971;33:293–300.
35. Knedel M, Haeckel R, Seidel D, Thiery J, Vonderschmitt DJ, Haenseler E. Analytical performance of the random access analyser Hitachi 737. A multicentre evaluation. J Clin Chem Clin Biochem 1986;24:409–32.
36. Marx AM, Gressner AM. Evaluation of the Kodak Ektachem slide assays for iron and lactate. J Clin Lab Anal 1991;5:86–9.
37. Wissenschaftliche Tabellen Geigy, Band 1, p. 87; Ciba-Geigy Ltd., Basel 1977.

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