

## FULMINANT LIVER FAILURE IN ASSOCIATION WITH THE EMETIC TOXIN OF *BACILLUS CEREUS*

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### ABSTRACT

**Background** A 17-year-old boy and his father had acute gastroenteritis after eating spaghetti and pesto that had been prepared four days earlier. Within two days, fulminant liver failure and rhabdomyolysis developed in the boy and he died. The father had hyperbilirubinemia and rhabdomyolysis but recovered. We investigated the cause of these illnesses.

**Methods** Bacteria were isolated and characterized by conventional methods, and bacterial toxins were quantified by immunoassays and cell-culture techniques. The effect of the isolated toxin on the rates of oxidation of various substrates was analyzed in rat-liver mitochondria.

**Results** Autopsy of the boy's liver revealed diffuse microvesicular steatosis and midzonal necrosis that suggested impaired  $\beta$ -oxidation of liver mitochondria due to a mitochondrial toxin. There was no evidence of ingestion of heavy metals, halogenated compounds, hepatotoxic drugs, or staphylococcal enterotoxin. However, high concentrations of *Bacillus cereus* emetic toxin were found both in the residue from the pan used to reheat the food and in the boy's liver and bile. *B. cereus* was cultured from the intestinal contents and the pan residue. The emetic toxin isolated from the *B. cereus* cultures was found to be a mitochondrial toxin.

**Conclusions** Fulminant liver failure developed after the ingestion of food contaminated with the *B. cereus* emetic toxin. The toxin inhibits hepatic mitochondrial fatty-acid oxidation, indicating that it caused liver failure in this patient. (N Engl J Med 1997;336:1142-8.)

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THE principal causes of fulminant liver failure are viral infections and drugs.<sup>1</sup> Food poisoning is rarely implicated, and most documented cases arise from the consumption of toxic mushrooms such as *amanita*, *lepiota*, and *gyromitra* species.<sup>1-5</sup> Foodborne bacterial toxins most often cause acute gastroenteritis and are only rarely associated with liver injury.

Microvesicular steatosis of hepatocytes, normally associated with acute fatty liver of pregnancy, valproate- or hypoglycin-induced toxicity, and Reye's syndrome,<sup>1,6-8</sup> results from reduced fatty-acid metabolism by hepatic mitochondria, which can be caused by impaired  $\beta$ -oxidation or impaired activity of the mitochondrial electron-transport chain.<sup>7,8</sup> The development of microvesicular steatosis is often associ-

ated with severe liver injury and is frequently fatal in the absence of liver transplantation.<sup>1,9</sup>

Although bacterial food poisoning is common,<sup>10</sup> and has an economic impact,<sup>11</sup> it is rarely fatal in previously healthy persons. *Bacillus cereus* is widely recognized as a foodborne pathogen<sup>12</sup> that causes a self-limiting gastroenteritis requiring only symptomatic treatment.<sup>13,14</sup> The symptoms are mediated by exotoxins, including a diarrheal toxin (enterotoxin) and an emetic toxin (cereulide<sup>15</sup>). In the three reported cases of fatal *B. cereus* food poisoning,<sup>16-18</sup> liver steatosis was observed, but not fulminant liver failure.

We describe a patient who died of fulminant liver failure after eating food contaminated with *B. cereus* and its toxins.

### CASE REPORT

Gastrointestinal symptoms developed in a previously healthy 17-year-old boy and his father (a physician) 30 minutes after they ate spaghetti with homemade pesto. The food had been prepared four days earlier and refrigerated, although on several occasions it had been left at room temperature for one or more hours before being reheated in a pan. The food had an unusual smell but was eaten completely, with the son consuming more than his father. Both had also eaten the food on the day it was prepared and the next day without having any symptoms.

Thirty minutes after consuming the food, the father had abdominal pain followed by diarrhea, but his overall condition remained satisfactory with symptomatic treatment (antiemetics and charcoal). In contrast, the son had no diarrhea and vomited the initial dose of charcoal despite antiemetic treatment. His condition gradually deteriorated during the next two days, and he became listless. During this period, he was treated symptomatically by his father with aspirin (total dose, 1 g), acetaminophen (total dose, 1 g), thiethylperazine, meclizine, and domperidone. When he became somnolent, he was admitted to a district hospital, where he was found to be icteric and afebrile, with tachycardia but otherwise normal cardiac function, a blood pressure of 115/70 mm Hg, and pain in the upper right quadrant of the abdomen. His prothrombin ratio was 12 percent (normal value, 80 to 100 percent), with a serum aspartate aminotransferase concentration of 2140 U per liter (normal value, <18), an alanine aminotransferase concentration of 5270 U per liter (normal value, <22), an alkaline phosphatase concentration of 378 U per liter (normal range, 60 to 170), and a creatine kinase concentration of 2560 U per liter (normal range, 10 to 50) with a normal MB fraction. His serum bilirubin concentration was 7.0 mg per deci-

