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Occurrence of H-antigen z_{66} of R phase in cultures of *Salmonella* serovar typhi originated from Indonesia

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SUMMARY

Eighteen strains of *Salmonella choleraesuis* subsp. *choleraesuis* serovar Typhi (*S. typhi*) isolated from blood of patients in Japan who had visited Indonesia and returned before the onset of typhoid fever were found to possess the H-antigen z_{66} reported by Guinée *et al.* (1981) but had the naturally occurring H-antigen d. They were not lysed by any of the phages of the Vi phage typing system. After passage through semi-solid medium containing H-z_{66} antiserum, H-antigens of 11 of 18 cultures with H-z_{66} phage changed to H-j and those of 2 others to H-d, while the remaining 5 were immobilized. With cultures in the H-d or H-j phages, change of the H-antigens did not occur when they were cultured in semi-solid medium containing homologous H-antiserum. These phase induction experiments as well as colony examination of original cultures suggest that H-z_{66} phase is unstable and tends to change to the H-j phase. It also suggest that the original H-z_{66} cultures in which change of H-antigen to H-d or H-j occurred without difficulty probably represented a mixed population of cells in the H-z_{66} and H-d or H-j phases. Since none of 856 isolates from Japan or from imported cases of typhoid fever from Southeast Asia other than Indonesia exhibited the H-z_{66} antigen, it was concluded that the focus of typhoid fever caused by *S. typhi* in H-z_{66} phase was probably in Indonesia.

INTRODUCTION

It is known that culturing salmonella in the presence of homologous H-antisera for a long period of incubation can result in profound antigenic changes which differ from those of natural phase variation. Such induced H-antigen changes were called R-phase by Kauffmann (1966) and as a whole it does not occur in natural isolates. *Salmonella typhi* is known as a strictly human pathogen which possesses the monophasic H-antigen d. In 1936, Kauffmann (1936) demonstrated an R-phase which was irreversible to naturally occurring H-phase d by culturing strains of *S. typhi* by prolonged incubation in semi-solid medium containing H-d antiserum; he designated it H-j. On the other hand, Guinée *et al.* (1981) reported that isolates of *S. typhi* from patients with typhoid fever in Indonesia possessed a new H-antigen which did not react with either H-d nor H-j antisera. They regarded this H-antigen as the second R-phase of *S. typhi* and designated H-z_{66}. The present study was carried out to investigate the occurrence of H-z_{66} phase in 874 cultures of *S. typhi* isolated in Japan.

MATERIALS AND METHODS

The 874 cultures studied were isolated from blood cultures from patients with typhoid fever in Japan during the period 1974–80. After preliminary identification and phage typing, they were maintained on slopes of Dorset egg medium with rubber stoppers at room temperature until examined in this study. The methods used for the biochemical and serological examination of the cultures were those described by Edwards & Ewing (1972). H-antisera for the two R-phases, H-j and H-z_{66},were produced by immunizing rabbits with formalin-killed antigens prepared from reference strains with these R-phases received from the WHO Collaborative Centre for Reference and Research on *Salmonella* (Institut Pasteur, Paris). The two anti-R-phase sera and H-d antiserum were absorbed reciprocally with cultures of the other two phases to avoid cross-reactions with heterologous antigens. H-antigens of each strains were prepared from actively motile cultures obtained by passage several times through a semi-solid medium. Changes in H-phases were carried out in the usual way by addition of the appropriate absorbed H-antisera to the semi-solid medium. Each time the phases changed, the cultures were plated out and well-isolated single colonies selected for examination. If change of the H-antigen did not occur after single colonies were cultured in semi-solid medium containing antisera, the organisms were subcultured serially in similar tubes over period of 3 months to confirm that the cultures were stable and that other antigens would not appear.

