A New Type of Flagellar Variation associated with New Antigens in the Salmonella Group

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SUMMARY

A new type of phase variation which changes in one direction only has been noted in some members of the Salmonella group. This was found in association with three hitherto undescribed flagellar antigens, z_{43} , z_{45} and z_{46} , which are characterized. These occurred naturally with particular frequency in Salmonella group E and in cultures which possessed an alternate g, s, t phase. Two of these antigens, z_{43} and z_{45} , were found less frequently in other O groups and in organisms possessing other alternative phases. Although the antigens in question can be readily transformed to the alternate phases of the organisms in which they occur, these alternate phases could not be changed to z_{43} , z_{45} or z_{46} .

INTRODUCTION

The classification of the Salmonella group is based on the antigenic structure of the somatic and flagellar antigens of the serotypes of which it is composed. The structure is indicated by arabic numerals for somatic antigens, alphabetical letters for flagella in phase 1 and numerals or letters for flagella in phase 2. Salmonellas may fail to produce flagella; they may produce but one type of flagella, when they are called monophasic; or they may produce two types of flagella, being known as diphasic. Diphasic types may occur naturally, either in phase 1, phase 2, or a mixture of both. In the laboratory there are simple cultural techniques by which a diphasic organism in phase 1 may be changed to phase 2 and vice versa. One organism has flagella of one type only but, if it is diphasic, has the ability to produce progeny with flagella of a different type; the phase change occurs easily in either direction. The Salmonella group is divided into subgroups based on somatic O antigens. For example, subgroup E is composed of serotypes containing O antigen 3; many of the organisms which are the subject of this communication are classified in group E.

RESULTS

Recently, many investigations have been made into the occurrence of salmonellas in home-produced and foreign animal feeding-stuffs and fertilizers. Some of these

materials have been imported from countries whose salmonella problem has received little attention, so there is no information as to the salmonella serotypes present in man and animals in the country from which they have come. In addition to new salmonella serotypes composed of known antigens, three undescribed flagellar antigens have been found to which the symbols z_{43} , z_{45} and z_{46} have been assigned. These three antigens were found in organisms of O group E, in association with flagella having the formula g, s, t. The particular interest of these new flagella was the ease with which the change from the natural z_{43} , z_{45} or z_{46} phase to the g, s, t phase occurred, but the complete inability, by using all known techniques, to reverse this change. The ability of each culture to revert from single colonies of the g, s, t phase to z_{43} , z_{45} or z_{46} forms was studied. In many of the cultures, multiple colonies were examined by serial transfer in g, s, t antiserum. In all, more than 150 such colonies were transferred periodically in this manner for 6 months. In no instance was reversion of the g, s, t antigens accomplished. It was evident that there was a very marked difference in the variation rate of the g, s, t phases and that of the z_{43} , z_{45} , and z_{46} phases.

The z_{43} flagellar antigen

Among motile cultures belonging to group E_4 , O = 1, 3, 19, were a number which were agglutinated only in low dilution by *Salmonella wichita* phase 2 (z_{37}) antiserum and were unaffected by all other flagella (H) antisera then in use. An H antiserum prepared from one such culture agglutinated all similar cultures isolated in the United States and some of those isolated in England. The symbol z_{43} was assigned to the H antigens of these cultures. The z_{43} antiserum was absorbed with *S. senftenberg* to remove O agglutinins and added to semisolid medium for isolation of other phases which might be present in the organisms. When inoculated into this medium, all cultures which possessed z_{43} antigens migrated readily through it, and from the spreading growth organisms having H antigens g, s, t were isolated in each instance. In addition to 33 such cultures belonging to O group E_4 , four others were recognized, all members of group E_1 , O = 3, 10, and three cultures isolated from one lot of tankage (which is a protein by-product of abattoirs) had the T_1 (transitional) antigens described by Kauffmann (1956). The antigenic formulae of representative cultures were 1, 3, 19:g, s, t: z_{43} , 3, 10:g, s, t: z_{43} and T_1 :g, s, t: z_{43} .