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liter (119  $\mu\text{mol}$  per liter; normal value,  $<1$  mg per deciliter [17  $\mu\text{mol}$  per liter]), and the serum creatinine concentration was 4.9 mg per deciliter (431  $\mu\text{mol}$  per liter; normal range, 0.6 to 1.3 mg per deciliter [53 to 115  $\mu\text{mol}$  per liter]). He had metabolic acidosis (arterial pH, 7.27), a normal hemoglobin concentration, normal white-cell and platelet counts, and an erythrocyte sedimentation rate of 2 mm per hour. Fulminant hepatic failure, rhabdomyolysis, and acute renal failure were diagnosed, and the patient was transferred immediately to the University Hospital of Zurich.

The father continued to have episodes of abdominal pain and diarrhea but was otherwise in a satisfactory condition. His serum bilirubin concentration was 3.3 mg per deciliter (57  $\mu\text{mol}$  per liter), the aspartate aminotransferase concentration was 55 U per liter, the alanine aminotransferase concentration was 18 U per liter, the creatine kinase concentration was 1920 U per liter (normal value,  $<270$ ), and the prothrombin ratio was 91 percent (normal value,  $>70$  percent). Two weeks later, the symptoms had resolved, and all laboratory values were within the normal ranges.

On examination at University Hospital, the son was not able to walk or respond to simple commands, but his pupils reacted to light. He had severe, intermittent extensor spasms. His prothrombin ratio remained low (12 percent); coagulation factor II, V, and VII activities were 22, 10, and 11 percent of normal, respectively; the plasma ammonia concentration was 250  $\mu\text{g}$  per deciliter (147  $\mu\text{mol}$  per liter; normal value, 27 to 82  $\mu\text{g}$  per deciliter [16 to 48  $\mu\text{mol}$  per liter]), and the serum lactate concentration was 14.4 mmol per liter (normal range, 0.6 to 2.4). Urinalysis revealed red cells, hemoglobinuria, myoglobinuria, and proteinuria. No hepatic toxins such as acetaminophen, carbon tetrachloride, amatoxins, amphetamine, opiates, or cocaine were detectable in plasma or urine. Multiple blood cultures for bacteria and fungi were negative, as were serologic assays for hepatitis A, B, C, and E, Epstein-Barr virus, and herpesvirus.

The patient was immediately scheduled for liver transplantation, and supportive treatment for fulminant liver failure including antibiotic prophylaxis was initiated. Despite this treatment, brain edema developed with increased intracranial pressure, and the patient died the day after hospitalization.

Postmortem findings included diffuse edema and oligemia of the brain, thrombotic microangiopathy in small renal arteries, and vacuolar degeneration of renal tubular epithelia. The abdominal cavity contained 100 ml of clear ascitic fluid. The liver was yellowish and enlarged (weight, 1800 g). Microscopical examination revealed that the architecture of the liver was intact. There was diffuse microvesicular steatosis of the whole liver parenchyma that was especially prominent in Rappaport's zone II, with hepatocytic necrosis in the same zone (Fig. 1). In addition, there were plugged bile ducts but no major cellular infiltrates.

The patient's parents provided informed consent.

