the development of satisfactory methods of sterilization.

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Unusual Antigenic Variation in an Enteric Bacterium. (17944)

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The bacterium to be described is represented by 8 cultures, all of which were isolated by Hinshaw and McNeil(1) in their study of salmonellosis of reptiles. The 8 cultures were isolated from the viscera of as many fence lizards *(Sceloporus occidentalis)* captured along the banks of a stream in Davis, California. According to a personal communication from Dr. Hinshaw, the viscera of each animal were ground, placed in the tetrathionate broth of Kauffmann for enrichment and plated on brilliant green agar. Although the organism was isolated repeatedly from lizards captured at intervals in the same locality, there was no evidence that it produced disease. From the meager information available, this organism would seem to be commonly present in the lizards in that locality.

The bacterium was a motile rod which possessed the usual characteristics of the *Enterobacteriaceae.* It produced hydrogen sulfide, was methyl red positive and Voges-Proskauer negative, failed to form indol or to liquefy gelatin, and gave negative reactions in Stern's glycerol-fuchsin broth. The organism promptly produced acid from d-tartrate and mucate but did not attack l- or i-tartrate. Citrate was utilized as evidenced by growth on Simmons' medium but in fluid medium some of the cultures did not completely utilize the substance. Acid and gas were produced from glucose, arabinose, xylose, rhamnose, trehalose, maltose, sucrose, raffinose, sorbitol, dulcitol and mannitol within 24 hours. Cellobiose was fermented after 5 days. Lactose, inositol, and salicin were not attacked. The cultures possessed a well developed avidity for sucrose, after overnight incubation marked acidity and moderate gas formation were observed in sucrose broth. These characteristics indicate that the organism is an intermediate coliform bacterium which is not readily classifiable in any of the presently recognized genera of the *Enterobacteriaceae.* It was recognized by Hinshaw and McNeil that the cultures were agglutinated by O serum for group B *Salmonella* but no further observations concerning their antigenic characteristics were recorded. Upon examination it was found that the strains were agglutinated to the titre of group B serum and possessed antigens IV, XII_1, XII_2. Mirror absorption tests with serums prepared from *Salmonella abortus-equi* and from the lizard strains established the identity of the O antigens of the two types.

The first cultures received were poorly motile and continuous passage through semi-solid agar was necessary to maintain the cultures in a condition in which they were flocculated well by H serums. The reactions obtained with Pc 231 were typical of those of all the cultures studied and are recorded in Table I. It was found that prompt agglutination occurred in polyvalent *Salmonella* serum and in *Salmonella typhi* serum (d). In addition, the cultures were flocculated slowly and in low dilution in serum for phase 1 of *Salmonella reading* (e,h). Thus it seemed that the cultures possessed antigens d,e,h and that antigen d was the major component while

1. Hinshaw, W. R., and McNeil, E., *J. Bact.,* 1947, v53, 715.

TABLE I.
H Antigens of Pc231.

Serums	Antigens						
	S. typhi (d)	*S. reading* phase 1 (e,h)	*S. glostrup* phase 2 (e,n,z_{15})	Pc231 (d,e,h)	Pc231 (d)	Pc231 (e,h)	Pc231 (e,n,z_{15})
S. typhi (d)							
Unabsorbed	10000	0	0	5000	5000	0	0
Absorbed by							
Pc231 (d,e,h)	200			0	0		
Pc231 (d)	200			0	0		
S. reading (e,h)							
Unabsorbed	0	10000	500	500	0	10000	500
Absorbed by							
Pc231 (e,h)		0	0	0		0	0
S. glostrup (e,n,z_{15})							
Unabsorbed	0	500	10000	0	0	1000	10000
Absorbed by							
Pc231 (e,n,z_{15})		0	0			0	0
Pc231 (d,e,h)							
Unabsorbed	5000	200	0	5000	5000	200	0
Absorbed by							
Pc231 (d)	0	100		50	0	200	
S. typhi (d) +							
S. reading (e,h)	0	0		500	500	0	
S. oregon (d) +							
S. reading (e,h)	0	0		0	0	0	
Pc231 (e,h)							
Unabsorbed	0	5000	500	1000	0	10000	500
Absorbed by							
S. reading (e,h)		0	0	0		0	0
Pc231 (e,n,z_{15})							
Unabsorbed	0	1000	20000	0	0	2000	20000
Absorbed by							
S. glostrup (e,n,z_{15})		0	0			0	0
Pc231 (d)							
Unabsorbed	10000	0	0	20000	20000	0	0
Absorbed by							
S. typhi (d)	0			500	500		
S. oregon (d)	0			0	0		

antigens e,h were present in small amount. In order to determine whether this antigenic complex was separable under ordinary conditions of culture, the various strains were plated and numerous colonies examined. In all, more than 400 single colony cultures were studied. With one exception all the single colony isolations behaved as did the parent strains. One colony from Pc 219, the first culture studied, failed to flocculate in d serum but reacted strongly in e,h serum, as would a typical e,h phase. From these results it may be assumed that the lizard strains are monophasic, have the formula IV,XII : d,e,h in which the major H antigen is d and that rarely, under usual conditions of culture, variants occur which have the formula IV,XII : e,h and which appear to be stable.

To investigate more fully the variational potentialities of the cultures they were examined by a modification of the Gard technic devised by Edwards and Bruner(2). When the organisms were grown in semisolid agar with agglutinating serums, phases having antigens d; e,h; and e,n,z_{15} were isolated. The

2. Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta. Cir.* 54, 1942.