The z_{45} flagellar antigen

Among the E_4 group cultures isolated in England were seven which were not agglutinated by z_{43} serum. An H serum prepared from one of these agglutinated four of the cultures. The H antigens of these cultures were designated as z_{45} . One culture had the T_1 antigen. One culture of group E_1 contained z_{45} antigen. Finally, it was found that the stock culture of *Salmonella halmstad* (3, 15:g, s, t) contained a z_{45} phase. From all of the cultures which contained z_{45} antigens, g, s, t phases were easily isolated by passage of the cultures through semisolid medium which contained z_{45} serum. The antigenic formulae of representative cultures was 1, 3, 19:g, s, t: z_{45} , T_1 :g, s, t: z_{45} , 3, 10:g, s, t: z_{45} and 3, 15:g, s, t: z_{45} .

The z_{46} flagellar antigen

One culture of group E_4 isolated in England contained neither z_{43} nor z_{45} antigen. An antiserum prepared from it agglutinated the homologous organism in high

dilution but did not agglutinate z_{43} or z_{45} forms. To the antigen thus revealed the symbol z_{46} was assigned. This culture also yielded a g, s, t phase when passed through semisolid medium containing z_{46} antiserum. The formula was 1, 3, 19:g, s, t: z_{46} .

With the exception of three cultures isolated from one lot of infected poultis which received feed contaminated with a salmonella strain of the same type, 1, 3, 19:g, s, t: z_{43} , all cultures were isolated from feeds, feed ingredients, or fertilizers. The sources of the cultures are summarized in Table 1.

Table 1. *Biochemical reactions and sources of cultures*

Antigenic formula	No. of cultures	H ₂ S	Tartrate			Citrate	Mucate	Source
			D	L	I			
1, 3, 19:g, s, t: z_{43}	2	+	+	—	+ ⁸⁻¹⁰	+	+	Bone meal, degreased bones*
	23	+	+	×	+ ²⁻⁵	+	+	Animal feeds and ingredients, poultis**
	7	—	+	×	+ ²⁻⁵	+	+	Feed ingredients**
	1	+ ²	+	×	+ ⁵	+	+ ²	Feeding meat and bone*
T ₁ :g, s, t: z_{43}	3	+	+	×	+ ²⁻⁵	+	+	Tankage (one sample)**
3, 10:g, s, t: z_{43}	3	+	+	×	+ ²⁻⁵	+	+	Meat by-products**
	1	—	+	×	+ ⁴	+	+	Meat by-products**
T ₁ :g, s, t: z_{45}	1	+	+	+	—	+	+	Bone chips (India)*
1, 3, 19:g, s, t: z_{45}	3	+	+	+	—	+	+	Bone meal, hoof and horn meal*
	1	+	+	—	—	+	+	Bone meal*
	2	+	+	+ ⁵⁻⁷	—	+	+	River Humber (England)
3, 10:g, s, t: z_{45}	1	+	+	+	—	+	—	Fertilizer (Pakistan)*
3, 15:g, s, t: z_{45}	1	+	+	+	—	+	+ ⁴	<i>S. halmstad</i> , stock culture
1, 3, 15:g, s, t: z_{45}	1	+	+	+	—	+	+	Bone meal*
1, 3, 19:g, s, t: z_{46}	1	—	+	—	—	+	+ ³	Bone meal*
1, 3, 19:g, s, t	34	+	+	×	×	+	+	
	2	+	+	+	—	+	+	Divers sources**
	31	—	+	+	—	+	+ ²⁻⁴	
	220	+	0	0	0	0	0	Divers sources*
	2	—	0	0	0	0	0	

0 Not tested.

+ Positive in 1 day. Superscript numbers indicate day of incubation on which positive reaction first observed.

× Late and irregular.

— negative.

