

Food Poisoning Due to Toxic Substances Formed by Strains of the Cloacae-Aerogenes Group*

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AN increasing variety of bacterial species including *B. cloacae*, *B. proteus*, and staphylococci, is being found to incite gastrointestinal disturbances following the consumption of food containing these microorganisms or their toxic products, as is exemplified by the work of Buchanan and Megrail,¹ Bengston,² Baerthlein,³ Ilsley,⁴ Dack and his coworkers,⁵ Jordan,⁶ Ramsey and Tracy,⁷ and Barber.⁸

Early in October, 1930, an outbreak of gastroenteritis attributed to cream-filled pastry occurred, the outstanding epidemiological features of which were described in *Public Health News* of New Jersey⁹ and *Health News* of New York.¹⁰ At least 125 cases were reported to have developed on 2 successive days in an area approximately 20 miles square, those occurring on the first day being confined chiefly to the southern part of the district. The symptoms were said to have been manifested in every case within a few hours after the eating of either cream puffs or chocolate éclairs manufactured by a wholesale bakery. A few of those who ate the pastry noticed a metallic or gaseous taste, but most of them did not report any unpleasant taste or odor. The illness was characterized by violent and prolonged nausea and vomiting, followed by a profuse diarrhea and, in some instances, cramps affecting the leg muscles. No rise in temperature was noted. The symptoms generally subsided in from 4 to 10 hours. The description of the cases occurring on the second day indicated that they were markedly more severe than those of the first. In fact, the administration of morphine was required in many instances.

The bakery was visited by a member of the laboratory staff on the day following the outbreak. It was in a concrete building which

* Read before the Laboratory Section of the American Public Health Association at the Sixtieth Annual Meeting at Montreal, Canada, September 17, 1931.

appeared to be so constructed that few rats and mice could gain entrance. Traps, but no poisons, were used. The work rooms and all utensils were scrupulously clean, and containers had properly fitting covers. The method of preparing the cream filling was as follows: Eggs, cornstarch, and milk powder were mixed, poured into a sugar solution previously heated, and cooked with constant stirring until thick. The material was heated to approximately 100° C., but was kept at this temperature for only a very short time, then left in a covered, tin mixing-bowl, at room temperature, for 4 or 5 hours before the shells were filled. The pastries were kept in a refrigerator overnight and distributed by automobile truck on the following morning.

The eggs were obtained from a firm which handles "cracked eggs," which are removed from the shells, put in tin cans, and distributed to bakeries, restaurants, etc. The eggs used on the second day were said to have been taken from a half-filled can which had been in the refrigerator overnight. None of this lot was available for examination and the can in which they were shipped had been discarded.

Six specimens were secured of cream puffs and chocolate éclairs, some of which were portions of those eaten by individuals who were ill. Unfortunately, only those distributed on the second day were available. Fecal specimens were obtained from 7 patients. Examination of the filling failed to show evidence of chemical poisons. None of the species generally associated with enteric disease were found in any of the specimens. *Staphylococcus aureus* was present in large numbers in the cream filling of the 6 pastry specimens and was also found in 2 fecal specimens.

In a study of a representative strain of these organisms isolated from the filling, a monkey was fed with 5 c.c. of a 48-hour culture grown in medium containing ingredients similar to those used in the pastry filling; a guinea pig and a mouse were inoculated intraperitoneally with 2 c.c. and 1 c.c. respectively of a filtrate from it; a rabbit was inoculated intravenously with 3 c.c. of this filtrate, and another with the same amount of the filtrate from a broth culture. None of the animals showed any evidence of illness.

From 5 fecal specimens and from the filling of 5 cream puffs or éclairs large numbers of nonmotile, Gram-negative, encapsulated bacilli were isolated, the growth products of which proved toxic for certain animals. The biochemical reactions indicate that the strain belongs to the cloacae-aerogenes group, although it does not correspond

exactly to any species for which a description could be found. With one possible exception, no evidence of motility has been demonstrated. Two stained films of one culture, however, have shown monotrichous

