THREE TRIPHASIC ARIZONA SEROTYPES (23:23:30:42; 26:23:30:40; and 1, 33:24:25:39)

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The literature concerning the occurrence of multiphasic cultures of Enterobacteriaceae was reviewed briefly by Fife, Edwards and Sakazski (1962). The purpose of the present paper is to describe three additional triphasic Arizona cultures.

MATERIALS AND METHODS

The cultures to be described were isolated from the feces of apparently normal snakes (*Agkistrodon halys blomhaffi*). The cultures used in the investigation were standard cultures of the *Salmonella* and Arizona groups and the sera employed were derived from the standard cultures. The methods used were those described by Edwards and Ewing (1962).

RESULTS

The cultures were motile members of the Enterobacteriaceae and possessed properties typical of the Arizona group. Indol was not formed, the methyl red test was positive and the Voges-Proskauer test negative, growth occurred on Simmons' citrate agar, hydrogen sulfide was produced, urease and phenylalanine deaminase were not produced, no growth occurred in Moeller's KCN medium, and gelatin was liquefied slowly. Lysine and ornithine decarboxylase and arginine dihydrolase were produced. When tested in organic acid media by the method of Kauffmann and Petersen (1956), D-tartrate, citrate, and mucate were acidified only after 48 to 72 hours incubation. The cultures utilized malonate.

Glucose, arabinose, xylose, lactose, maltose, trehalose, mannitol, and sorbitol were fermented promptly with gas production. Rhamnose was fermented promptly by all cultures except 2111-60 which produced acid from the sugar only after three days incubation. Fermentation of cellobiose was late and irregular. Sucrose, raffinose, inositol, and salicin were not fermented.

The serologic properties of the cultures were as follows:

Culture 2055-60 (CDC 3064-61) possessed the antigenic formula 23:23:30:42. When first examined this culture contained not only the aforementioned triphasic type, but a monophasic Arizona type (13:22:-) as well. Later, two additional cultures of 23:23:30:42 were recognized. This serotype was agglutinated to the titre of Arizona

O group 23 serum and in absorption tests removed all agglutinins from the serum. When colonies of this type first were examined, some colonies of the H 23 phase and others of the H 30 phase were found. These two flagellar phases each reverted spontaneously to the other and the culture could be changed from one phase to the other at will through growth in semisolid medium containing appropriate flagellar sera. When the culture was inoculated into medium which contained both H 23 and H 30 sera, instead of being immobilized as would a diphasic culture, the organism spread rapidly through the medium. Cultures made from this spreading growth, although actively motile, were not agglutinated by sera for the then recognized H antigens of the *Salmonella* and Arizona groups. Serum just prepared from an unknown phase of a quaterphasic culture of *Salmonella mikawashima* then under study (Edwards, Sakazaki and Kato, 1962) agglutinated the third phase of 2055-60 to titre. This antigen was assigned the *Salmonella* H symbol z_{50} and in the Arizona group became H antigen 42. Since this antigen previously had not been found in the Arizona group, and since at that time little was known concerning its occurrence in the *Salmonella* group, an intense search was made to determine whether the antigen occurred naturally in 2055-60 or whether it might be an artifact which appeared during growth in agglutinating sera like the j and z_5 antigens of salmonellae. Among 190 colonies examined, one colony of the H 42 phase was found. Later, when additional cultures of this serotype were examined, H 42 phases were isolated without difficulty by simple colony selection and without recourse to serum. Likewise, z_{50} colonies were found to occur naturally in cultures of *S. mikawashima*. In reciprocal absorption tests the z_{50} antigen of *S. mikawashima* and the H 42 antigen of 2055-60 were found identical. When placed in semisolid medium which contained H 23, H 30, and H 42 sera, the organism was immobilized, indicating that no further H phases were present.

The H 23 phase of 2055-60 was agglutinated to half the titre of H 23 serum prepared from a standard strain (3209-54) and in absorption tests reduced the titre of the serum from 20,000 to 200. The H 30 phase removed all agglutinins from a standard H 30 serum. When isolated from single colonies, the H 23, H 30, and H 42 phases each could be reversed readily to either of the others and this process apparently could be continued indefinitely. Thus the organism possessed three reversible phases, all of which occurred naturally.

Culture 2415-60 (CDC 3068-61) possessed the antigenic formula 26:23:30:40. The type was represented only by one culture. The organism was agglutinated to the titre of O group 26 serum and in absorption tests removed all agglutinins from the serum. When first examined with H sera the organism was agglutinated by specifically absorbed H 30 serum and by H 40 serum, but not by absorbed H 23 serum. When placed in semisolid medium containing H 30 and H 40 sera, the organism migrated readily through the medium and the H 23 phase was obtained without difficulty. In absorption tests, the H 23 phase removed all agglutinins from H 23 serum and the H 30 phase exhausted H 30 serum of agglutinins. The H 40 phase was agglutinated to the titre of H 40 serum derived from the standard H 40 strain, 2907-58, but in absorption tests failed to remove agglutinins from it. It did, however, remove agglutinins from H 40 serum prepared from culture 2253-60 (CDC 3061-61). The relationships of these H 40 antigens were delineated by Fife, Edwards and Sakazaki (1962) and it was demonstrated that the H 40 antigen was divisible into two entities, 40a, 40b and 40a, 40c. The H 40 of 2415-60 was identical with H40a, 40c. Single colonies of each of the three phases easily could be changed to either of the other two by growth in appropriate

sera and such changes could be continued in series at will.

Culture 2111-60 (CDC 3060-61) possessed the antigenic formula 1, 33 : 24 : 25 : 39 and the type was represented by only one culture. Arizona serotype 24 : 24 : 31 was isolated from the same snake. Attention should be called to the relationship of 2111-60 to the quartrephasic Arizona type 1, 33 : 24 : 25 : 39 : 40 of Fife, Edwards and Sakazaki (1962). Culture 2111-60 differed from that type only in that it failed to ferment sucrose and raffinose and lacked the H 40 phase. Like the quartrephasic type it failed to remove completely the agglutinins from H 24 serum derived from the test strain for that antigen but did remove all agglutinins from O serum 1, 33 and H sera 25 and 39. Each phase easily could be changed to either of the other two and these changes could be carried on in series. When the organism was placed in semisolid medium which contained agglutinating sera for the phases H 24, H 25, and H 39, it was immobilized.

The organism occurred originally in the H 24 phase and when placed in semisolid medium containing serum for that phase, the H 25 phase emerged. When the H 25 phase thus obtained was placed in semisolid medium containing H 25 serum, the H 39 phase was recovered. When the H 39 phase was cultivated in homologous serum it reverted to the H 24 phase. Since all three phases were recognized antigens that occurred naturally in known Arizona types, no particular effort was made to detect colonies of each phase in the original culture. It is noteworthy that Arizona H 39 is identical with *Salmonella* H antigen z_{47} found in the quartrephasic cultures of *S. mikawashima* by Edwards, Sakazaki and Kato (1962).

SUMMARY

Three triphasic Arizona types (23 : 23 : 30 : 42 ; 26 : 23 : 30 : 40 ; and 1, 33 : 24 : 25 : 39) isolated from snakes (*Agkistrodon halys blomhaffi*) are described. All contained only H antigens known to occur in nature and the H phases were easily reversible at will.

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