* Isolated in England from products not imported from U.S.A.

** Isolated in U.S.A. or isolated in England from products imported from U.S.A.

Biochemical reactions

Both H₂S-positive and H₂S-negative cultures were included in the group and Dr F. Kauffmann (personal communication) suggested that it might be possible to separate them into *Salmonella newcastle* and *S. senftenberg* types. *S. newcastle* was an H₂S-negative type described by Warren & Scott (1930) which later was shown by Kauffmann & Mitsui (1930) to be identical with the H₂S-positive *S. senftenberg* type although Kauffmann (1941) found that they differed in their reactions on organic acids. Following this suggestion, the cultures were tested for H₂S production and action on organic acids. A number of monophasic cultures were included for comparison. The results are given in Table 1. The thirty-one H₂S-negative monophasic cultures listed in the table conformed to the biochemical pattern of *S. newcastle*

while the thirty-four H_2S positive cultures which gave delayed positive reactions in L-tartrate were typical of the *S. senftenberg* type.

Both H_2S positive and H_2S negative types occurred among the 1, 3, 19:g, s, t:z₄₃ forms, and the organic-acid reactions of the 1, 3, 19:g, s, t:z₄₅ forms were not uniform; further, the 3, 10:g, s, t:z₄₃ cultures were not identical in their reactions. Thus, it was not possible to correlate biochemical and serological results and divide the E_4 organisms into *Salmonella newcastile* and *S. senftenberg*.

Agglutination tests

The results of agglutination reactions of z₄₃, z₄₅ and z₄₆ antigens and their respective sera are given in Table 2. Phase 2 of *Salmonella wichita* (z₃₇) was included since it is slightly related to z₄₃. *S. senftenberg* (g, s, t), *S. simsbury* (z₂₇), and an 'induced'

Table 2. *Agglutination reactions*

Antigens	Source	Sera						
		g, s, t	z ₂₇	z ₃₄	z ₃₇	z ₄₃	z ₄₅	z ₄₆
g, s, t	<i>S. senftenberg</i> 3007*	25,600	< 100	< 100	< 100	< 100	< 100	< 100
z ₂₇	<i>S. simsbury</i> 11077*	< 100	3,200	< 100	< 100	< 100	< 100	400
z ₃₄	Induced phase of <i>S. senftenberg</i> CDC. 629*, obtained by passage through g, s, t serum	1,600	< 100	6,400	100	< 100	< 100	100
z ₃₇	<i>S. wichita</i> CDC. 6093-52*	< 100	< 100	200	3,200	400	< 100	< 100
z ₄₃	<i>S. senftenberg</i> Col. Sal. 2545/57*	< 100	< 100	100	800	12,800	< 100	200
z ₄₅	<i>S. senftenberg</i> Col. Sal. 655/58*	< 100	< 100	< 100	< 100	< 100	12,800	< 100
z ₄₆	<i>S. senftenberg</i> Col. Sal. 2634/57*	< 100	< 100	< 100	< 100	< 100	< 100	6,400

* These numbers indicate the particular strain of salmonella used. The prefix indicates the laboratory in which the strain was first investigated—CDC meaning the Enteric Bacteriology Laboratories, Communicable Disease Center, U.S.A., Col. Sal. the Salmonella Reference Laboratory, Colindale, England. Strains with no prefix are those reported in the original descriptions of such serotypes.

phase of *S. senftenberg* (z₃₄) also were included since all are connected in one way or another with the g, s, t complex (Edwards & Moran, 1947). It was evident that z₄₃, z₄₅, and z₄₆ were distinct antigens which occurred naturally and which had little relationship one to another or to other known salmonella antigens. Absorption tests proved that the three z₄₃ forms isolated in England from products not imported from the U.S.A. were identical. Likewise, all z₄₃ forms isolated in the United States, or isolated in England from imported American products, were identical, even though some belonged to group E_1 , some to group E_4 , and some had T_1 antigens. The genetic significance of the various subgroups of group E is now in doubt since it is well known that lysogenicity plays a large part in their characterization. Experience indicates that T_1 antigens may be derived from either E_1 or E_4 cultures as well as from other O groups. The z₄₃ antigens of the British and American strains, though closely related, were not identical. After cross-absorption a slight residue of agglutinins remained in the antiserum derived from the British strains and a considerable residue in the antiserum of the American strains.

