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Endogenous Alcohol Production by Intestinal Fermentation in Sudden Infant Death

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Summary. In some cases of sudden infant death syndrome (SIDS) the intestinal flora was found to be dominated by *Candida albicans.* Microbiologic investigations of the various organs showed the occasional presence of different Candida species, but not in the form of massive growth as in sepsis. There is no basis to assume that the activity of yeasts, first of all of *Candida albicans,* is a contributory factor in the occurrence of SIDS.

Candida albicans was shown to produce alcohol from glucose at a rate of maximally 1 mg of alcohol per gram of intestinal content per hour. It is concluded that the intestinal production of alcohol in vivo from cases showing a *Candida albicans* dominated intestinal flora will not be able to surpass the normal alcohol metabolizing capacity of the liver. Thus, measurable concentrations of alcohol in the blood from such cases cannot be expected.

Key words: Sudden infant death, *Candida albicans* – Intestinal alcohol production

Zusammenfassung. In einigen Fällen vom Syndrom des plötzlichen Säuglingstodes (SIDS) wurde befunden, daß die Darmflora von *Candida albicans* dominiert war. Mikrobiologische Untersuchungen der verschiedenen Organe zeigten gelegentlich Vorkommen von verschiedenen Candida-Arten, aber nicht in der Form von massivem Wachstum wie bei Sepsis. Es besteht kein Grund zur Annahme, daß die Wirksamkeit von Hefen, in erster Linie von *Candida albicans,* ein mitwirkender Faktor beim Vorkommen von SIDS ist.

Es wurde festgestellt, daß *Candida albicans* Alkohol aus Glukose in einer Geschwindigkeit von höchstens 1 mg Alkohol pro Gramm Darminhalt je Stunde produziert. Daraus wird gefolgert, daß die Darmproduktion von Alkohol in vivo in Fällen, die eine von *Candida albicans* dominierte Darmflora aufweisen, die normale alkoholmetabolisierende Fähigkeit der Leber nicht übertreffen wird. Meßbare Konzentrationen von Alkohol im Blut sind daher in solchen Fällen nicht zu erwarten.

Schlüsselwörter: Plötzlicher Säuglingstod, *Candida albicans* – Alkoholproduktion, im Darm

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Introduction

It has been known for many years that certain strains of yeasts and bacteria are able to produce alcohol in the body. Postmortem alcohol production by these organisms is a well known source of false values in alcohol analysis of samples from dead bodies and thus a problem in forensic medicine. Gormsen [2] reported a blood concentration of 2‰ in a case of a 3-month-old dead infant, where the blood contained alcohol-producing micro-organisms. Endogenous alcohol production in the living body may also give rise to problems in legal medicine. The term 'auto-brewery syndrome' was introduced by Kaji et al. in 1976 [3] who claimed that an overgrowth of *Candida albicans* in the gastrointestinal tract resulted in a blood alcohol concentration exceeding 2‰.

A high frequency of *Candida albicans* was previously found by Geertinger [4] in cases of typical crib death (SIDS = sudden infant death syndrome). We therefore investigated the possibility of intestinal alcohol production in SIDS and its possible significance in the etiology of SIDS, and examined the magnitude of alcohol production by pure strains of *Candida albicans* to evaluate the importance of possible endogenous alcohol production in legal medicine.

Case Report

Material

Eight consecutive cases of typical SIDS brought to the Institute of Forensic Medicine in Copenhagen in December 1980 and January 1981 were studied. The age of the infants varied from 2 to 5 months, they had been born between late August and beginning of November 1980. The pregnancies and births had been normal.

All infants had been breast-fed during the first 2 weeks and four were still breast-fed at the time of their death. A 5-week routine control by the family doctor had shown the infants in normal development and with no signs of illness or malformations. All eight infants were found suddenly dead having shown no previous alarming symptoms. In each case the circumstances were examined by the police who found no sign of violence or maltreatment.

