EIGHT NEW ARIZONA SEROTYPES ISOLATED FROM REPTILES WITH SPECIAL REFERENCE TO A NEW ARIZONA H ANTIGEN

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Since the Arizona group was first delineated by Edwards, West and Bruner (1947), many additional antigens and serotypes were recognized and members of the group have been found in a variety of pathological conditions of man and animals. Current knowledge of the biochemical, serological, and ecological characteristics of the group was summarized by Edwards, Fife, and Ramsey (1959). Through studies covering several years the Arizona group has emerged as a distinct entity within the family Enterobacteriaceae, closely allied both biochemically and serologically to the genus Salmonella. Since the isolation of the original Arizona strains by Caldwell and Ryerson (1939) it has been known that organisms of this group occurred in reptiles but it was not until LeMinor, Fife, and Edwards (1958) recovered Arizona strains from 43% of 310 apparently normal snakes that their prevalence in reptiles was recognized. In the present paper eight newly recognized serotypes isolated from the feces of apparently normal snakes are described.

MATERIALS AND METHODS

The eight cultures to be described were isolated from the feces of as many snakes. Isolation of the cultures was carried out using DHL agar plate according to Sakazaki, Namioka, Osada, and Yamada (1960) after enrichment with selenite broth. The methods used in the biochemical and serological study of the bacteria were described by Edwards and Ewing (1955), Edwards, Fife and Ramsey (1959), and Sakazaki, Namioka and Ishii (1959):

RESULTS

With the exceptions cited below, the cultures possessed biochemical reactions considered
typical of the Arizona group, i.e., they were motile gram negative rods which failed to produce indol, were methyl red positive and Voges-Proskauer negative, grew readily on Simmons’ citrate agar, produced hydrogen sulfide, reduced nitrates to nitrites, did not produce urease or grow in KCN medium, gave positive decarboxylase reaction in lysine, arginine, and ornithine media, did not acidify D-tartrate, citrate, or mucate media promptly when tested by the method of Kauffmann and Petersen (1956), gave positive reactions in malonate broth, and liquefied gelatin. Acid and gas were produced promptly from glucose, xylose, arabinose, rhamnose, lactose, trehalose, maltose, mannitol, and sorbitol. Sucrose, raffinose, dulcitol, adonitol, inositol and salicin were not fermented. Action on cellobiose was delayed and irregular. The exceptions noted to this pattern of reactions were as follows: culture 2025–59 produced indol, culture 2125–59 promptly acidified citrate and mucate broths, and culture 2219–59 promptly fermented sucrose. Such aberrant reactions have been noted in other Arizona serotypes. The serological properties of the organisms are described below.

Culture 2025–59 (CDC 730–60) was agglutinated to the titer of Arizona O 1,4 antiserum and O factor 4 serum. In absorption tests the titer of the serum was reduced from 2,000 to 100. The H antigens were diphasic and phase 1 was agglutinated to the titer of, and removed all H agglutinins from, Arizona H22 serum. Phase 2 was agglutinated to the titer of Arizona H21 serum and removed all H agglutinins from the serum in absorption tests. The antigenic formula of the culture was 1,4:22:21. Arizona O 1,4 antigen is closely related to Salmonella O antigen 53. Arizona H antigens 22 and 21 are related to Salmonella H antigens k and z35 respectively.

Culture 2123–59 (CDC 733–60) was agglutinated to the titer of Arizona O 5 serum and in absorption tests reduced the titer of the serum from 2,000 to 200. The H antigens were diphasic and phase 1 and 2 agglutinated to the titer of Arizona H23 and H30 sera, respectively and removed all H agglutinins from the sera in absorption tests. The antigenic formula of the culture was 5:23:30. Arizona O antigen 5 is closely related to Salmonella O antigen 48 while Arizona H antigens 23 and 30 are related to Salmonella H antigens 1, z13 and 1,5, respectively.

Culture 2125–59 (CDC 735–60) was strongly agglutinated by Arizona O group 9 serum. O group 9 is divisible into two parts, 9a, 9b and 9a, 9c. The O antigens of culture 2125–59 were identical with those of the type strain of the 9a, 9b subgroup. The H antigens were diphasic and the antigens of phase 1 and 2 were identical with those of the type strain of Arizona H26 and H25, respectively. The antigenic formula of the culture is 9:26:25. The antigens of this culture are unrelated to described Salmonella antigens except that the O antigens are slightly related to Salmonella O antigen 50 which is identical with Arizona O antigen 9a, 9c.

