Phase Variation of Andrewes in *Salmonella enteritidis* Bioserotype Paratyphi-A

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The natural occurrence of a strain of *Salmonella enteritidis* bioserotype Paratyphi-A is reported, in which the flagellar antigens segregated readily into normal phase 2 antigens and mixtures of normal phase 1 and phase 2 antigens, and in which phase variation of Andrewes was demonstrated with ease.

*Salmonella enteritidis* bioserotype (bioser) Paratyphi-A (*Salmonella paratyphi*A) is an important pathogen of man which produces an enteric fever syndrome rather than acute enteritis. This microorganism no longer occurs frequently in the United States (2, 5), but it is imported occasionally. Therefore, bacteriologists should be familiar with it (8).

Bruner and Edwards (1) were able to produce induced phases in a number of strains of bioser Paratyphi-A by cultivation of the microorganisms in the presence of antisera. The H (flagellar) antigen phase 1,5 was induced in many, but not all, of the cultures examined (1). This phase was known to occur in various serotypes of *Salmonella*, but never had been demonstrated in bioser Paratyphi-A (1). All of the induced phases (z2; z1; and 1,5) were stable during the 2-year period that they were maintained in the laboratory (1). The stability of the induced antigens, as well as of the naturally occurring H antigen a, was contrasted to the instability of the phases of normally diphasic serotypes of *Salmonella*, in which phase variation of Andrewes is effected easily (see reference 4). Bruner and Edwards (1) demonstrated reversibility of the phase a and phase 1,5 flagellar antigens in some of the cultures examined by growth in the presence of antisera. However, this was not accomplished with ease, since, in another publication, Edwards et al. (3) emphasized that it was very difficult to effect reversion of induced 1,5 forms of bioser Paratyphi-A to the original form and that attempts to do so failed in most instances.

Edwards et al. (3) described a strain of bioser Paratyphi-A that was monophasic in the second phase (1,2,12: -1,5). This culture was isolated in Egypt from the urine of a patient who was ill with enteric fever, and the isolation constituted the first recorded instance of the natural occurrence of phase 2 of bioser Paratyphi-A. These investigators (3) proved that this particular culture was antigenically identical with the 1,5 phase previously induced in strains of bioser Paratyphi-A (1). However, the strain was stable, and reversion from phase 2 to phase 1 was not demonstrated in it.

**MATERIALS AND METHODS**

The culture to be described was isolated in Hong Kong from aspirated bile from a patient afflicted with gangrenous cholecystitis. The methods used in its characterization were the same as those described earlier (4, 6, 10).

**RESULTS**

The biochemical reactions given by culture 1470-71 were typical of those of bioser Paratyphi-A (4, 7, 8, 11). Nitrate was reduced to nitrite, and production of hydrogen sulfide was apparent in Triple Sugar Iron and peptone iron agar media. The amount was equivalent to that produced by typical strains of *Salmonella typhi* in these media. Indole was not formed, the methyl red reaction was positive, the Voges-Proskauer test was negative, and growth did not occur in Simmons' citrate medium. Neither urease nor phenylalanine deaminase was produced, and growth did not occur in medium containing potassium cyanide. Malonate and mucate were not utilized, and gelatin was not liquefied in nutrient medium (22 C) nor in the Kohn test (4, 9). Definite evidence of decarboxylation of lysine was not obtained during 4 days of incubation, but tests were somewhat doubtful on the fourth day and became positive on the sixth day of incubation.
tion. Delayed reactions of this sort have been described (9, 12). Evidence of production of arginine dihydrolase was apparent after 3 days, and ornithine was decarboxylated rapidly. Acid and gas were produced within 24 hr from glucose, mannitol, arabinose, rhamnose, maltose, and trehalose. Dulcitol and sorbitol were fermented after 48 hr, glycerol in 3 to 5 days, and acid was produced from cellobiose after 16 days of incubation. Lactose, sucrose, adonitol, raffinose, xylose, alpha-methyl glucoside, erythritol, and esculin were not fermented. The strain was not lipolytic, did not grow on sodium acetate medium, did not yield a positive reaction of Stern's glycerol fuchsin medium, and failed to acidify Jordan's tartrate medium. When tested by the method of Kauffmann and Petersen (4, 9), citrate, D-, iso-, and L-tartrate were negative. Beta-galactosidase was not produced, and the indophenol oxidase test was negative.

Antigen suspensions prepared with culture 1470-71 were agglutinated to the homologous titer (1:1,280) of O antiserum produced with bioser Paratyphi-A (1, 2, 12) and removed all agglutinins from that antiserum in absorption tests. The occurrence of O antigens 1, 2, and 12 in this strain also was demonstrated by agglutination in antiserum for O antigen groups 4, 5, 12; 9, 12; 1, 3, 19 and in absorbed single-factor antiserum for O2.