A detailed analysis of the z₄₅ antigens was not done. Antiserum was prepared from only one culture and when this was absorbed with the other z₄₅ phases it was

evident that at least three distinct but closely related antigens were present in the z_{45} complex. These distinct antigens could not be correlated with the O antigens of the cultures.

DISCUSSION

It was stated that g, s, t phase could be isolated without difficulty from all organisms which occurred in nature in z_{43} , z_{45} or z_{46} phases. In some instances, both g, s, t and z_{43} phases were evident in cultures when they were received for typing. From such cultures colonies were isolated which were agglutinated either by g, s, t antiserum or z_{43} antiserum. Those which were agglutinated by z_{43} antiserum were readily transformed to g, s, t by passage through semisolid medium to which homologous antiserum was added. On the contrary, when the progeny of colonies which had g, s, t antigens were similarly treated they were immobilized and no change in H antigens occurred. In each instance in which a culture that was agglutinated by g, s, t antiserum was thought to have changed to z_{43} or z_{45} by passage through g, s, t antiserum, it was possible to plate the original culture and isolate z_{43} or z_{45} colonies. In other words, it was necessary for overt z_{43} or z_{45} antigens to be present in the cultures if they were to migrate through g, s, t antiserum.

The question naturally arose as to whether one was dealing with three different serotypes of group E₄, characterized respectively by antigens z_{43} , z_{45} and z_{46} , and whether these should be given distinct designations in the Kauffmann-White Schema. In this connexion, it should be emphasized that reversion of the g, s, t phases to z_{43} , z_{45} or z_{46} did not occur; if one is so unfortunate as to isolate a colony with g, s, t antigens it is not possible to determine whether the strain in question is truly monophasic, or whether an irreversible g, s, t form of a diphasic bacterium has been selected. Rapid fermentation of L-tartrate seems to be a characteristic of forms having z_{45} antigens, but even the small number of cultures examined was not uniform in this respect. Strains having z_{43} antigens utilized L-tartrate, though some were slow, whereas strains having z_{45} antigens failed to utilize it. Hydrogen sulphide production is of no value in their differentiation and, in fact, H₂S-positive and H₂S-negative forms were isolated from the same feed sample. It must be concluded that the biochemical reactions ordinarily used do not suffice to distinguish cultures which possess z_{43} , z_{45} or z_{46} antigens. One can but speculate as to how many of the supposedly monophasic cultures of *Salmonella senftenberg* were derived from cultures such as those described above. Considering these facts, the writers would classify all these cultures as *S. senftenberg*, but it must be kept in mind that cultures of *S. senftenberg* may fail to be agglutinated by g, s, t antiserum and may be completely in a z_{43} , z_{45} , or z_{46} form when isolated. The same facts apply to cultures of groups E₁ and E₂ which previously have been characterized as having only the H antigens g, s, t.

In addition to the cultures described here A. B. Moran & P. R. Edwards (private communication) noted z_{43} antigen in a culture of *Salmonella kingston* (4, 12, 27:g, s, t: z_{43}) and we found the same antigen in a culture of *S. worthington* having the formula 1, 13, 23, 37:z:1, w: z_{43} ; also we encountered a culture of *S. havana* which had the formula 13, 23, 36:f, g: z_{45} . As in the cultures of group E, once the cultures were reisolated from single colonies of other phases, they could not be forced to revert to the z_{43} or z_{45} form. These observations indicate that z_{43} and z_{45} antigens eventually may be found rather widely distributed throughout the Salmonella group.

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