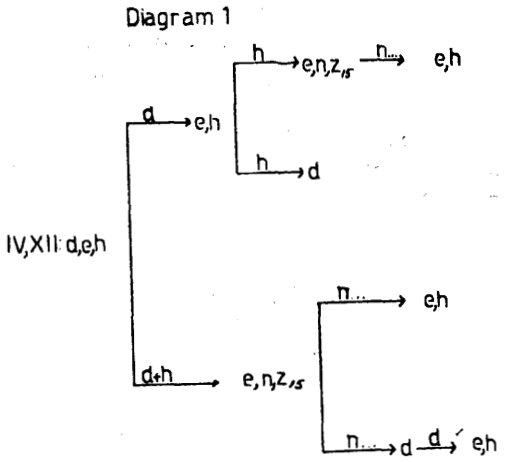

FIG. 1.

Symbols on arrows indicate serums in which cultures were grown.

d—*S. typhi* serum unabsorbed.

h—*S. reading* phase 1 serum absorbed by *S. abortus-equi*.

n . . .—*S. abortus-equi* serum absorbed by *S. reading*, phase 1.

variations observed in the cultures are summarized in Fig. 1. The results are a composite of those obtained with all the cultures but most of the changes shown were obtained with each strain. Pure d phases were obtained only from two cultures, but e,h and e,n,z_{15} phases were obtained from all. The experiments were done with carefully selected single colony cultures and each time antigenic change occurred the cultures were plated and single colonies selected for subsequent work.

When the original cultures (d,e,h) were placed in e,h serums, the organisms spread slowly through the medium and the antigenic characters of the spreading growth were unchanged. Apparently the e,h component was so slightly developed that the corresponding serum had no immobilizing effect. When the cultures were placed in d serum, they were immobilized. After one or 2 days incubation filmy bulbs appeared which spread through the medium and this spreading growth was composed of typical e,h phases. If the original cultures were placed in both d and e,h serums no evidence of antigenic change was seen until the cultures were transferred 2 or 3 times, when bulbs composed of e,n,z_{15} phases appeared. Subsequent changes were accomplished with even greater difficulty; often the organisms were carried through long series of transfers in serum mediums before evidence of change was noted.

The salient characters of the d,e,h; d; e,h; and e,n,z_{15} phases are given in Table I. For the sake of brevity only a few of the many absorption tests performed are included. It is evident that the d antigen of the lizard strains, like the d antigens of many *Salmonella* types, is not identical with the d of *S. typhi*. *Salmonella oregon* removed all d agglutinins from serums prepared from the d phase of the lizard strains but reciprocal tests not included in the table demonstrated that the lizard cultures did not have the entire d complex of *S. oregon*. An interesting observation was that the d antigen of the IV,XII : d,e,h cultures was identical with the d of a sucrose fermenting lizard culture described by Edwards, Moran and Bruner(3) which had antigens VI,VIII : d,i. The pure d phases were identical with the d antigen of the d,e,h phases, as shown by suitable absorption tests.

The e,h and e,n,z_{15} phases of the lizard strains were identical with phase 1 of *S. reading* and phase 2 of *Salmonella glostrup* respectively. So far as could be determined, the phases obtained by growth in serums were stable under usual conditions of culture. No changes were observed in them during a 2 year period and serums prepared from them contained no minor components for other antigens.

The cultures described here are similar in many ways to *Salmonella salinatis* (IV,XII : d,e,h-d,e,n,z_{15}) of Edwards and Bruner(4). They differ from the latter form in that they ferment sucrose and are monophasic. Further, it was never possible to demonstrate the natural occurrence of loss variants in *S. salinatis* nor could antigen d ever be recovered from e,h or e,n,z_{15} phases induced by growth in serum. Stable d phases were not found in *S. salinatis*. The question naturally occurs as to whether the lizard cultures should be

3. Edwards, P. R., Moran, A. B., and Bruner, D. W., *J. Bact.*, 1948, v60, 529.

4. Edwards, P. R., and Bruner, D. W., *J. Bact.*, 1942, v44, 289.

placed in the genus *Salmonella* or whether *S. salinatis* should be removed from the genus. Against the latter view is the fact that it easily is possible to induce *Salmonella sandiego* (IV,XII : e,h-e,n,z_{15}), a commonly occurring *Salmonella* type, from *S. salinatis*. Although they differ in biochemical properties, the IV,XII : e,h variants which appear naturally in the lizard strains are antigenically identical with the monophasic *Salmonella* forms described by Cherry, Barnes and Edwards(5). The classification of cultures such as those described here is an arbitrary matter and must be the subject of international agreement.

The isolation of three stable phases from one culture is most unusual and has not previously been reported. From these phases it was possible to prepare three excellent diagnostic serums for *Salmonella* typing. The fact that no monophasic *Salmonella* possessing antigens e,n,z_{15} has yet been found makes that form of the lizard strains quite useful and it has been used for routine production of typing serum.

Summary. Eight sucrose fermenting cultures isolated from lizards were found to have the antigenic formula IV,XII : d,e,h. From them it was possible to obtain forms having the formulas IV,XII : d; IV,XII : e,h; and IV,XII : e,n,z_{15}. The IV,XII : e,h form occurred spontaneously as a loss variant under the usual conditions of culture. The variants were quite stable and could be used for the production of diagnostic serums.

5. Cherry, W. B., Barnes, L. A., and Edwards, P. R., *J. Bact.*, 1946, v51, 235.

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