A Group of Paracolon Organisms Having Apparent Pathogenicity*

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THERE is a large number of heterogeneous coli-like organisms which, having certain physiological characteristics unlike typical *Escherichia,* have been grouped together as paracolon bacilli.[1] Taxonomically they may be considered as occupying a position intermediate between the typical coliforms and the salmonellas.[2] A few sub-groups of paracolon organisms have been described and their importance noted.[2-4] It is the purpose of this communication to describe briefly the characteristics of another sub-group of paracolon bacilli isolated during a small outbreak of gastroenteritis.

FEATURES OF THE EPIDEMIC

On May 13, 1944, it became apparent that an unusual number of cases of diarrhea were occurring in certain wards of a United States Naval Hospital and investigative procedures were instituted; clinical and epidemiological information was obtained by interviewing 219 patients and staff members. The illness was relatively mild. Only 1 of the 52 patients who were attacked exhibited blood in the stool, while 1 other had fever that was attributable to the disease. In almost all cases recovery was complete within 12 hours; 92.3 per cent of the cases had diarrhea, the number of diarrheal stools per case averaging 4.8. Percentages of patients with other signs and symptoms were: abdominal cramps, 75.0; nausea, 50.0; and vomiting, 11.5.

Statistical analysis implicated corn pudding as the probable vehicle of the etiologic agent. It was found that this "left-over" food had been substituted for rice in the evening meal the previous day and that it was sent on food carts to the wards affected. As is the case all too frequently, no samples of the corn pudding were available for bacteriologic study. The incubation period averaged approximately 12 hours after eating the suspected food.

BACTERIOLOGIC STUDIES

Stool or rectal swab specimens for culturing were secured from 17 of the 52 patients; both types of specimens were obtained from 2 subjects and all 4 yielded positive results. All specimens were plated directly onto Difco S S agar and B-B-L Desoxycholate-Citrate agar and also were inoculated into the modified tetrathionate enrichment medium of Kauffmann.[5] After 18 to 24 hours' incubation at 37° C. apparently non-lactose-fermenting colonies were transferred from the plates to agar slants, and plates of S S agar were streaked from the tetrathionate broth cultures. The following morning suspicious colonies were picked from these plates. In all, 28 single-colony

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cultures of the organisms to be described were obtained from 12 of the 17 patients. No other cultures thought to bear an etiologic relationship to the outbreak were found.

Fermentative characteristics of the cultures were studied in glucose, lactose, sucrose, mannitol, xylose, maltose, rhamnose, dulcitol, arabinose, inositol, trehalose, salicin, sorbitol, cellobiose, adonitol, levulose, galactose, mannose, inulin, glycogen, raffinose, milk, and d-tartrate. In addition, the Imvic reactions and hydrogen sulfide production were determined and motility was studied in semi-solid agar.[5] The results of these studies are as follows:

Acid and gas produced in 1 day (all cultures): glucose, mannitol, maltose, rhamnose, dulcitol (one strain remained negative for 23 days), arabinose, trehalose, sorbitol, levulose, galactose and mannose.

Delayed production of acid or acid and gas (all cultures): lactose (2 to 6 days); cellobiose (4 to 9 days).

No visible reaction in 21 days or more (all cultures): inositol, adonitol, inulin, glycogen, and raffinose.

Variable reactions: sucrose, 8 strains were negative for 21 days but the balance produced acid and gas in 1 day; xylose, 3 strains produced no change in 21 days while the remainder produced acid and gas in 1 day; salicin, 11 strains formed a slight amount of acid or acid and gas in 10 to 21 days but the other cultures remained negative for at least 3 weeks.

In B-C-P milk all strains produced a slight acidity that increased slowly during the period of incubation and eventually 5 cultures produced coagulation; all cultures tested were positive in d-tartrate. All cultures produced large amounts of H_2S, were actively motile, and the Imvic reactions for those studied were $-+-+$; Koser's citrate solution was the medium used in the last test. Gelatin was not liquefied by any culture during 10 days' incubation. As can be seen, observations made early in the period of incubation of the cultures show a similarity to the characteristics of the *Salmonella* group but further incubation elicits the traits typical of the paracolon organisms.

In order to test the pathogenicity of the cultures, 5 different strains were injected intraperitoneally into white mice in a dosage of 0.5 ml. of saline suspension prepared from agar slants. Three groups of 3 mice each received sucrose-positive strains and 2 groups of 3 mice each received sucrose-negative cultures. Thirteen mice were dead within 24 hours after injection and the other 2 were obviously ill. Cultures made from the heart's blood of the 9 mice which were autopsied yielded pure cultures of organisms having biochemical reactions identical with those injected.

Drug susceptibility of 1 sucrose-negative and 1 sucrose-positive strain was demonstrated by complete inhibition of growth of the organisms when streaked onto plates of agar containing 5 mg. per cent of sulfathiazole; 1 mg. per cent of the drug in agar resulted in 70 to 90 per cent inhibition of growth.

SEROLOGIC STUDIES

Antisera were prepared in rabbits for one of the sucrose-negative and one of the sucrose-positive strains, using formalinized broth cultures as antigens. Sera with high titers for homologous H antigens were obtained. Of some interest, however, is the fact that while use of the sucrose-negative strains resulted in a very high titered serum for the O antigen, the sucrose-positive strain was weakly antigenic in the single rabbit used. Alcohol-heat prepared O antigens[6] of 23 of the 28 strains reacted strongly with appropriate dilutions of both sera. Strangely enough, 5 sucrose-negative strains reacted only with the sucrose-positive serum. For-

malinized broth cultures of 24 strains used as H antigens [6] agglutinated well in a 1:1,000 dilution of serum for the sucrose-negative strain, while 4 strains reacted only with serum for the sucrose-positive strain; no strain reacted with both sera at a dilution of 1:1,000. It appears, therefore, that these epidemic strains are serologically related; their exact relationship to each other and to other Enterobacteriaceae will depend upon extensive antigenic analyses.

Blood samples obtained from 10 of the patients on the second day and from 5 of the same patients on the tenth day following the outbreak failed to reveal significant agglutinin titers when the sera were tested against 3 of the strains. Unfortunately it was not possible to continue these tests further but it is unlikely that agglutination titers would have shown appreciable increases since the illness was transient and there was no evidence of blood invasion.

INCIDENCE AND DISTRIBUTION

Subsequent to the recognition of this paracolon sub-group additional strains have been identified; they have been isolated from subjects who were asymptomatic at the time of culturing, from sporadic cases of diarrhea, and at least one other epidemic. In the two latter categories evidences of etiologic relationships were strong. Further details concerning the known distribution of the organisms will be given in a later report.

SUMMARY AND CONCLUSIONS

A group of biochemically and serologically related organisms was isolated from patients during a small outbreak of mild gastroenteritis; the disease was self-limited and of short duration. The organisms that appear to have been the etiologic agents possess characteristics that at present classify them as paracolon bacilli; until a more systematic scheme of classification is evolved these cultures are designated as paracolon (Pc.) 100-5-13. Clinical, epidemiological, biochemical and serological features are described briefly.

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