THE OCCURRENCE OF THREE FLAGELLAR PHASES IN ARIZONA SEROTYPES

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Since Andrewes (1922) described "specific" and "nonspecific" flagellar antigens in several *Salmonella* types, the diphasic state has been recognized in hundreds of *Salmonella* serotypes and has been recognized also in the Arizona group (Edwards and West, 1945). Similar variations have been found in the *Escherichia freundii* (Citobacter) group by Edwards (1946), in the Hafnia group by Deacon (1952), and in the *Aerobacter cloacae* (Cloaca) group by Sakazaki and Namioka (1960).

Salmonella types which had complex phases and which gave rise to simpler types through loss variation were reported. Edwards and Bruner (1942) described *S. salinatis* (4, 12: d, e, h: d, e, n, z₁₅) which could be changed irreversibly to *S. san diego* (4, 12: e, h: e, n, z₁₅) while Edwards, Kauffmann and Huey (1957) found that *S. montgomery* (11: d, a: d, e, n, z₁₅) was changed to *S. luciana* (11: a: e, n, z₁₅). Both of the changes resulted from the permanent loss of the common major antigenic constituent of phases 1 and 2 through cultivation in semisolid medium which contained d serum. Further, Edwards (1950) observed spontaneous irreversible segregation of e, h phases from more complex d, e, h phases. "Artificial" or induced phases often have been obtained by cultivation of salmonellae in homologous flagellar antisera. Thus, Kauffmann (1936) obtained j phases by cultivation of *S. typhi* in d serum and Edwards and Bruner (1939) and Bruner and Edwards (1941) obtained induced phases from *S. abortus equi* and *S. paratyphi A*. Such induced phases were reverted to naturally occurring antigens only with difficulty if, indeed, reversion was accomplished.

It is known that *Salmonella* types with similar H antigens yield similar induced phases. For example, salmonellae which contain antigen d tend to produce j phases when cultivated in homologous H antisera, and organisms which contained b antigens tend to yield z₃₃ phases (Edwards and Moran, 1946; Lederberg and Edwards, 1953). These induced antigens function as phase 1 if the organism which yielded them originally was diphasic.

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For instance, *S. paratyphi B* when cultivated in b and 1, 2 antisera yields a diphasic form having the H formula $z_{33} : 1, 2$. Further, such induced phases replace phase 1 of other types in transduction experiments (Lederberg and Edwards, unpublished data). Thus, while various phases have been induced in enteric bacteria, these apparently were artifacts which occurred rarely, if at all, in nature and resulted from changes produced in the natural antigens. These changes were not additive changes but were substitutions and in no instance was the number of flagellar phases of the organisms increased. Diphasic organisms, after change of one of the phases to an induced antigen, still exhibited variation only between two points of reference, as did the original culture.

It was not until recently that *Enterobacteriaceae* which possessed three naturally occurring H antigens were found. Edwards and Fife (1960) recognized an Arizona serotype which had the antigenic formula 5, 29 : 33 : 21 : 40. The three H phases, 33, 21, and 40, were known to occur naturally in other serotypes and were completely reversible at will in all directions. Later, Taylor and Lee (1960) recognized a culture of *S. worthington* which had the antigenic formula 1, 13, 23 : z : 1, w : z_{43} . The third phase was known to occur naturally in a number of cultures of *S. senftenberg*. The z_{43} phase could be changed to z or 1, w at will and natural phase variation occurred between the z and 1, w components but neither of these was induced to revert to z_{43} which was irretrievably lost. LeMinor and Edwards (1960) working with *S. hamilton* (3, 15 : z_{27}) found that upon cultivation in semisolid medium which contained z_{27} serum a 3, 15 : e, h : 1, 2 form indistinguishable from *S. goerlitz* was obtained. Here again, the e, h and 1, 2 phases were easily reversible but it was only with difficulty in many trials that z_{27} was recovered upon three occasions from the 1, 2 phase. From the foregoing summary it is apparent that organisms which have three natural phases have been found only rarely and that only the triphasic Arizona type recognized by Edwards and Fife contained three natural, readily reversible flagellar antigens. The purpose of the present paper is to describe two additional Arizona serotypes which possessed three reversible phases.

MATERIALS AND METHODS

The two cultures to be described were isolated from the feces of normal snakes by the methods using DHL agar plate according to Sakazaki, Namioka, Osada and Yamada after enrichment with selenite broth. The methods used in the biochemical and serological examination of the culture were those described by Edwards and Ewing (1955), Edwards, Fife and Ramsey (1959), and Sakazaki, Namioka and Ishii (1959).

RESULTS

The biochemical properties of the two cultures were those of typical members of the Arizona group as detailed by Edwards, Fife and Ramsey (1959) except that culture 2134-59 produced indol. Both cultures produced acid and gas from lactose within 24 hours. The serological characteristics of the cultures were as follows :

Culture 2127-59 (CDC 736-60) was agglutinated strongly by Arizona O group 5 serum in slide tests and in tube tests reacted to the titer of the serum. From absorption tests it was evident that the O antigens of 2127-59 were not identical with those of the test strain of O group 5 since the titer of the serum was reduced only 50% after absorption. However, since the organism possessed no antigenic relationships to other Arizona O groups, the culture was classified simply as a member of O group 5 rather than to introduce further complexities into the Arizona scheme by the establishment of additional O

subgroups.

