"BACILLUS MUCOSUS CAPSULATUS" IN INFANTILE DIARRHEA1

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Recently an epidemic of diarrhea occurred in the nursery of a children's home. An organism classified as *Bacillus mucosus capsulatus* was present in large numbers in cultures of the stools of the sick infants. Following convalescence of the patients the organisms were not recovered from the stools. The identity of the bacterium isolated in this epidemic has not been established, but it is definitely one of the mucoid encapsulated bacilli. The epidemic is described because there has been an increasing tendency to regard such organisms as possible intestinal pathogens ² and because the correlation between the recovery of the organism and disease was striking.

DESCRIPTION OF THE EPIDEMIC

St. Anthony Home, which is situated in New Haven, accepts long-term placements and has a complement of about 70 children ranging in age from newborns to 5 years. At the time of the epidemic the nursery contained 24 infants from 6 weeks to 10 months of age.

During the month of September, 1944, 18 of the 24 infants developed diarrhea. Within 8 days from the onset of the epidemic these 18 babies were showing clinical signs of the illness.

The disease process first manifested itself by an increase in the number of stools. Subsequently they became loose but were not expelled explosively and did not contain large amounts of liquid. The color changed from a normal yellowbrown to a chalky, gray-white. At this point they possessed a distinctly musty odor. The odor was unusual in that it has not been observed in association with other diarrheal stools. Within 10 days from the onset of the illness the frequency, color, and the odor had returned to normal in the majority of the cases. Neither symptoms of an

upper respiratory infection nor stomatitis was present. Uncomplicated cases did not develop a significant temperature elevation. Moderate irritability and lethargy characterized the behavior of the babies. None appeared prostrated by the illness.

Treatment in the Home consisted only in the limitation of fluids and caloric intake by mouth with maintainance of fluid intake by clysis. In the more severely ill patients nothing was given by mouth for as long as 96 hours. Though the minimum fluid requirement per day was set at 100 ml. per kgm. of body weight, this level was not always attained due to the inability of some patients to absorb the desired amount of the clysis fluid. Three patients were admitted to the Pediatric Service of the New Haven Hospital to adjust the acid-base balance by intravenous therapy.

During convalescence from the diarrhea a fourth infant was hospitalized with an overwhelming pneumococcal infection. Type 14 pneumococci were present in the rhinopharynx, spinal fluid, and blood. Only one stool culture was taken, and no pathogens were recovered.

MATERIALS AND METHODS

At least 3 anal swab cultures were taken on each infant with the exception of the 1 baby from whom only 1 such culture was taken. Dry swabs were used at first. However, with the use of swabs moistened with beef infusion broth the number of organisms recovered on culture was materially increased. Material was grown on Endo's and Shigella and Salmonella-SS-agar. Colonial growth was considered optimum after 48 hours of incubation at 37° C. A gram-negative, nonmotile, encapsulated organism producing an especially mucoid colony, particularly on SS agar, was recovered in large numbers from 22 of the 24 babies (Figure 1). It was not recovered from 1 of the infants showing no signs of the illness, nor from the infant who died. On the basis of the cultural and morphological characteristics it was tentatively classified as a member of the Friedländer Group, Bacillus mucosus capsulatus. The babies in the nursery were recultured 1 month after the illness, and only 1 was demonstrated to be carrying the organism. This infant was the last to develop clinical evidence of

¹ This study was aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

² Personal communication from Dr. Friend Lee Mickle, Bureau of Laboratories, Connecticut State Department of Health.

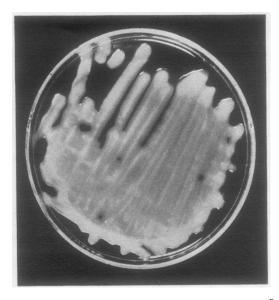


Fig. 1. A Subculture on SS Agar of the Organism Recovered from the Stools of the Infants with Diarrhea

the disease. A repeat culture on this patient 12 weeks later did not reveal the presence of the mucoid organism.

Nose and throat cultures, grown on rabbit's blood agar plates, were obtained from seven babies—the 4 who first became ill and the 3 who were hospitalized because of diarrhea. Neither the commonly recognized pathogens nor organisms resembling those of the Friedländer Group were present.