Autopsy Findings

The autopsies were performed 16–96 h post mortem (p.m.), all by the same pediatric pathologist. Gross examination showed no malformation or infection. The findings at the autopsy were as in a typical case of SIDS [1, 4, 8]: hyperemic lungs, petecchiae on the pleura and on the external and cut surfaces of the thymus, and varying degrees of emphysema and atelectases of the lungs, probably the results of attempts of resuscitation. There were no obstructions of the airways. Microscopic sections were taken from all organs. In six cases few islands of extramedullary hematopoiesis in the liver and in six cases increased chromaffin tissues in the adrenal medulla and retention of periadrenal brown fat were demonstrated. In six cases steatosis of the liver was found. The degree of steatosis was graded 0 – ++++, + meaning a few (less than 1%) hepatocytes involved, ++++ severe steatosis (i.e., 30%–50% hepatocytes involved). The findings are summarized in Table 1.

Microbiological Technique

The autopsies were performed avoiding contamination, and material for bacteriologic and mycologic cultures were taken from brain, tonsils, lungs, liver, spleen, kidneys, middle ear, stomach, small and large intestines, blood from the heart, and cerebrospinal liquor from the cisterna magna.

Postmortem material (about 2 mg)—with the exception of the middle ear—was crushed aseptically and streaked on the surface of corn meal agar (pH 6.5), Sabouraud agar (pH 4.0), and blood agar plates (pH 7.3). The plates were incubated at 25° C and 37° C.

Table 1. Histological findings and BAC in eight SIDS

Journal no.	Sex	Age (months)	Histological findings			Alcohol in blood
			Liver fatty change	Liver hemato-poiesis	Adrenal medullary hyper-plasia	
F 338/80	M	2	0	+	+	0
F 342/80	F	2¾	+	+	+	0
D 563/80	F	3	++	+	+	0
D 580/80	F	2¼	++	0	0	0
F 352/80	M	2¼	0	+	+	0
D 22/81	F	4	++	+	+	0
F 15/81	M	3¼	+	0	0	0
F 23/81	F	4¾	++	+	+	0

Table 2. The alcohol production in mg per day (24 h) per gram of contents from various parts of the gastrointestinal tract: *s* = stomach, *i* = ileum, *c* = colon

Journal no.	Con-tents from	Incubation time in days						Yeasts[a]	
		0	1	2	3	4	6		
F 338/80	i	0.0	0.0	0.0		0.0		0	
	c		0.0	0.0		0.0			
F 342/80	s	0.0	0.0	0.0					
	i		0.6	21.8	23.2	7.0		+++	*(Candida albicans)*
	c		1.7	1.2	9.5	17.5		++	
D 563/80	i	0.0	4.5	0.7			0.0	0	
	c		0.0	0.0			0.0	+	*(Candida rugosa)*
D 580/80	i	0.0	1.8	0.0	0.0	0.0		0	
	c		0.8	0.0	0.9	0.0			
F 352/80	i	0.0	1.2	0.4	1.3	0.0		0	
	c		2.3	0.2	0.0	0.0		++	*(Candida parapsi-losis)*
D 22/81	s	0.0	0.0	0.0					
	i		0.4	0.0	3.3	24.2		0	
	c		0.0	0.0	0.0	1.0			
F 15/81	s	0.0	0.0	0.0					
	i		0.8	1.5	1.4	0.3	1.0	+	*(Candida albicans)*
	c		1.3	1.4	3.3	19.5	12.2	+++	
F 23/81	i	0.0	2.6	0.0				0	
	c		0.0	0.0					

[a] 0 = no, + = slight, ++ = moderate, +++ = heavy infection

Table 3. The production of alcohol by a pure culture of *Candida albicans* and of wine yeast

		Incubation time in days (24 h)		
		Day 1	Day 2	Day 3
Candida albicans incubated at 37° C	S-substrate:	19.8 mg/d	18.7 mg/d	4.0 mg/d
	R-substrate:	10.0 mg/d	9.3 mg/d	9.8 mg/d
		Incubation time in hours		
		0.5 h	1 h	2 h
Wine yeast incubated at 26° C	S-substrate:	48 mg/h	25 mg/h[a]	
	R-substrate:	70 mg/h	61 mg/h	35 mg/h[a]

[a] The fall in the hourly alcohol production is due to the decrease of glucose in the substrate

The middle ear material was cultured in a flask containing 100 ml filtered broth with added 0.5 *N* lactid acid, penicillin, and streptomycin (pH 6.5), for 24–48 h. Secondary inoculation on corn meal, Sabouraud, and blood agar plates was undertaken if growth occurred. The plates were incubated at 37° C for 48 h. Yeast growth was assessed by the number of colonies as follows: + a few colonies (1–10 colonies per culture); ++ a number of colonies (11–25 colonies per culture); +++ many colonies (more than 25 colonies per culture).