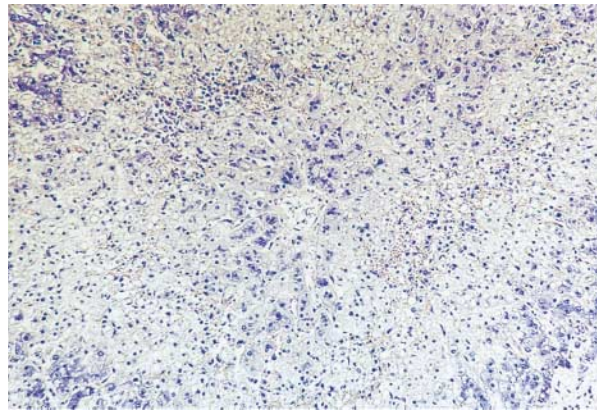
## METHODS

### Isolation and Characterization of Bacteria

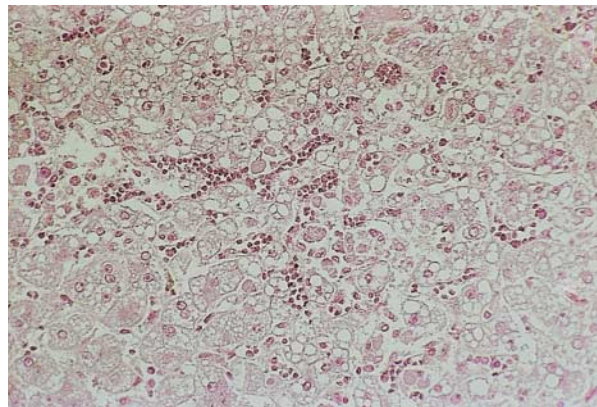
Bacteria were isolated and characterized according to routine laboratory methods,<sup>19,20</sup> and *B. cereus* selective agar (Unipath, Basingstoke, United Kingdom) was used for isolation and enumeration.<sup>21</sup> The identity of the isolates was confirmed on the basis of spore morphology; motility; hemolysin, catalase, and lecithinase production; ability to reduce nitrate; sensitivity to  $\gamma$ -phage and penicillin; and ability to ferment glucose, xylose, arabinose, mannitol, and salicin.<sup>20</sup>

### Detection, Isolation, and Characterization of *B. cereus* Toxins

A Vero-cell assay<sup>22</sup> was used for the determination of cytotoxicity. Enterotoxin was measured by reverse passive latex agglutination (BCET-RPLA, Unipath) and by an enzyme-linked immunosorbent assay (BCE-VIA, Tecra Diagnostics, Roseville, New South Wales, Australia). Emetic toxin was detected by the vacuolation assay of



A



B

**Figure 1.** Histologic Findings in Liver Tissue.

Panel A shows a liver lobule in which hepatocytic necrosis and hemorrhage are present predominantly in Rappaport's zone II (Giemsa stain,  $\times 32$ ). There are no major cellular infiltrates in the portal fields (e.g., in the lower right-hand corner). Panel B shows a section of Rappaport's zone II in which hepatocytes appear swollen and are partially necrotic (Gram's stain,  $\times 80$ ). Most have microvesicular steatosis with central nuclei.

Hughes et al.,<sup>23</sup> which uses HEP-2 cells, or a modified version of the assay in which cultured HEP-G2 cells were used. The results were quantified according to the method of Agata et al.,<sup>24</sup> except that we used 70 percent ethanol at 90°C to extract the toxin from rice cultures and clinical specimens and freeze-dried the extracts.

The emetic toxin was isolated from rice-culture filtrates of *B. cereus* by ethanolic extraction at 90°C and purified by reverse-phase chromatography.<sup>15</sup> Purified emetic toxin was characterized on the basis of its ability to induce vacuolation in HEP-2 and HEP-G2 cells, its resistance to proteolysis, and its profile on nuclear magnetic resonance spectroscopy.<sup>15</sup>

### Toxicity of *B. cereus* Emetic Toxin in Isolated Rat-Liver Mitochondria

To determine the toxicity of *B. cereus* emetic toxin, rat-liver mitochondria were isolated as described previously.<sup>25</sup> The effect of the toxin on the oxidation rate of various substrates was determined with a Clarke type of oxygen electrode.<sup>26</sup> State 3 oxidation

**TABLE 1. MICROBIOLOGIC FINDINGS IN AND TOXIN PRODUCTION BY *B. CEREUS* STRAINS ISOLATED FROM THE PATIENT, THE PESTO, AND THE PAN USED TO HEAT THE PESTO.\***

SOURCE	MICROBIOLOGIC FINDINGS	<i>B. CEREUS</i> LEVEL	VERO-CELL ASSAY FOR CYTOTOXICITY†	ENTEROTOXIN		HEP-2-CELL ASSAY FOR EMETIC TOXIN‡
			CFU/g	RPLA ASSAY†	ELISA	
Pan residue	Gram-positive bacilli	++§	NT	NT	+++	480±60
Pesto¶	Gram-positive bacilli and cocci	400	180±60	1024	++	—
Patient						
Small intestine	High levels of gram-positive cocci and bacilli	650	480±160	96±32	NT	—
Colon	High levels of gram-positive cocci and bacilli	9200	160	>2048	NT	—
Controls**	—	—	5120	256	+	50±20

\*RPLA denotes reverse passive latex agglutination, ELISA enzyme-linked immunosorbent assay, CFU colony-forming units, NT not tested, minus signs not detectable, the plus sign detectable, two plus signs intermediate levels, and three plus signs high levels.

†Each value represents the reciprocal of the highest dilution of 1 g of source material that gave a positive result.