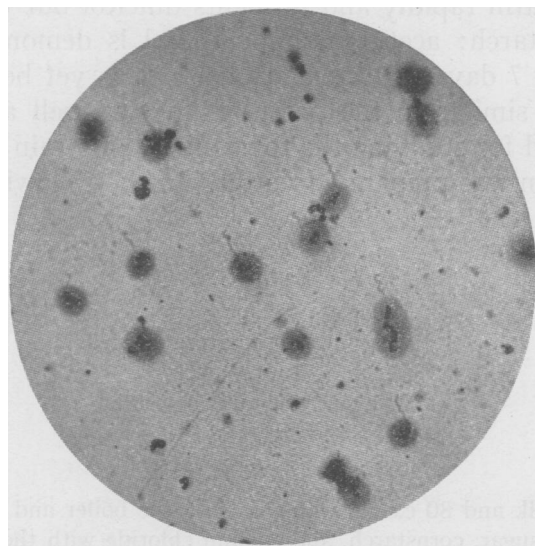


PLATE I—ORGANISMS SHOWING CAPSULES AND MONOTRICHOUS FLAGELLA. $\times 1,000$ DIAMETERS.

flagella (see plate I). Dextrose and lactose are fermented, at least three times as much gas being produced in the former and about twice as much in the latter as is formed by *B. coli*. Saccharose, maltose, mannitol, glycerol, inositol, and starch are also fermented, while dulcitol and adonitol are not. The majority of the strains liquefy gelatin very slowly, requiring from 8 to 14 weeks at from 35° to 37° C. Litmus milk is acidified and coagulated; indol is not produced. Acetyl-methyl-carbinol can be demonstrated in dextrose broth (Voges-Proskauer positive) after 24 and 48 hours' incubation, but not after a longer period; the reversion of reaction in 0.5 per cent dextrose-phosphate-peptone solution (methyl-red negative) is rapid, occurring within 24 hours. As our experience would indicate that this group of organisms is not frequently found, in considerable numbers at least, in human feces, the presence of the particular strain described in 5 of 7 of the stools from persons ill following the eating of the cream puffs is of significance.

Antiserum produced with 1 strain (No. 328) isolated from the cream-puff filling agglutinates to its full titer the other 9 strains. With one exception, no appreciable reaction with other available strains of the cloacae-aerogenes group was obtained. A culture isolated in the branch laboratory in New York City from cream-pie

filling, to which were attributed 2 cases of food poisoning in 1 family, was agglutinated to one-half the titer of the antiserum. This culture is motile and differs slightly from No. 328 in biochemical reactions. It liquefies gelatin rapidly and ferments dulcitol but fails to ferment glycerol and starch; acetyl-methyl-carbinol is demonstrable in dextrose broth for 7 days. Its toxicity has not as yet been determined.

A medium similar to the pastry filling, as well as beef-infusion broth, was used for studying the toxigenicity of strain No. 328. The custard medium was prepared according to the following formula:

CUSTARD MEDIUM

225 c.c.	Milk
100 c.c.	Water
53 gm.	Saccharose
15 gm.	Cornstarch
0.5 gm.	Sodium chloride
1	Egg
3 c.c.	Vanilla extract

Place the milk and 80 c.c. of water in a double boiler and heat until a scum forms. Mix the sugar, cornstarch, and sodium chloride with the remainder of the water and add the well beaten egg. Combine with the hot milk and cook slowly until thick. Distribute in flasks or tubes and sterilize in an autoclave at 115° C. for 20 minutes.

Unless otherwise stated, all cultures of No. 328 used for feeding or inoculation were incubated for 24 hours, while filtrates tested for toxicity were obtained from 48-hour cultures. No evidence of toxicity for mice or guinea pigs was observed following the feeding of custard-medium culture or subcutaneous inoculation with broth culture. Intraperitoneal inoculation with 1 c.c. of broth culture was followed in mice by diarrhea within 1 hour, and by death of both mice and guinea pigs within 24 hours; animals of both species remained alive and well after similar inoculation with culture filtrates.

A monkey was fed approximately 5 c.c. of a 48-hour culture in custard medium, subsequent to which diarrhea, but no other symptoms of illness, was observed. Two hens fed custard-medium culture and 1 inoculated intravenously with broth culture developed diarrhea within from $\frac{1}{2}$ to 3 and 6 hours, respectively. Rabbits inoculated intravenously with from 0.1 c.c. to 5 c.c. or fed with from 2 c.c. to 5 c.c. of filtrate from the custard-medium culture developed diarrhea within from 15 minutes to 6 hours, but usually recovered.

Similar results were obtained with this material after it had been heated to 100° C. Broth-culture filtrates caused diarrhea when given intravenously, but no symptoms were observed after feeding such

filtrates to rabbits. A kitten fed about 20 c.c. of a 24-hour culture grown in the custard medium showed no ill effects.

The epidemiology clearly points to cream puffs and chocolate éclairs as the source of intoxication. Since the cream filling was said to have been prepared each morning, a common vector is indicated. All the ingredients except the eggs and water were dry and it was understood that portions of material from the same lots were used both before and after the outbreak.