Identification of the yeasts was based on the methods given in Lodder's "The Yeasts" [5].

Biochemical Technique

The synthesis of alcohol (ethanol) in the contents of the intestines and the stomach was investigated by incubating 1 g of intestinal content with 10 ml of a glucose-containing substrate at 37° C. We used two different substrates to be sure to meet the demands for optimal growth by the micro-organisms of the gastro-intestinal tract and especially those of *Candida albicans.*

The *S-substrate* contained per liter: ammonium sulphate, 5 g; monosodium phosphate, 0.33 g; dipotassium phosphate, 0.92 g; glucose, 10 g; and trace amounts of the minerals Ca, Mg, Zn, Mo, Mn, Fe, Cu, B, of the vitamins thiamine, riboflavin, pyridoxine, niacin, biotin, inositol, folic and pantothenic and p-aminobenzoic acid, and of the amino acids asparagine, histidine, methionine, tryptophane. The pH of the substrate was 4.0.

The *R-substrate* contained per liter: ammonium chloride, 4.1 g; disodium phosphate, 1.1 g; and glucose, 20 g. The pH of the substrate was 8.0.

Each day a sample was removed from each incubated suspension for alcohol analysis, which was performed enzymatically according to the ADH technique.

Results

Microbiologic Findings

With material from the eight children yeasts were only demonstrated in single organs, but not in the form of massive growth as in sepsis.

The most frequently occurring yeasts was *Candida albicans,* the other yeasts demonstrated were *Candida glabrata, Candida parapsilosis, Candida rugosa,* and *Saccharomyces cerevisiae.* Only in one child (F 23/81)—in the middle ear—there were two yeasts (*Candida albicans* and *Candida glabrata*) demonstrated simultaneously. In two children (F 352/80 and D 563/80) *Candida albicans* was demonstrated in the middle ear and *Candida parapsilosis* in the liver and large intestine, respectively, of the one child, and *Candida rugosa* was demonstrated in the middle ear and tonsils of the other child.

Bacteriologic screening did not reveal pathogenic bacteria in this series except in one case where a few *Staphylococci aurei* were cultured from the tonsils.

It appears from this investigation that there is no basis to assume that the presence of yeasts would be a contributory factor in the occurrence of sudden, unexpected death of infants.

There is apparently a certain discrepancy with respect to the occurrence of yeasts in this postmortem material in comparison with that described by Geertinger [4], a discrepancy that can perhaps be the result of a later improvement in hygiene.

Biochemical Findings

In all our experiments, even in those with pure cultures of micro-organisms, we found essentially the same amounts of alcohol produced whether the incubation was performed with the R-substrate or with the more elaborate S-substrate. The only difference between the effects of the two substrates seemed to be that the amount of alcohol produced in the S-substrate during the first days of incubation was sometimes slightly reduced during the following days indicating a tendency to further oxidation of the alcohol formed. For the sake of clarity, therefore, only the results obtained with the R-substrate are listed. The results in Table 2 show clearly that *Candida albicans* is able to produce alcohol and that this production is proportional to the degree of infection (cf. Nos. F 15 and F 342). Alcohol production may, however, occasionally occur in cases (D 22) where *Candida albicans* was not present. The maximal daily production of alcohol did not exceed 24 mg/g of intestinal content or 1 mg/g/h. The alcohol production of a pure strain of *Candida albicans* was measured after heavy infection of the substrates from a plate culture of the fungus. The results in Table 3 show that *Candida albicans* produces alcohol from glucose, but that it does so at a slow rate only. The maximal daily production is thus of the same order of magnitude (20 mg/day) as that found for the intestinal contents. On the contrary, the alcohol production by an equal amount of wine yeast is several orders of magnitude higher, i.e., 60–70 mg/h or 1,500 mg/day. It is noteworthy that the alcohol production of the pure culture of *Candida albicans* reached its maximum on day 1 of incubation, whereas this maximum was delayed to days 3 or 4 in the experiments with the intestinal contents. The explanation for this delay is probably that the intestinal contents had been kept frozen until the incubation could be performed. The time elapsing between death and the performing of autopsy (16–96 h) did not seem to be of any significance.

