

of the PABA treated gerbilles with their respective homologous strains of *R. orientalis*, cross-immunity tests were performed with heterologous strains. Inocula were prepared and administered in the same manner as described for the immunity tests. Simultaneous titrations of the infectious material were made in normal gerbilles with dilutions of 10^{-1} through 10^{-8} . The test period of 21 days remained the same.

Of the 24 gerbilles immune to the Ceylon strain, 9 were tested with the Karp strain, 8 with the Calcutta, and 7 with the Imphal strain. One death occurred in the group challenged with the Calcutta strain, 15 days after the inoculation. This gerbille showed evidence of marked weight loss, but the autopsy was otherwise negative. No rickettsiae were seen in peritoneal smears.

Three gerbilles immune to the Calcutta strain were challenged with Karp strain inoculum and survived.

The 30 Karp-immune gerbilles were divided into 3 groups: 9 were inoculated with the Ceylon strain, 10 with the Calcutta strain, and 11 with Imphal strain infectious material. There were no deaths among these gerbilles.

The 9 gerbilles immune to the Imphal strain were divided into 3 groups of 3 which

were tested with the Ceylon, Calcutta, and Karp strains, respectively. All of the gerbilles survived.

The gerbilles which were tested with the Karp strain received 316,000 lethal doses; those tested with the Calcutta strain received 31,600 lethal doses; while those receiving Ceylon and Imphal infectious material survived 2,140 and 3,160 lethal doses, respectively. Thus, it is apparent that a solid immunity against heterologous strains of *R. orientalis* was present in these gerbilles. It is possible, of course, "interference phenomenon" may have exerted an effect of undetermined degree in these tests.

Summary. Gerbilles which were infected with tsutsugamushi disease and survived through treatment with PABA were tested 6 to 9 months later and found to be immune to homologous strains of *R. orientalis*. This is interpreted as evidence that the mode of action of para-aminobenzoic acid in scrub typhus is rickettsiostatic.

Cross-immunity tests revealed that these gerbilles were also immune to heterologous strains of *R. orientalis*.

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Natural Occurrence of an "Induced" Antigen in Salmonella Cultures.*

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It is known¹⁻⁶ that H antigens of *Salmonella* can be markedly changed by cultivating the organisms in serum containing agglutinins for the flagellar components. Excepting *S. abortus-equi*⁴ and *S. paratyphi A*,⁵ all antigens

so obtained have had little or no relationship to naturally occurring H antigens of the genus, and have been referred to as induced

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¹ Kauffmann, F., *Z. Hyg.*, 1936, **119**, 104.

² Gnosspelius, A., *Z. Hyg.*, 1939, **121**, 529.

³ Kauffmann, F., and Tesdal, M., *Z. Hyg.*, 1937, **120**, 168.

⁴ Edwards, P. R., and Bruner, D. W., *J. Bact.*, 1939, **38**, 63.

⁵ Bruner, D. W., and Edwards, P. R., *J. Bact.*, 1941, **42**, 467.

⁶ Edwards, P. R., and Bruner, D. W., *J. Bact.*, 1942, **44**, 289.

or artificial forms. Whether these are minor components of the flagellar complex brought into prominence, or artifacts produced by action of the serum, has been the subject of some discussion. Hitherto, such forms have not been recognized in nature, although Eriksson and Malmström⁷ found agglutinins for an induced phase of *S. newport* in the serum of a patient infected with that type. The present note records the occurrence in nature of antigens apparently identical with those obtained by cultivation of *Salmonella* in agglutinating serum.

Since April, 1945, 14 cultures received from Connecticut, Maryland, Florida, Illinois, California and Uruguay, were apparently monophasic variants of *S. minnesota*, which had the formula XXI, XXVI:b. Phase 2 (e,n,x,...) could not be demonstrated. When stabbed into semi-solid agar containing b serum, the cultures were either confined to the site of inoculation or produced one or 2 small bulbs of spreading growth which yielded a form flocculated no longer by serums for the known H antigens of the genus but by serums for certain induced antigens. By continued transfer in b serum, such forms were obtained from 13 of the cultures. While this work was in progress, 2 cultures isolated from sewage by Mr. A. A. Hajna were received. These cultures also had O antigens XXI, XXVI and gave H reactions similar to those cited above. Serums were prepared from one of the induced forms of the XXI, XXVI:b cultures and one of the sewage cultures. Reciprocal agglutination and absorption tests indicated that the forms obtained by growth in b serum were identical with the cultures from sewage. A symbol (z_{33}) was assigned the H antigens of these forms. By cultivation of the z_{33} form of the XXI, XXVI:b cultures in z_{33} serum, e,n,x,... phases were obtained from 5 of them. One reverted to b. The 2 sewage cultures (XXI, XXVI: z_{33}) were cultivated in z_{33} serum and an e,n,x,... phase was obtained from one. The z_{33} and e,n,x,... forms are quite resistant to change. As yet a change from b directly to e,n,x,... has not been accomplished. The

changes brought about in the 2 naturally occurring forms may be summarized as follows:

XXI, XXVI:b $\rightleftharpoons$ XXI, XXVI: z_{33} $\rightarrow$ XXI, XXVI:e,n,x...

XXI, XXVI: z_{33} $\rightarrow$ XXI, XXVI:e,n,x...

Since so little is known concerning phylogeny and evolution in the *Salmonella*, it is difficult to interpret these results. A complex parent containing b, e,n,x... and z_{33} could be postulated for *S. minnesota* (XXI, XXVI:b-e,n,x...) and the XXI, XXVI:b and XXI, XXVI: z_{33} forms. On the contrary it is possible that the XXI, XXVI:b cultures are loss variants of *S. minnesota* which give rise to XXI, XXVI: z_{33} forms under certain environments. The fact that in the progression b $\rightarrow$ z_{33} $\rightarrow$ e,n,x... only z_{33} gave rise to the e,n,x... phase which would normally be expected in these cultures, indicates that z_{33} is not an artifact or denatured antigen, but a minor constituent brought into prominence. Other antigens hitherto observed only in cultures exposed to agglutinating serum probably also occur in nature. These observations agree with those of Monteverde and Leiguarda⁸ and of Edwards⁹ that *S. ballerup* (XXIX[Vi]: z_{14}) can be changed to a form resembling *S. hormaechei* (XXIX[Vi]: z_{30}, z_{31}). They are further supported by unpublished results of the writers, who succeeded in changing all of 9 cultures of *S. simsbury* (I, III, XIX: z_{27}) to forms serologically indistinguishable from *S. senftenberg* (I, III, XIX:g,s,t...). It is not yet possible to say whether the transformability of these apparently distinct types means that serologically divergent strains of *Salmonella* may arise by processes analogous to induced variation or whether these apparently distinct types are merely loss variants of complex parents. In the transformation of *S. salinatis* (IV, XII: d,e,h-d,e,n, z_{15}) to *S. sandiego* (IV, XII: e,h-e,n, z_{15})⁶ and in the appearance of XXIX[Vi]: z_{30} and XXIX[Vi]: z_{31} variants of *S. hormaechei*,⁹ only loss variation seems operative. In any event, a re-examination of some of the apparently distinct *Salmonella* types is indicated.

⁷ Eriksson, E., and Malmström, F., *Z. Hyg.*, 1939, **121**, 629.

⁸ Monteverde, J. J., and Leiguarda, R. H., *Nature*, 1944, **153**, 589.

⁹ Edwards, P. R., *J. Bact.*, in press.