Five of the babies with diarrhea were studied in more detail. Agglutinins and precipitins for the organism could not be demonstrated in their blood during convalescence. Capsular swelling of their strains was not obtained with Friedländer A and B typing serums. Barritt's (1) modification of the Voges-Proskauer test using alpha-naphthol was carried out. The organisms were V. P. positive. The methyl red test was used as it was originally described by Clark and Lubs (2), incubating the cultures for 5 days at 30° C. They were methyl red negative. Simmons' (3) modification of Koser's (4) citrate test, that of adding bromthymol blue and agar, was used. The citrate test was also used without the agar being added.3 The organisms were found to be citrate positive by both methods. Litmus milk became acid and was coagulated by the organisms. Lactose was fermented with the production of acid and gas. Mice were killed by dilutions varying from 10-1 to 10-4 (Table I). Samples of stools from each of 2 patients were separately inoculated on to the scarified corneas of 2 rabbits.4 The tests were negative.

TABLE I
Studies of characteristics of the organisms

| Quellung | V.P. | M.R. | Citrate | Lactose | Mouse virulence |
|----------|----------|------------------------------------|-------------------------------------|--|---|
| | + | _ | + | + | 10-1 |
| _ | + | _ | + | + | 10-1 |
| _ | + | _ | + | + | 10-2 |
| _ | + | _ | + | + | 10-3 |
| _ | + | _ | + | + | 10-1 |
| | Quellung | Quellung V.P. - + - + - + - + - + | Quellung V.P. M.R. - + + + + + + - | Quellung V.P. M.R. Citrate - + - + - + - + - + - + - + - + - + - + | Quellung V.P. M.R. Citrate Lactose - + - + + + - + - + </td |

DISCUSSION

Little is found in the literature on the association of such an organism with epidemics of diarrhea.

In 1913 a "peculiar bacillus" was recovered (5) from 31 of 103 children with summer diarrhea. It was tentatively grouped as a pneumobacillus. Such a classification implies that the organism was similar to the Friedländer bacillus. Unfortunately no description was given of the bacterium.

In 1926 it was reported (6) that an organism was found belonging to the *mucosus capsulatus* group in 5.5 per cent of 200 stool samples examined. These findings have since been quoted by others (7). No description, however, was given of the organism called *mucosus capsulatus*, and the data are reported in such a manner that it is impossible to analyze them with respect to agegroups and the frequency with which the organism was recovered from cases of diarrhea or from healthy carriers.

In 1932 an epidemic of diarrhea was reported (8) in a newborn nursery. The organism recovered from the stools was described as "culturally and morphologically that of mucosus capsulatus" and was frequently associated with a non-hemolytic streptococcus. No further description of the organism was made. Perhaps the best evidence for pathogenicity, though indirect, comes from the fact that upon removal from duty of 3 nurse maids who were found to harbor mucosus capsulatus in their throats, the epidemic was drawn to a rapid conclusion.

Stool cultures are taken on infants and children on the Pediatric Service who have diarrhea of even mild degree. The reports on these cultures for the past 6 years (1939 to 1945) have been reviewed. Eleven hundred and thirty cultures were taken on 571 patients. Cultures were obtained

³ This technique was employed by Miss Osterman (11) in her study of encapsulated gram-negative organisms.

⁴ Dr. Herbert A. Wenner, of the Section of Preventive Medicine, Department of Internal Medicine, Yale University School of Medicine, kindly carried out this study.

either by taking a sample from the stool or by anal Endo's agar was used throughout this period. Several other culture media were also used for short periods throughout the first 3 years of the 6-year period. Shigella and Salmonella agar was used in conjunction with Endo's agar during the last 3 years. In this laboratory a gram-negative, nonmotile, encapsulated bacillus which produces a markedly mucoid colony has been placed in the Friedländer Group and is called Bacillus mucosus capsulatus. Such an organism was recovered from 18 patients, about 3 per cent. One patient was admitted twice with a 2-day interval between admissions. Bacillus mucosus capsulatus was recovered on both occasions. Fourteen of the patients were under 1 year of age, 13 of whom were less than 7 months. All were under 12 years (Table II).