‡Titers are given as the reciprocal of the highest mean (±SD) dilution (of 3 to 20 tests) of rice-culture filtrates corresponding to 1 g of dry rice (approximately 3 g of cooked rice) that caused vacuolation of HEP-2 cells. When HEP-G2 cells were used in this assay, values obtained for positive controls and pan-residue isolates were 5±2 and 340±120, respectively.

§There was insufficient material for a precise quantification.

¶No spaghetti from the implicated meal was available for testing.

||Only low levels of *B. cereus* (<100 CFU per gram) were found in gastric juice. These strains were therefore not assayed for toxins.

\*\*Positive *B. cereus* control strains F4433/73 and F4108/89 were obtained from the Food Hygiene Laboratory, Central Public Health Laboratory, London.

**TABLE 2. DETECTION OF *B. CEREUS* EXOTOXINS IN SPECIMENS FROM THE PATIENT AND THE PAN USED TO HEAT THE PESTO.\***

SOURCE	VERO-CELL ASSAY FOR CYTOTOXICITY†	ENTEROTOXIN		HEP-2-CELL ASSAY FOR EMETIC TOXIN‡
		RPLA ASSAY†	ELISA	
Pan residue§	NT	NT	NT	960±320
Patient				
Small intestine	160	256	+	50±12
Colon	80	<2	+	—
Plasma	NT	NT	NT	11±2
Liver	NT	NT	NT	22±4
Bile	NT	NT	NT	560±60

\*RPLA denotes reverse passive latex agglutination, ELISA enzyme-linked immunosorbent assay, NT not tested, plus signs detectable, and the minus sign not detectable.

†Each value represents the reciprocal of the highest dilution of 1 g of source material that gave a positive result.

‡Titers are given as the reciprocal of the highest mean (±SD) dilution (of 3 to 15 tests) of 1 g of source material that caused vacuolation of HEP-2 cells. When HEP-G2 cells were used in this assay, the value obtained for bile was 640±160.

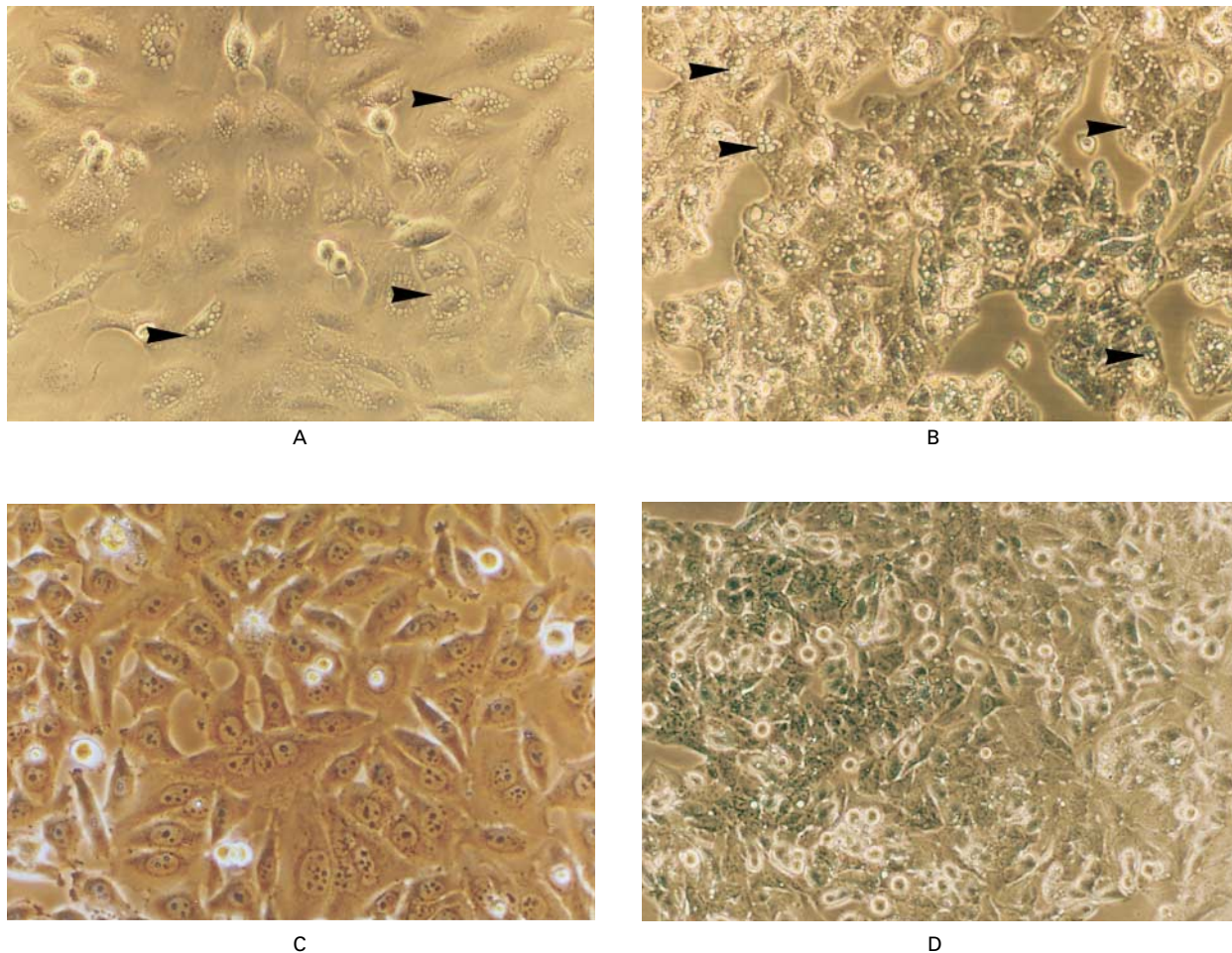
§Except for a low level of activity in the pesto sample in the Vero-cell assay, *B. cereus* toxins were absent in the pesto as well as in gastric juice.