Discussion

It is apparent from our results that *Candida albicans* is a reliable but slow producer of alcohol from glucose, the production of alcohol from wine yeast being a 100 times faster. The pure culture of *Candida albicans* was found to produce alcohol at a rate of 10–20 mg per day (24 h), and the intestinal contents infected with *Candida albicans* produces maximally 24 mg of alcohol per gram of content per day or 1 mg/g/h. To evaluate this production rate, we may transcribe it to 1 g/kg/h and we may further assume that the intestinal contents of an adult person

may amount to maximally 1 kg. Since the metabolizing capacity of the liver of an adult person is 8 g of alcohol per hour, the trapping of the intestinally produced alcohol (maximally 1 g/h) by the liver will be complete so that none of the alcohol will reach the general circulation. In conformity with this, the blood from the present cases proved to be free of alcohol.

Sprung et al. [7] has reinvestigated the problem of the endogenous blood alcohol concentration (BAC) with a highly specific and sensitive method for BAC determination. They found the endogenous BAC to be on an average 0.1–0.2 mg/l and never exceeding 0.8 mg/l, and this was valid for normal individuals as well as for patients suffering from various metabolic diseases. Lundquist and Wolthers [6] found the endogenous BAC in normal, fasting subjects to be on an average 1 mg/l. From this figure and from an alcohol oxidation capacity of the liver of 7 g/h they calculated the continuous production of alcohol in the whole body to be about 0.15 g/h, which they considered to be produced in the intestines by bacterial activity. An abnormal intestinal flora dominated by *Candida albicans* may, as shown by the present investigations, lead to an alcohol production, which is supposed to amount to maximally 1 g/h in an adult individual. Such a rate of production of alcohol should not cause the endogenous BAC to exceed 10 mg/l or 0.01‰, provided that the liver is functioning normally with a metabolizing capacity of 7–8 g of alcohol per hour. Consequently, the slow production of alcohol in a *Candida albicans* infected gastrointestinal tract cannot be expected to cause any tissue damage or alterations in an otherwise normally functioning organism.

Our conclusion is, therefore, that an endogenic alcohol production by intestinal fermentation can be of no significance in the aethiology of SIDS.

In contrast to our findings, Kaji et al. [3] claim to have found in a case with overgrowth of *Candida albicans* in the gastrointestinal tract an alcohol production resulting in a BAC exceeding 2‰, for which they apply the term auto-brewery syndrome. However, the BAC was measured indirectly on the respiratory air, and as the patient besides her Candida infection suffered from stomatitis, the specificity of the alcohol determination was hardly sufficiently specific.

References

1. Adelson L (1963) Specific studies of infant victims of sudden death. GPOPHS 412:1–40
2. Gormsen H (1954) Alcohol production in the dead body. J Forensic Med 1:314–315
3. Kaji H, Asanuma Y, Ide H, Saito N, Hisamura M, Murao M, Toshida T, Takahashi K (1976) The auto-brewery syndrome — the repeated attacks of alcohol intoxication due to the overgrowth of Candida albicans in the gastrointestinal tract. Materia Med Pol 29:1–7
4. Geertinger P (1968) Sudden death in infancy. American Lecture Series no 704. Thomas, Springfield, USA
5. Lodder I (1970) The yeasts. A taxonomic study. North-Holland, Amsterdam Oxford
6. Lundquist F, Wolthers H (1958) The kinetics of alcohol elimination in man. Acta Pharmacol Toxicol 14:265–289
7. Sprung R, Bonte W, Rüdell E, Domke M, Frauenrath C (1981) Zum Problem des endogenen Alkohols. Blutalkohol 18:65–70
8. Valdes-Dapena M (1982) The pathologist and the sudden infant death syndrome. Am J Pathol 106:118–131

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