TABLE II

Summary of positive stool cultures for bacillus mucosus capsulatus

(1939 to 1945)

| Age | Number of patients | | |
|---------------------|--------------------|--|--|
| Under 1 year | 14 | | |
| 1 year | 1 | | |
| 4 years | 1 | | |
| 6 years | 1 | | |
| 6 years 11 years | 1 | | |
| | | | |

Five of the 18 infants and children were admitted with diarrhea as the presenting disease. The remaining 13 included: nephritis (3), low calcium tetany (2), meningococcal meningitis (1), celiac disease (1), pyelitis (1), congenital obliteration of the bile ducts (1), cleft palate (1), feeding problem (1), prematurity (1), and bronchiolitis (1). However, all had diarrhea at the time the organism was recovered, and recognized enteric pathogens were demonstrated in only 1 case. This 2-months old baby had 1 stool culture positive for Shigella sonnei, and 4 subsequent cultures were positive for Bacillus mucosus capsulatus.

In order to determine the frequency with which this organism is found in children of ages comparable to those of the group involved in the epidemic, cultures were taken for the most part on all babies ranging in age from newborns through 1 year who were admitted to the Pediatric Service from December, 1944, through May, 1945. Swabs previously moistened with beef infusion broth were used to obtain the anal cultures which were grown for 48 hours on SS agar. Two cultures were taken on each infant on successive days. One hundred and seven patients were so cultured. Organisms from 2 patients gave the same cultural, morphological, and biochemical characteristics as did the organisms recovered from the infants involved in the epidemic. Organisms from 3 additional patients had the same microscopically morphological characteristics but differed from the others in that they were less mucoid and fermented the lactose in the SS agar more readily. The 5 babies did not have diarrhea.

The evidence in favor of the pathogenicity of the organism recovered from the babies during the epidemic rests on the facts that it was recovered in large numbers from the infants during their illness, and that its presence could not be demonstrated following convalescence. From the data presented this organism in an uncommon inhabitant of infants' stools. Salmonella and Shigella organisms were not demonstrated in the cultures. The virus with which some workers (9) were dealing in their epidemic was probably excluded by the above-mentioned studies. The pattern of onset and spread throughout the group would have been different had this epidemic been due to food contamination.

The present cultural, morphological, pathogenic, and biochemical means of identification of the gram-negative, encapsulated bacilli from the intestinal tract are not adequate to classify them accurately. In a comparative study of organisms of the Friedländer and coli-aerogenes group it has been observed (10, 11) that known Friedländer organisms gave biochemical reactions characteristic of the coli-aerogenes group and that some strains which had been identified by the usual biochemical criteria as Aerobacter aerogenes were found to possess antigens in common with the 3 Friedländer types, A, B, and C.

For the moment the organism is being designated *Bacillus mucosus capsulatus* for want of a better name. If the biochemical tests could be accepted as final the organism would be classi-

fied as Aerobacter aerogenas.⁵ Due to the recognized inaccuracy of the above-mentioned methods of identification serological studies will be performed.

SUMMARY

During September, 1944, 18 of 24 infants in the nursery of a children's home developed diarrhea. The age of the infants ranged from 6 weeks to 10 months. A gram-negative, nonmotile, encapsulated bacillus was recovered from the stools of 22 of the 24 infants in the nursery. This produced an especially mucoid colony, particularly on SS agar. The organism was not present following convalescence. It was impossible to classify accurately the bacterium recovered, but it is tentatively called *Bacillus mucosus capsulatus*. Efforts to establish its pathogenicity were unsuccessful; *i.e.*, agglutinins and precipitins were not present in the blood of the babies.

Five hundred and seventy-one children on the Pediatric In-patient Service of the New Haven Hospital in the past 6 years had stool cultures because of evidence of diarrhea. Eighteen patients (about 3 per cent) had Bacillus mucosus capsulatus in their stools; in 5 of the patients diarrhea was the presenting symptom. Recently a control series of cultures was obtained from infants under 1 year of age. Out of 107 patients 2 had Bacillus mucosus capsulatus in their stools.

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⁵ Such tests were also carried out by the Bureau of Laboratories, Connecticut State Department of Health, and the organism was placed in this category.