rates (in which adenosine diphosphate and substrate are present) and state 4 oxidation rates (in which only substrate is present) were determined as described by Estabrook,<sup>27</sup> and the respiratory-control ratio was calculated by dividing the state 3 rates by the state 4 rates.

## RESULTS

The rapid onset of symptoms after the meal strongly suggested foodborne intoxication. However, postmortem examinations of tissues, plasma, urine, and bile failed to detect halogenated compounds (carbon tetrachloride, chloroform, chloral hydrate, and α-chloralose), alcohols, aflatoxins, toxic concentrations of metals (arsenic, gold, copper, iron, thallium, mercury, and 37 other metals), or drugs (acetaminophen, salicylates, coumarins, cocaine, and colchicine). No hepatotoxic plants, fungi, or amanita spores could be identified in the pesto or in the patient's intestinal contents after death.

Bacterial toxins causing abdominal pain, nausea, and emesis after an incubation period of 30 minutes in the absence of fever include *Staphylococcus aureus* and *B. cereus* enterotoxins. Very low levels of *S. aureus* were detected in the pesto (<100 colony-form-



**Figure 2.** Phase-Contrast Microscopy of Cultured HEP-2 Cells (Panel A) and HEP-G2 Cells (Panel B) Exposed to Emetic Toxin Derived from *B. cereus* Strains Isolated from the Pan Residue and Control Cultures of HEP-2 Cells (Panel C) and HEP-G2 Cells (Panel D) Treated only with Physiologic Saline.

Most cells exposed to emetic toxin formed multiple vacuoles (arrowheads in Panels A and B), which correspond to enlarged mitochondria. Vacuoles were absent or rare in the respective control preparations. Similar results were obtained for cells exposed to extracts of the pan residue, body fluids (blood, plasma, and bile), intestinal contents, and liver extracts. (Panels A and C,  $\times 114$ ; Panels B and D,  $\times 66$ .)

ing units per gram), but *S. aureus* enterotoxins A, B, C, and D and enterobacter, campylobacter, listeria, and salmonella species were not detected in either the food remnants or clinical specimens. *Escherichia coli* could not be detected in blood and food remnants. However, *B. cereus* strains were isolated from the pesto, from ileum and colon contents, and from residue in the pan that was used to reheat the implicated food and cleaned with tissue paper afterward (Table 1). The strains isolated from the pan residue grew well at both 10°C and 50°C and produced both enterotoxin and emetic toxin when grown on cooked rice.

An ethanolic extract of the pan residue was strongly positive for *B. cereus* emetic toxin, which was also de-

tected in high concentrations in the bile obtained 17 hours post mortem and in lower concentrations in plasma, liver, and intestinal contents (Table 2). In comparison, bile samples from control subjects (17 control bile samples obtained post mortem and 2 bile samples obtained before death) were negative for emetic toxin. *B. cereus* diarrheal enterotoxin was detected in the intestinal contents and in the pesto.