The data suggest, therefore, that the etiological agent might have been introduced by means of the eggs, which may have been contaminated from the shells, the persons removing them from the shells, or some other source. Although the mixture was cooked after the eggs were added, it is conceivable that a portion of the custard, especially that adherent to the kettle near the top, may not have been heated sufficiently to destroy the bacteria. Furthermore, if the toxin of the organisms of the cloacae-aerogenes group described had already been formed, it might not have been rendered inactive by the method of cooking employed.

While the pastry was said to have been kept in the refrigerator overnight, ample time elapsed for incubation of the bacteria during the 4 or 5 hours the cream filling was left at room temperature before the pastry shells were filled, and again the following day when the finished products were being distributed by truck, the temperature being unusually high for that season.

The highest standards of cleanliness cannot prevent contamination of food from dust, utensils, egg shells, and other similar sources which are known to harbor numerous so-called saprophytic microorganisms. Certain of these bacteria, in themselves innocuous, under favorable conditions produce substances which, if ingested, may prove markedly toxic. The frequent reports of outbreaks of food poisoning following the consumption of pastries containing cream-custard filling suggest that this material—possibly owing to the egg content—furnishes an excellent medium for the development of harmful bacterial products. Although the subjection of these foods to temperatures necessary for sterilization is not practicable, the production of toxic substances can be averted by continuous refrigeration during the brief period before distribution to the consumer.

The handling and refrigeration of "cracked eggs" may need more careful supervision. It seems important that steps be taken to control the preparation and distribution of all cream-custard food products, as well as to disseminate information to the public to insure necessary precautions on the part of the consumer.

REFERENCES

1. Buchanan, E. B., and Megrail, Emerson. Two outbreaks of food poisoning probably due to *B. cloacae*. *J. Infect. Dis.*, 44: 235, 1929.
2. Bengston, I. A. The Proteus group of organisms with special reference to agglutination and fermentation reactions and to classification. *J. Infect. Dis.*, 24:428, 1919.
3. Baerthlein, Karl. Ueber ausgedehnte Wurstvergiftungen, bedingt durch *Bacillus proteus vulgaris*. *München. med. Wchnschr.*, 69: 155, 1922.
4. Ilsley, M. L. Food poisoning probably caused by *Bacillus proteus*. *J. A. M. A.*, 90: 292, 1928.
5. Dack, G. M., Cary, W. E., Woolpert, Oram, and Wiggers, Hazel. An Outbreak of food poisoning proved to be due to a yellow hemolytic staphylococcus. *J. Prev. Med.*, 4: 167, 1930.
6. Jordan, E. O. The Production by staphylococci of a substance causing food poisoning. *J. A. M. A.*, 94: 1648, 1930.
7. Ramsey, R. J., and Tracy, P. H. Food poisoning probably caused by orange colored staphylococcus from udders of apparently healthy cows. *Proc. Soc. Exper. Biol. & Med.*, 28: 390, 1931.
8. Barber, M. A. Milk poisoning due to a type of *Staphylococcus albus* occurring in the udder of a healthy cow. *Philippine J. Sc.*, 9: 515; 1914.
9. Shaw, I. H. Cream and custard filled bakery products as causes of human illness. *Pub. Health News*, Dept. Health, State of New Jersey, 16: 67, 1931.
10. Outbreak of gastroenteritis. *Health News*, New York State Dept. Health, 7: 193, 1930.

Dental Research

AN important symposium on the cause and nature of tooth decay was held June 23 in Pittsburgh, at the Frick Training School, under the auspices of the Pittsburgh Section of the International Association for Dental Research. The main papers were presented by Dr. H. E. Friesell, Dean of the University of Pittsburgh School of Dentistry, and Dr. J. J. Enright, a research specialist of Mellon Institute. These contributions gave the first complete account of the results of the comprehensive scientific investigation of tooth decay or dental caries that has been in progress at the University of Pittsburgh during the past nine years.

In the reading and discussion of the papers by Drs. Friesell and Enright it was brought out that the new additions to dental knowledge resulting from this broad investigation were as follows: first, the determination of the effects of acids on tooth enamel under natural conditions; secondly, the discovery of the relation of the calcium phosphate content of the saliva to tooth decay; thirdly, the identification of the lactobacilli that cause tooth decay; and, fourthly, the description, in a preliminary way, of the results of a clinical study that demonstrated that an oral prophylactic program efficient in reducing the massive lactobacilli infection of the dental zone decreased by 66 per cent the amount of new tooth decay developing in a year.