RESULTS AND DISCUSSION

The 874 cultures studied were biochemically typical of *S. typhi* possessing the O antigens 9 and 12 and the Vi antigen. They gave positive reactions in tests for H_2S (peptone-iron agar), lysine decarboxylase, d-tartrate (Kauffmann–Petersen broth), and fermentation of trehalose, mannitol, and sorbitol, but negative reactions for citrate utilization (Simmons agar), ornithine decarboxylase, gas production from glucose, and acid production from L-arabinose, rhamnose, inositol, and mucate. All but two of them were motile.

At first 803 motile cultures isolated in Japan during 1979–80 were examined and 9 of them were agglutinated by H-z_{66} antiserum; the remaining 794 cultures were agglutinated by H-d antiserum as usual. The nine cultures in H-z_{66} phase were isolated from patients who had visited Indonesia before the onset of typhoid fever. They were lysed by phages I and IV but not by any of the phages of the Vi phage-typing system. Then, an additional 69 cultures of *S. typhi*, all of which had been isolated from cases of typhoid fever originated in Southeast Asia during 1974–8 and which were untypable by the Vi phage typing system, were examined for their H antigens. It was found that 6 of the 69 cultures were agglutinated only by H-z_{66} antiserum and further 3 cultures were strongly by H-z_{66} and weakly by H-j antisera, while the remaining 60 strains reacted only with H-d antiserum.

Guinée *et al.* (1981) reported that all of their 11 cultures in naturally occurring R-phase were first agglutinated by H-z_{66} antiserum, and when they were inoculated onto Gard's plates containing H-z_{66} antiserum, 7 of them developed the H-j phase, while 4 reverted to the H-d phase. All the cultures were

Table 1. *Induced H-antigen variation in cultures of* Salmonella typhi *from cases returning from Indonesia*

No. of cultures	H-phase	Changed H-phase after passage through medium with homologous antiserum			
		z_{66}	j	d	Immobilized
15	z_{66}	—	8	2	5
3	z_{66}, (j)	—	3	—	—
11	j	—	—	—	11
8	d	—	—	—	8

immobilized on Gard's plates containing H-z_{66} antiserum in combination with either H-j or H-d antiserum. Their findings were confirmed in the present study: a single colony of each of the 18 cultures agglutinated by H-z_{66} antiserum was cultured into semi-solid medium containing H-z_{66} antiserum. As shown Table 1, the H-antigens of 11 of the 18 cultures changed to H-j and 2 to H-d within 48 h of incubation, while the remaining 5 strains were immobilized. When H-j phase cultures – including all those that changed from H-z_{66} phase and 3 which were originally in the H-j phase – were cultured in semi-solid medium that contained H-j antiserum, all of them were immobilized and remained non-motile even after 3 months. These results suggest that the H-z_{66} phase is relatively unstable and tends to change to H-j whereas the H-j phase is stable and irreversible as reported by Kauffmann (1936). Moreover, when 8 H-d phase cultures including 3 from the original H-z_{66} strains were cultured in semi-solid medium containing H-d antiserum, all of them remained non-motile in the medium for 3 months, thus confirming the stability of the naturally occurring phase H-d. Four of the original H-z_{66} isolates, two of which changed to H-d and two to H-j phase, were cultured on soft nutrient agar plates. More than 400 colonies from each of these cultures were examined and colonies with H-z_{66} and either H-d or H-j antigens were found. From these results, it seems probable that the original H-z_{66} cultures from which H-d or H-j phases dissociated without difficulty represented a mixed population of H-z_{66} phase cells as well as H-d or H-j phase cells.

Cases of typhoid fever infected abroad were first recognized in Japan in 1974, and a total of 195 isolates of *S. typhi* were obtained from these cases during 1974–80. H-antigen analysis revealed that 18 of these cultures were in the H-z_{66} or H-$z_{66,(j)}$ phases, and these unusual isolates first occurred in 1976. Guinée *et al.* (1981) reported that all of their cultures in phase H-z_{66} were received from Indonesia during 1979–80. It is therefore interesting to note that all 18 cultures in R-phase reported in the present paper were also isolated from patients who had visited Indonesia. The results suggest that the focus of typhoid fever caused by this unusual form of *S. typhi* is probably in Indonesia.

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