The suspected causative strain obtained from the pan residue was grown on cooked rice, and *B. cereus* emetic toxin was isolated from rice-culture filtrate and characterized by nuclear magnetic resonance spectroscopy and on the basis of its resistance to heat and proteolysis.<sup>15</sup> The toxin was identified on the basis of its ability to induce vacuole formation in HEP-2

**TABLE 3.** TOXICITY OF PURIFIED *B. CEREBUS* EMETIC TOXIN IN ISOLATED RAT-LIVER MITOCHONDRIA.\*

SUBSTRATE	CONCENTRATION OF <i>B. CEREBUS</i> EMETIC TOXIN	STATE 3 OXIDATION RATE	STATE 4 OXIDATION RATE	RESPIRATORY- CONTROL RATIO
	$\mu\text{g/ml}$	nanaotoms/mg/min		
L-Glutamate, 20 mmol/liter	0	102 $\pm$ 26	12 $\pm$ 5	8.5 $\pm$ 2.8
	10	81 $\pm$ 17†	29 $\pm$ 7†	2.8 $\pm$ 0.4†
	25	70 $\pm$ 19†	51 $\pm$ 11†	1.4 $\pm$ 0.2†
Succinate, 20 mmol/liter	0	214 $\pm$ 38	49 $\pm$ 15	4.4 $\pm$ 0.7
	10	124 $\pm$ 34†	62 $\pm$ 15†	2.0 $\pm$ 0.5†
	25	114 $\pm$ 24†	99 $\pm$ 19†	1.2 $\pm$ 0.1†
Ascorbate, 7.2 mmol/liter‡	0	40 $\pm$ 4	28 $\pm$ 11	1.4 $\pm$ 0.5
	10	60 $\pm$ 4†	43 $\pm$ 5†	1.4 $\pm$ 0.2
	25	57 $\pm$ 8†	43 $\pm$ 6†	1.3 $\pm$ 0.2
Palmitoyl-coenzyme A, 160 $\mu\text{mol/liter}$ §	0	99 $\pm$ 12	23 $\pm$ 7	4.3 $\pm$ 1.0
	10	49 $\pm$ 11†	35 $\pm$ 10†	1.4 $\pm$ 0.3†
	25	46 $\pm$ 3†	35 $\pm$ 4†	1.3 $\pm$ 0.1†
Palmitoylcarnitine, 80 $\mu\text{mol/liter}$ ¶	0	108 $\pm$ 12	19 $\pm$ 7	5.7 $\pm$ 1.6
	10	61 $\pm$ 3†	34 $\pm$ 2†	1.8 $\pm$ 0.2†
	25	73 $\pm$ 18†	64 $\pm$ 16†	1.1 $\pm$ 0.1†

\*Mitochondria were isolated and investigated as described in the Methods section. The respiratory-control ratio was obtained by dividing state 3 oxidation rates by state 4 oxidation rates. Plus-minus values are the means ( $\pm$ SD) of five mitochondrial preparations.

† $P < 0.05$  for the comparison with values for control samples containing no emetic toxin, by analysis of variance and Student's t-test for correlated samples with Bonferroni's correction.

‡The incubations also contained 240  $\mu\text{mol}$  of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine per liter.

§The incubations also contained 5 mmol of L-malate per liter and 2.5 mmol of L-carnitine per liter.

¶The incubations also contained 5 mmol of L-malate per liter.

cells (Fig. 2A), a human laryngeal-carcinoma cell line.<sup>23</sup> Emetic toxin also induced vacuolation in HEP-G2 cells, a human liver-carcinoma cell line (Fig. 2B), suggesting that this toxin may affect the liver in vivo.

To provide direct evidence that the emetic toxin of the *B. cereus* species isolated from the pan residue was a mitochondrial toxin, its effect on oxidative metabolism of isolated rat-liver mitochondria was investigated. As shown in Table 3, emetic toxin had two effects on mitochondrial oxidation of various substrates, including fatty acids but not ascorbate. First, it decreased state 3 oxidation rates, indicating inhibition of the electron-transport chain. Second, it markedly increased state 4 oxidation rates, leading to a decrease in the respiratory-control ratios, indicating the uncoupling of oxidative phosphorylation.

### DISCUSSION

Two patients, father and son, had gastrointestinal symptoms, including nausea and emesis, followed by liver injury and rhabdomyolysis, after eating food contaminated with *B. cereus* and its toxins. The father made a complete recovery, but his son died of fulminant liver failure.

Histologic examination of the boy's liver showed diffuse microvesicular steatosis, a finding indicative

of decreased mitochondrial  $\beta$ -oxidation.<sup>7</sup> The primary causes of microvesicular steatosis include acute fatty liver of pregnancy, Reye's syndrome, valproate-induced hepatotoxicity, intoxication with hypoglycin, and inherited disorders of mitochondrial energy metabolism.<sup>7</sup> With the exception of Reye's syndrome, these possibilities were ruled out on the basis of the case history and laboratory tests, which were negative for toxins. Reye's syndrome was considered because the youth was treated with 1 g of aspirin. However, the rapid onset of the symptoms after consumption of the implicated meal, the appearance of similar symptoms in his father, and the absence of signs of a viral infection rendered this diagnosis improbable.<sup>6,8</sup> Acetaminophen-induced intoxication was also ruled out because of the small dose ingested (1 g) and the histologic findings.

