Further Studies on One Type of Paracolon Organism*

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FOR many years continental bacteriologists, including the English, have considered the paracolon organisms as a group intermediate between the coliform and *Salmonella* bacteria. Topley and Wilson (1938) say of the paracolon group, "Certain of these species are definitely pathogenic for man. Others are under suspicion in this respect. Others again are almost certainly non-pathogenic." Stuart *et al.* (1943), after studying 465 paracolon cultures, isolated for the most part from gastroenteritis patients over a period of 4 years, came to identical conclusions. These investigators found that the paracolon group, like the coliform bacteria, could be divided into three sections: paracolon *Aerobacter*, paracolon intermediates, and paracolon *Escherichia* according to their IMViC reactions. Each section was again divided into a number of biochemical types according to their carbohydrate reactions.

Of all cultures studied biochemical type 32011,† a paracolon *Aerobacter*, offered the best evidence for pathogenicity. Thirty-one of the 35 strains investigated were isolated from gastroenteritis patients and 4 from food handlers in one or another institution in which the strain had been isolated during so-called food poisoning epidemics. In the course of 5 weeks in the Rhode Island Hospital 4 patients listed as "typhoid suspects" yielded almost pure cultures of biochemical type 32011 from the feces and one a pure culture from the blood.

At the April, 1942, meeting of the New England Health Institute the senior author read a paper on the paracolon group, emphasizing the probable pathogenicity of biochemical type 32011 (hereinafter called bio-type). Within one year 114 strains of this organism were received from a number of New England laboratories.

Bio-type 32011 ferments glucose, maltose, mannitol, and frequently salicin, in 24 hours. Strong acid reactions (brom cresol purple indicator) and 20 to 30 per cent gas are produced in glucose and, when positive, in salicin. In maltose, however, a moderate acid reaction with or without a bubble of gas and in mannitol a moderate to strong acid reaction and a bubble to 10 per cent gas are produced in 18 to 24 hours. Lactose and sucrose are fermented slowly or not at all; some strains ferment one or another or both of these carbohydrates in about 6 days and other strains only after 3 to 4 weeks. Some strains ferment salicin rapidly, others slowly, and some are negative. Growth, usually of the beaded type, occurs on citrate agar in 5 to 30 days

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† Biochemical type 32011 was previously described by Stuart, *et al.*, 1943. After their paper was published it was found that cultures with the biochemical reactions of 32011 could be divided into several different serological types as shown in Table 1. Biochemical type 32011—serological type 32611, for example, means that cultures of serological type 32611 have all the biochemical reactions of 32011 but differ antigenically from 32011.

and the Voges-Proskauer reaction is positive though usually weak with freshly isolated strains. A great majority of strains are motile. The only nonmotile paracolon *Aerobacter* encountered among 343 cultures studied in this laboratory to date were not only bio-type 32011 but also sero-type 32611 (Table 1). (One previously reported nonmotile paracolon *Aerobacter*, bio-type 32821, Stuart, *et al.* (1943), was found to be bio-type 32011 and sero-type 32611.) Because of the relatively slow action on maltose and mannitol in 18 to 24 hours bio-type 32011 almost always can be distinguished easily from other paracolon types of any section.

Table 1 shows that 58 strains were sero-type 32011 while 21, 15, 9, 9, 7, 6, and 2 were sero-type 26311, 44311, 32611, 63511, 56211, 70811, and 64811, respectively. All eight sero-types possessed interlocking somatic, and in some instances flagellar, antigens. Three sero-types had minor, unlabeled somatic antigens in common with *Salmonella choleraesuis* and *Salmonella rubislaw*.

Twenty-two of the 149 strains did not belong to any of the eight sero-types. These strains, however, possessed antigens in common with one or another of the eight sero-types. Fourteen of them agglutinated to high titers with one or another of the eight antisera (five to the homologous titer) but in no instance was the homologous titer of any antiserum reduced upon adsorption.

