

carried on to determine the changes which occur in muscles of other parts of the body, of the effects produced by temperatures exceeding 101°F, and the changes which occur later than 5 hours after the reaction. It is also important to determine whether similar alterations develop in fevers of other types.

Since the calf muscle temperature was always several degrees lower than the systemic temperature and since it actually decreased during the production of fever, it appears that the increased body temperature under the conditions described cannot be attributed to any activity in the calf muscles. This was true in spite of evidence of markedly increased muscular activity during the chill period.

Since the circulation of the skin is improved during the reaction following the intravenous injection of typhoid vaccine, such injections may be beneficial in the treatment of ulcers. However, since the calf muscle circulation is not increased, the use of this method of treatment for relief of intermittent claudication appears to have no physiological basis.

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### Reversibility of the *Alpha* and *Beta* Phases of *Salmonella typhi*.\*

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By the cultivation of *Salmonella typhi* in broth containing anti-serum derived from the specific phase of *Salmonella muenchen*, Kauffmann<sup>1</sup> was able to isolate a variant which possessed altered flagellar antigens and which was no longer agglutinable in *S. muenchen* antiserum. The variant was obtained from only one culture and was not reversible. It was designated as a *beta* phase of the theretofore supposedly monophasic typhoid bacillus. Through cultivation in immune serums, *beta* phases were also obtained by Kauffmann and Tesdal<sup>2</sup> from *Salmonella schleissheim* and by Gard<sup>3</sup> from *Salmonella abortus-canis*. None of these induced phases were reverted to the

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<sup>1</sup> Kauffmann, F., *Z. f. Hyg.*, 1936, **119**, 103.

<sup>2</sup> Kauffmann, F., and Tesdal, M., *Z. f. Hyg.*, 1937, **120**, 168.

<sup>3</sup> Gard, S., *Z. f. Hyg.*, 1938, **121**, 139.

naturally occurring phases of the bacilli and it is questionable whether they represented phase-variation as it is observed in naturally diphasic species, or whether they were mutants produced by exposure to antisera.

It was found by Bruner and Edwards<sup>4</sup> that the technic of Wassén<sup>5</sup> was very effective in the isolation of suppressed specific phases in the so-called monophasic nonspecific *Salmonella* types. Therefore 7 cultures of *S. typhi* in our possession were examined by this method. The organisms were cultivated in agglutination-tubes containing semi-solid agar to which had been added sufficient *S. muenchen* antiserum to confine the growth of the normal, or *alpha*, phase of the bacilli to the line of inoculation. The medium was inoculated by stabbing at one side of the tube. Outgrowths from this line represented variation in the flagellar antigens of the bacilli. By this method *beta* phases similar to the one described by Kauffmann<sup>1</sup> were obtained from all the cultures on the first trial. Subsequently, the experiment was twice repeated with the same results.

The variants were transferred several times in semi-solid agar containing *S. muenchen* antiserum to free them of the *alpha* phase. They were then examined by agglutination and agglutinin-absorption to insure their purity and identity, after which they were placed in semi-solid agar containing serum derived from a *beta* phase of the typhoid bacillus in an effort to revert them. Two *beta* phases derived from each of the 7 cultures at different times were tested for reversion. The only reversion noted was in one *beta* phase of the Watson strain. Under the influence of suitable antisera this culture was highly variable and could be transformed from one phase to the other at will. No reversion was noted in the absence of antiserum, indicating that the variations observed were not due to impurity of the *beta* phase.

To further establish the reversibility of the *beta* phase of the Watson strain, it was plated in semi-solid agar and well isolated colonies fished to agar-slants and to semi-solid agar containing antiserum derived from the *beta* phase of *S. typhi*. Of 65 colonies thus examined, 2 yielded the *alpha* phase when placed in contact with *S. typhi beta* serum. The agar-slant cultures inoculated from the same colonies were pure *beta* phases. It is quite evident, therefore, that certain *beta* phases derived from *S. typhi* are reversible when placed in contact with the corresponding antiserum. The phases thus reverted are typical *alpha* phases and differ in no way from the parent strain from which they were originally derived.

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<sup>4</sup> Bruner, D. W., and Edwards, P. R., *J. Bact.*, 1939, **37**, 365.

<sup>5</sup> Wassén, A., *Bull. mensuel de l'Office Internat. d'Hyg. publ.*, 1935, **27**, 1.