Intoxication with an unknown substance causing liver failure and rhabdomyolysis was suspected. There was no history of exposure to heavy metals or solvents or of drug abuse. The rapid onset of symptoms after eating in both father and son strongly suggested a foodborne toxin. Since the food had been made several days earlier and stored improperly, a bacterial toxin was suspected. In view of the short incubation period, *S. aureus* and *B. cereus* toxins were the most probable

candidates. Staphylococcal intoxication could not be demonstrated in our patient. However, *B. cereus* exotoxins were detected in high concentrations in the residue from the pan used to heat the food and in intestinal contents, bile, and tissues from the boy.

Although the *B. cereus* enterotoxin and emetic toxin were likely to have caused the gastrointestinal symptoms, our results indicate that the emetic toxin was responsible for the liver failure and possibly the rhabdomyolysis. The emetic toxin is a cyclic peptide<sup>15</sup> that is unusually resistant to heat and proteolysis. Ultrastructural studies in cultured Hep-2 cells exposed to emetic toxin have shown that the vacuoles observed by light microscopy correspond to swollen mitochondria, suggesting mitochondrial toxicity.<sup>28</sup> Our studies in isolated rat-liver mitochondria showed that emetic toxin produced by the *B. cereus* strain recovered from the pan residue impaired mitochondrial fatty-acid metabolism. These findings are in agreement with a recent report showing uncoupling of oxidative phosphorylation in isolated mitochondria in the presence of *B. cereus* rice-culture filtrate.<sup>28</sup> Inhibition of mitochondrial fatty-acid metabolism explains the histologic findings of microvesicular steatosis in the liver and suggests that the emetic toxin was directly responsible for acute liver failure. Since mitochondrial toxicity may not be confined to hepatocytes, rhabdomyolysis may have been caused by the same mechanism.

The father had milder symptoms than his son, and he ingested only about half as much of the contaminated food. Furthermore, he had diarrhea, responded well to treatment with antiemetics, and did not vomit the charcoal. Since aspirin inhibits mitochondrial  $\beta$ -oxidation,<sup>29</sup> it is possible that in the son, who was treated with 1 g of aspirin, it had an additional inhibitory effect on hepatic mitochondrial fatty-acid metabolism. As has been suggested for valproate-induced hepatotoxicity, the boy may have had an underlying defect in mitochondrial  $\beta$ -oxidation.<sup>30</sup> However, such a predisposition was not apparent from the family history.

The extremely high concentration of emetic toxin in the bile suggests biliary excretion and enterohepatic circulation. Charcoal may therefore be effective not only for primary detoxification, but also for the accelerated elimination of absorbed toxin. As in other intoxications, high doses of charcoal should be administered repeatedly.<sup>31</sup> Since there is no specific treatment for mitochondrial damage, supportive therapy should be provided and liver transplantation considered in patients with fulminant liver failure.<sup>1,9</sup>