When first tested with H sera, the culture reacted only with Arizona H39 serum. When a single colony of that phase was placed in semisolid medium which contained H39 serum, the organisms recovered from the spreading growth were agglutinated both by H23 and by H30 sera. These sera are known to give cross reactions so the antigen was tested with the two sera which had been mutually absorbed. Again agglutination occurred in both. The form in question then was grown in one tube of semisolid medium which contained H39 and absorbed H23 sera and in a second tube which contained H39 and absorbed H30 sera. Spreading occurred in both tubes. From the first was isolated a form which behaved as a typical H30 phase and from the second a culture which seemed identical with H23. Closer examination of these two lines revealed that this was the case and that two distinct H antigens were involved. The results of agglutination and absorption tests with these antigens are given in Table 1. The data included in the table demonstrate the presence in the culture of three distinct H phases, one identical with the test strain of H23, the second identical with the test strain of H30, and the third identical with the test strain of H39.

Table 1. H antigens of 2127-59

Sera	Antigens					
	Arizona H23 (1109-55, phase 1)	2127-59 (phase 1)	Arizona H39 (1158-58, phase 2)	2727-59 (phase 2)	Arizona H30 (456-53, phase 2)	2127-59 (phase 3)
Arizona H23 (1109-55, phase 1)						
Unabsorbed	12,800	25,600	<106	<100	6,400	6,400
Absorbed by						
2127-59, phase 1	<100	<100	<100	<100	<100	<100
Arizona H39 (1158-58, phase 2)						
Unabsorbed	<100	<100	25,600	25,600	<100	<100
Absorbed by						
2127-59, phase 2	<100	<100	<100	<100	<100	<100
Arizona H30 (456-53, phase 2)						
Unabsorbed	3,200	3,200	<100	<100	25,600	25,600
Absorbed by						
2127-59, phase 3	<100	<100	<100	<100	<100	<100

After the presence of the three phases was proved, their reversibility was investigated. They were found to be fully reversible and any one of the three phases could be obtained from either of the other two by cultivation in the appropriate combinations of sera which had been absorbed to remove common agglutinins. Single colony isolation was interposed between each change of phase. Both antigens H23 and H39 occur in combination with phase 2 antigens in other types. Hence, both have been designated previously as phase 1 antigens. On the contrary, H30 occurs typically with phase 1 antigens and has been designated in many types as phase 2. Therefore, the antigenic formula of 2127-59 has been designated as 5: 23: 39: 30. Arizona O5 is closely related to *Salmonella* O antigen 48, Arizona H23 to *Salmonella* H antigen 1,_Z13, and Arizona H30 to *Salmonella* H antigen 1,5. Arizona H39 is identical with a new *Salmonella* H

Sera	Antigens						
	Arizona H33 (466-52, phase 1)	2134-59 (phase 1)	Arizona H25 (Pc 155, phase 2)	2134-59 (phase 2)	Arizona H38 (1995-57, phase 2)	Arizona H40 (2907-59, phase 2)	2134-59 (phase 3)
Arizona H33 (466-52, phase 1)							
Unabsorbed	25,600	25,600	<100	<100	<100	<100	<100
Absorbed by 2134-59, phase 1	<100	<100	<100	<100	<100	<100	<100
Arizona H25 (Pc 155, phase 2)							
Unabsorbed	<100	<100	6,400	6,400	<100	<100	<100
Absorbed by 2134-59, psase 2	<100	<100	<100	<100	<100	<100	<100
Arizona H38 (1995-57, phase 2)							
Unabsorbed	<100	<100	<100	<100	25,600	1,600	32,00
Absorbed by Arizona H40 (2907-59, phase 2) 2134-59, phase 3	<100 <100	<100 <100	<100 <100	<100 <100	6,400 1,600	<100 <100	400 <100
Arizona H40 (2907-59, phase 2)							
Unabsorbed	400	400	<100	<100	1,600	25,600	12,800
Absorbed by Arizona H38 (1995-57, phase 2) 2134-59, phase 3	<100 <100	<100 <100	<100 <100	<100 <100	<100 <100	3,200 1,600	1,600 <100

It was evident that one phase of the culture was identical with Arizona H33, the second with H25 and the third a complex phase related to H38 and H40 but identical with neither. The reversibility of these phases was next investigated and again it was found that each of the three phases could be changed to either of the others and that these changes apparently could be continued indefinitely. Here again, single colony isolation was interposed between each change in phase. The designation of the third phase involved an arbitrary decision since it was identical neither with H38 nor with H40. Since the component was agglutinated to half the titre of H40 serum but to a much lesser degree by H38 serum, the phase was designated simply as H40. Arizona H antigen 33 occurred previously in combination with phase 2 antigens and was designated as phase 1. H antigens 25 and 40 have occurred as phase 2 in other cultures. Culture 2134-59 arbitrarily was assigned the formula 28 : 33 : 25 : 40. Arizona O antigen 28 is identical with *Salmonella* O antigen 47 of *S. kaolack* and H antigen 33 is closely related to *Salmonella* H antigen i.

SUMMARY

Two Arizona cultures from snakes, each of which possessed three distinct flagellar antigens, are described. The first is represented by the antigenic formula 5 : 23 : 30 : 39 and the second by the formula 28 : 33 : 25 : 40. The phase designated as H40 in the second formula is not identical with that of the test strain but is related to both antigens H38 and H40.

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