Culture 2102–59 (CDC 732–60) possessed O antigens identical with those of the type strain of Arizona O group 15. Phases 1 and 2 of the diphasic H antigens were identical with Arizona H antigens 23 and 30, respectively. The antigenic formula of the strain was 15:23:30. Arizona O antigen 15 is identical with Salmonella O antigen 42 and, as noted above, Arizona H23 and H30 are closely related to Salmonella H antigens 1, z13 and 1, 5.

Culture 2152–59 (CDC 739–60) was agglutinated to the titer of, and removed all agglutinins from, Arizona O 16 serum. The two phases of the diphasic H antigens were identical with those of the type strain of Arizona H27 and Arizona H25. The antigenic formula of the culture was 16:27:25. Arizona O antigen 16 is identical with
Salmonella O antigen 38. The antigen of phase 1, H27, is closely related to Salmonella H antigen z19.

Culture 2219–59 (CDC 740–60) was agglutinated to the titer of Arizona O group 20 serum and in absorption tests removed all agglutinins from the serum. When isolated, the organism was flocculated to the titer of Arizona H24 serum and removed all H agglutinins from the serum in absorption tests. When placed in semisolid medium which contained H24 serum the organism migrated readily through the medium but cultures isolated from the spreading growth failed to agglutinate with available Arizona and Salmonella H sera. The organism was passed serially through medium containing Arizona H24 serum and after seven such passages a broth culture inoculated from the spreading growth was formalinized and used to prepare an H serum in rabbits. The resultant serum agglutinated the antigen used for injection in a dilution of 1–2,560 but agglutinated H24 antigen in a dilution of 1–5,120. Six further serial passages of 2129–59 through semisolid medium resulted in an antigen which, when injected into rabbits, yielded a serum which agglutinated the homologous phase at 1–5,120 and failed to agglutinate Arizona H24 in a dilution of 1–80. Further, the serum failed to agglutinate other Arizona H antigens and known Salmonella H antigens in dilutions of more than 1–160. Thus it was apparent that phase 2 of 2129–59 contained a new Arizona H antigen and to this the symbol H41 was assigned. The organisms of phase 2 were not so actively motile as the usual Arizona culture which has been passed through serial tubes of semisolid medium and this probably accounted for the relatively low H titer of the serum as contrasted to the usual H sera obtained with Arizona cultures. Distinct differences in the motility of the two phases of enteric bacteria have previously been noted (Edwards, McWhorter and Fife, 1954). The antigenic formula of the organism was 20 : 24 : 41. Arizona O antigen 20 is identical with Salmonella O antigen 35 and practically identical with E. coli O 111 : B4. The H antigens of phase 2 were not so actively motile as the usual Arizona culture which has been passed through serial tubes of semisolid medium and this probably accounted for the relatively low H titer of the serum as contrasted to the usual H sera obtained with Arizona cultures. Distinct differences in the motility of the two phases of enteric bacteria have previously been noted (Edwards, McWhorter and Fife, 1954). The antigenic formula of the organism was 20 : 24 : 41. Arizona O antigen 20 is identical with Salmonella O antigen 35 and practically identical with E. coli O 111 : B4. The H antigens of phase 1 are closely related to Salmonella H antigen r.

Culture 2132–59 (CDC 737–60) was agglutinated to the titer of Arizona O 23 serum and in absorption tests removed all agglutinins from the serum. Phase 1 and 2 of the diphasic H antigens were identical with those of the type strains of Arizona H24 and H31, respectively. The antigenic formula of the organism was 23 : 24 : 31. The O antigens are identical with Salmonella O antigen 47 of S. bergeri but not with the 47 antigen of S. kaolack. The H antigens 24 and 31 are closely related to Salmonella H antigens r and z, respectively.

Culture 2221–59 (CDC 741–60) possessed O antigens identical with those of the type strain of Arizona O group 25. The H antigens were diphasic and phase 1 and 2 were agglutinated to the titer of, and, removed all H agglutinins from Arizona sera H23 and H31, respectively. The antigenic formula of the organism was 25 : 23 : 31. Arizona O antigen 25 is closely related to Salmonella O antigen 16 while H phases 1 and 2 of 2221–59 are related closely to Salmonella H antigens 1, z13 and z, respectively.

SUMMARY

Eight new Arizona serotypes isolated from apparently normal reptiles are described. The types are represented by the antigenic formulas 1, 4 : 22 : 21 ; 5 : 23 : 30 ; 9 : 26 : 25 ; 15 : 23 : 30 ; 16 : 27 : 25 ; 20 : 24 : 41 ; 23 : 24 : 31 ; and 25 : 23 : 31. Attention is called to the serological relationships of each type to the Salmonella group. One type, 20 : 24 : 41, contained an undescribed H antigen (H41) which was not significantly related to previously recognized Arizona or Salmonella H antigens.
REFERENCES