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## REFERENCES

- Bernuau J, Rueff B, Benhamou JP. Fulminant and subfulminant liver failure: definitions and causes. *Semin Liver Dis* 1986;6:97-106.
- Klein AS, Hart J, Brems JJ, Goldstein L, Lewin K, Busuttill RW. Amanita poisoning: treatment and the role of liver transplantation. *Am J Med* 1989;86:187-93.
- Meunier B, Messner M, Bardaxoglou E, Spiliopoulos G, Terblanche J, Launois B. Liver transplantation for severe *Leptota helveola* poisoning. *Liver* 1994;14:158-60.
- Michelot D, Toth B. Poisoning by *Gyromitra esculenta* — a review. *J Appl Toxicol* 1991;11:235-43.
- Koppel C. Clinical symptomatology and management of mushroom poisoning. *Toxicon* 1993;31:1513-40.
- Mowat AP. Reye's syndrome — a continuing enigma. *Adverse Drug React Acute Poisoning Rev* 1987;6:211-30.
- Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol Ther* 1995;67:101-54.
- Treem WR. Inherited and acquired syndromes of hyperammonemia and encephalopathy in children. *Semin Liver Dis* 1994;14:236-58.
- Caraceni P, Van Thiel DH. Acute liver failure. *Lancet* 1995;345:163-9.
- Hennessy TW, Hedberg CW, Slutsker L, et al. A national outbreak of *Salmonella enteritidis* infections from ice cream. *N Engl J Med* 1996;334:1281-6.
- Cohen ML, Fontaine RE, Pollard RA, VonAllmen SD, Vernon TM, Gangarosa EJ. An assessment of patient-related economic costs in an outbreak of salmonellosis. *N Engl J Med* 1978;299:459-60.
- Kramer JM, Gilbert RJ. *Bacillus cereus* and other *Bacillus* species. In: Doyle MP. Foodborne bacterial pathogens. New York: Marcel Dekker, 1989:21-70.
- Terranova W, Blake PA. *Bacillus cereus* food poisoning. *N Engl J Med* 1978;298:143-4.
- Fermandjian C, Freymy JM, Lahalle C. *Bacillus cereus* pathogenicity: a review. *J Rapid Methods Autom Microbiol* 1993;2:83-134.
- Agata N, Mori M, Ohta M, Suwan S, Ohtani I, Isobe M. A novel dodecadepsipeptide, cereulide, isolated from *Bacillus cereus* causes vacuole formation in Hep-2 cells. *FEMS Microbiol Lett* 1994;121:31-4.
- Temper K. Exitus letalis nach Lebensmittelvergiftung durch *Bacillus cereus*. *Z Gesamte Hyg Grenzgeb* 1963;9:481-90.
- Takabe F, Oya M. An autopsy case of food poisoning associated with *Bacillus cereus*. *Forensic Sci* 1976;7:97-101.
- Dirnhofer R, Sonnabend O, Sonnabend W. Eine tödlich verlaufene Lebensmittelvergiftung durch *Bacillus cereus*. *Z Rechtsmed* 1977;80:139-51.
- Bouwer-Hertzberger SA, Mossel DAA. Quantitative isolation and identification of *Bacillus cereus*. In: Corry JEL, Roberts D, Skinner FA, eds. Isolation and identification methods for food poisoning organisms. No. 17. London: Academic Press, 1982:255-9.
- Kramer JM, Turnbull PCB, Munshi G, Gilbert RJ. Identification and characterization of *Bacillus cereus* and other *Bacillus* species associated with foods and food poisoning. In: Corry JEL, Roberts D, Skinner FA, eds. Isolation and identification methods for food poisoning organisms. No. 17. London: Academic Press, 1982:261-86.
- International Commission on Microbiological Specifications for Foods. Microorganisms in foods I: their significance and methods of enumeration. 2nd ed. Toronto: University of Toronto Press, 1978:119-20.
- Sandvig K, Olsnes S. Entry of toxic proteins abrin, modeccin, ricin, and diphtheria toxin into cells. I. Requirement for calcium. *J Biol Chem* 1982;257:7495-503.
- Hughes S, Bartholomew B, Hardy JC, Kramer JM. Potential application of a Hep-2 cell assay in the investigation of *Bacillus cereus* emetic syndrome food poisoning. *FEMS Microbiol Lett* 1988;52:7-12.
- Agata N, Ohta M, Mori M. Production of an emetic toxin, cereulide, is associated with a specific class of *Bacillus cereus*. *Curr Microbiol* 1996;33:67-9.
- Hoppel CL, DiMarco JP, Tandler B. Riboflavin and rat hepatic cell structure and function: mitochondrial oxidative metabolism in deficiency states. *J Biol Chem* 1979;254:4164-70.

- 26.** Krähenbühl S, Talos C, Reichen J. Mechanisms of impaired hepatic fatty acid metabolism in rats with long-term bile duct ligation. *Hepatology* 1994;19:1272-81.
- 27.** Estabrook RW. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Methods Enzymol* 1967;10:41-7.
- 28.** Sakurai N, Koike KA, Irie Y, Hayashi H. The rice culture filtrate of *Bacillus cereus* isolated from emetic-type food poisoning causes mitochondrial swelling in a HEp-2 cell. *Microbiol Immunol* 1994;38:337-43.
- 29.** Deschamps D, Fisch C, Fromenty B, Berson A, Degott C, Pessayre D. Inhibition by salicylic acid of the activation and thus oxidation of long chain fatty acids: possible role in the development of Reye's syndrome. *J Pharmacol Exp Ther* 1991;259:894-904.
- 30.** Krähenbühl S, Mang G, Kupferschmidt H, Meier PJ, Krause M. Plasma and hepatic carnitine and coenzyme A pools in a patient with fatal, valproate induced hepatotoxicity. *Gut* 1995;37:140-3.
- 31.** Levy G. Gastrointestinal clearance of drugs with activated charcoal. *N Engl J Med* 1982;307:676-8.