The flagellar (H) antigens of phase 1 of culture 1470-71 were flocculated to the homologous titer (1:25,600) of H a antiserum derived from a normal, monophasic strain of bioser Paratyphi-A and removed all agglutinins from it in absorption tests. The antigens of the second phase of this culture were agglutinated to the homologous titer (1:12,800) of H antiserum produced with a monophasic strain (228) of bioser Paratyphi-A, in which the second phase antigens (1, 5) had been induced (1), and removed all agglutinins from it. Further, the phase 2 antigens of culture 1470-71 were flocculated to the homologous titer (1: 12,800) of an antiserum derived from the phase 2 antigens (1, 5) of serotype Thompson (Berlin variant) and, in absorption tests, reduced the titer of the antiserum for the homologous microorganism to 1:400. When tested in absorbed single-factor H antiserum for H antigens 2, 5, 6, and 7, the phase 2 antigens of strain 1470-71 reacted only in factor 5 antiserum.

The growth from each of 190 single colonies from platings of culture 1470-71 was examined for agglutination (slide tests) in 1:100 dilutions of H a and H 1, 5 antiserum. All single colonies examined reacted in H a antiserum only. The progeny of 10 of the aforementioned single colonies were placed in individual tubes of semisolid agar medium that contained antiserum for H antigen a. When tested (tube agglutination tests in which antiseras were diluted 1:1,000), five of these single colonies yielded progeny that were agglutinated by 1, 5 antiserum only, whereas five other colonies produced progeny that were agglutinated by antiserum for both a and 1, 5. From this point, it was possible to reverse the phases of the bacteria in the 10 subcultures at will (a → 1, 5).

In addition, 10 of the above-mentioned single colonies from platings were inoculated into individual tubes of semisolid agar medium without antiserum. These subcultures were examined after storage for 6 months, at which time phase 2 (1, 5) was demonstrated in two of them.

**DISCUSSION**

Strictly speaking, it cannot be said that culture 1470-71 was a naturally occurring diphasic strain of bioser Paratyphi-A, since the second-phase antigens were not detected by examination of numerous single colonies from platings. However, culture 1470-71 was the first example known to the authors of the natural occurrence of a culture of bioser Paratyphi-A in which the flagellar antigens segregated readily into normal phase 2 antigens and mixtures of normal phase 1 and phase 2 antigens in which phase variation of Andrews was demonstrated with ease. Furthermore, normal phase variation took place in stored cultures. The mechanisms responsible for synthesis of the phase 2 antigens of the culture had not been lost, nor were they repressed to the usual extent in this strain. In the authors' opinion, it seems likely that H antigens 1, 5 are normal, but repressed, components of many strains of bioser Paratyphi-A.

**LITERATURE CITED**

1. Bruner, D. W., and P. R. Edwards. 1941. The demonstration of non-specific components in Salmonella paratyphi A by induced variation. J. Bacteriol. 42:467-478.
2. Center for Disease Control. 1970. Annual summary, Salmonella surveillance. Center for Disease Control, Atlanta, Ga.
3. Edwards, P. R., L. A. Barnes, and M. C. Babcock. 1950. The natural occurrence of phase 2 of Salmonella paratyphi A. J. Bacteriol. 59:135-136.
4. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co. Minneapolis.
5. Ewing, W. H. 1969. Excerpts from "An evaluation of the Salmonella problem." Center for Disease Control, Atlanta, Ga.
6. Ewing, W. H. 1970. Differentiation of Enterobacteriaceae by biochemical reactions. Center for Disease Control, Atlanta, Ga.

7. Ewing, W. H., and M. M. Ball. 1966. The biochemical reactions of members of the genus Salmonella. Center for Disease Control, Atlanta, Ga.

8. Ewing, W. H., M. M. Ball, S. F. Bartes, and A. C. McWhorter. 1970. The biochemical reactions of certain species and bioserotypes of Salmonella. J. Infect. Dis. 121:288-294.

9. Ewing, W. H., B. R. Davis, and P. R. Edwards. 1960. The decarboxylase reactions of Enterobacteriaceae and their value in taxonomy. Public Health Lab. 18: 77-83.

10. Ewing, W. H., and B. R. Davis. 1970. Media and tests for differentiation of Enterobacteriaceae. Center for Disease Control, Atlanta, Ga.

11. Kauffmann, F. 1966. The bacteriology of Enterobacteriaceae. E. Munksgaard, Copenhagen.

12. Moeller, V. 1955. Simplified tests for some amino acid decarboxylases and the arginine dihydrolase system. Acta Pathol. Microbiol. Scand. 36:158-172.