TABLE 1

Serological Types of Biochemical Type 32011

	Sero-types								
	32011	*26311*	*44311*	*63511*	*32611* °	*56211*	*70811*	*68411*	*Unknown*
Number of strains	58	21	15	9	9	7	6	2	22

° = All strains nonmotile

Of the 35 strains isolated from gastroenteritis patients as mentioned above, 32 were antigenically identical or closely related. As new cultures were obtained, however, it was evident that bio-type 32011 was serologically heterogeneous. Biochemical reactions were of no value in establishing different sero-types in this bio-type. Several strains of bio-type 32011 agglutinated in serum dilutions of 160 and a number of others in dilutions as high as 1280 of 32011 antiserum, but failed to reduce the homologous titer when used to adsorb the antiserum. Antisera were prepared from one culture agglutinating to 160 and from one agglutinating to 1280. Thus two new sero-types were established. By agglutination and adsorption tests with the three antisera, and the preparation of additional antisera, eight sero-types were identified in bio-type 32011.

Of a total of 149 strains of bio-type 32011, 127 (85.2 per cent) fell into one or another of eight sero-types.

Unfortunately, adequate information could not be obtained about the source of all of the 114 new strains. Eighty-six were from gastroenteritis patients (81 from feces and 5 from blood), 7 from food handlers, 2 from apparently normal contacts of patients, 1 from milk, 1 from pastry, and 17 from fecal specimens of unknown history. Eleven strains of sero-type 32011 and 3 of sero-type 26311 were isolated from patients listed as "typhoid suspects" which, with the 4 previously cited, make a total of 18 strains of bio-type 32011 isolated from patients suspected of having typhoid fever.

DISCUSSION

Adequate methods for the accurate determination of the toxicity and pathogenicity or aggressiveness of the *Enterobacteriaceae* are lacking. Despite this the pathogenicity for man of certain

Salmonella and *Shigella* species is well established if for no other reason than by the large mass of *in vivo* evidence. The pathogenicity of other species in these genera has been questioned. Because, for example, *Shigella alkalescens* (Andrews 1918) has been isolated occasionally from normal individuals, it usually is considered nonpathogenic. Both Neter (1942) and Weil (1943), in their reviews on *Shigella* agree that sufficient evidence has accumulated to show that under certain conditions this species can cause gastroenteritis and other infectious processes.

In view of the foregoing it seems that the pathogenicity of paracolon bacteria will not be established easily. Some bacteriologists will concede that a paracolon culture and a *Salmonella* type with identical somatic antigens will possess the same endotoxins, but the two cultures may have entirely different aggressive properties. For paracolon cultures such as bio-type 32011 having no major antigens in common with *Salmonella* the evidence for pathogenicity must rest on the sources and frequency of isolations.

Where epidemics have occurred in institutions a careful search has nearly always located a food handler carrying the organism. In specimens from food handlers plated on eosin-methylene-blue agar bio-type 32011 comprised only a small fraction of the flora while specimens from patients after onset of symptoms usually gave almost pure cultures of the organism. The loss of symptoms coincided with the disappearance of almost pure cultures from the feces.

The close association of the organism with gastroenteritis is shown by the following example: In a neighboring hospital 28 cases of mild to acute gastroenteritis occurred among patients and staff over a period of several hours on one day. A milk supply was the only common factor other than water. The 48 hour milk plates which had been counted the day before the epidemic had not been discarded, and isolations were made from these plates. Because of the loss of personnel due to the war only 2 fecal specimens from patients reached the laboratory. Several cases were reported in the city, emanating from a restaurant using the same milk supply as the hospital. A fecal specimen from one patient and cream filling from pastry were sent to the laboratory. From the cases in the hospital and city, the milk plates of the hospital, and from the cream pastry organisms of bio- and sero-type 32011 were isolated. Two laboratory infections have been caused by this organism, one in a student frequently working with bio-type 32011 and the other in a technician washing glassware, who had an acute gastroenteritis attack 4 days after a number of broth tubes of this organism had been placed by mistake in a basket marked " nonpathogenic."

Each year for the past 7 years fecal specimens from about 50 normal students have been examined. A few paracolon cultures have been isolated from these specimens but never bio-type 32011.

It hardly can be coincidence that 116 bio-type 32011 strains were isolated from known gastroenteritis patients and 11 from food handlers involved in epidemics, or that the same organism was isolated from milk or milk products involved in an epidemic or that 18 strains were isolated from typhoid suspects.

SUMMARY—Strong evidence has been found of the pathogenicity for man of one type of paracolon *Aerobacter*.

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