A NEW BACTERIAL VARIANT: THE NON-MOTILE H FORM

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In a young culture of flagellated bacteria grown under optimal conditions one expects that the flagella function spontaneously as motor organs, and that the degree of motility of the bacteria corresponds to the development of their flagellar apparatus, i.e. their H antigen. For example, Bridges & Taylor (1944) make the following recommendation: 'In testing for H agglutination...it is essential to ensure that the organism is motile, or in other words is provided with flagella....' From the absence of active motility under optimal conditions it is inferred that a flagellar apparatus does not exist, or that, at best, it is only poorly developed and that the strain in question is practically inagglutinable by H serum.

The present communication deals with a Salmonella strain the properties of which demand a revision of our knowledge of flagellar activity.

ISOLATION AND PROPERTIES OF THE STRAIN

In April 1945 a strain of *Salmonella paratyphi* B was isolated from the faeces of an old woman suffering from chronic constipation. On the original Endo agar plate the strain was present in numerous colonies, partly in the specific and partly in the group phase. Examined on the slide with the corresponding H-agglutinating sera (International Salmonella Centre, Copenhagen) the colonies, straight from the plate, were strongly and typically agglutinated. However, the growth of subcultures on freshly made meat-infusion agar slopes looked like that of O variants: there was no tendency towards a confluent growth, nor had the 'water of condensation' become whitish turbid, luxuriously grown with bacteria, as is so characteristic of fully flagellated strains. In fact, the microscopic examination of the slightly turbid water of condensation showed no active motility of the bacilli in the hanging drop.

It appeared remarkable that the colonies from the Endo agar plate had reacted so satisfactorily with the H sera of their corresponding phase, and that the subcultures should have lost the H antigen when grown on agar slants which are known to be much more favourable to its development. Indeed, this was not the case: both on the slide and in the tube these non-motile cultures were excellently H agglutinable. The flocculation was of the soft and voluminous type, characteristic of H agglutination; it occurred as speedily as that of other motile strains of *Salm. paratyphi* B; it was of equal intensity and titre, and corresponded to the phases of the original colonies.

Further colonies were picked off from the original plate and were tested for their motility and H agglutinability. Again, the same paradoxical phenomenon was observed, with the exception of one deviating strain which failed to react with any of the Salmonella H sera, although by its O antigen it also belonged to the B group. As this strain was non-motile, it could be either an O form or a strain with unknown H antigens, likewise paralysed in its motility.

In September 1945, another specimen of faeces of the same woman was examined. Eleven colonies of the Salmonella B group were picked off from the direct plating on Endo agar. Nine of these colonies were in phase 1 or 2 of the H antigen of *Salm. paratyphi* B, while two colonies failed to produce any H agglutination on the slide. On subcultures on agar slopes, seven of the nine H-agglutinable strains showed confluent growth, whitish turbid water of condensation and maximum motility. The remaining two H-agglutinable strains as well as the two H-inagglutinable strains grew like O variants and were completely non-motile. Test-tube agglutination of the seven motile and the two non-motile H-agglutinable strains failed to reveal any difference in the quality and intensity of their H agglutination.

On this occasion, therefore, it had been possible to isolate also normal motile strains and thus to obtain from the same specimen the following three variants:

(i) H-agglutinable, motile strains (normal H forms).

(ii) H-agglutinable, non-motile strains ('non-motile H forms').

(iii) H-inagglutinable, non-motile strains (later confirmed as apparent O variants).

A large number of colonies of these three groups grew from the enrichment medium (tetrathionate broth).

https://doi.org/10.1017/S002217240001411X Published online by Cambridge University Press

(i) Once a colony was picked off from a subculture of the first group and proved to be a motile H form it remained constant.

(ii) Cultures of the second group did not give off deviating forms: the non-motile H forms both from the first and the second isolation remained constant in their paradoxical behaviour of optimal development of H antigen in absence of all active motility. It is important to emphasize that the absence of motility did not in any way affect the phasic variation of the H antigen which took place to the same extent as in motile strains.

(iii) The third group, representing apparent O forms as defined by Felix (1924), repeatedly gave rise to normal H forms on further subculture on agar slants.

Staining of the flagella according to the method of Zettnow proved the non-motile H forms to be as richly flagellated as the normal H forms of *Salm. paratyphi* B that were isolated from the faeces of this or other patients. On the other hand, the non-motile and H-inagglutinable strains proved by the same staining method to be non-flagellated. The latter strains, when examined in a hanging drop preparation from the water of condensation, showed the bacilli to be arranged in bundles, a feature characteristic of true O forms, whereas the non-motile H forms did not show any regular arrangement of the bacilli.

All attempts to eliminate the 'paralysis' of the flagella were unsuccessful. The non-motile H forms remained non-motile when grown at 22° C., on liquid or semi-solid media, in ordinary meat-infusion broth with various peptones as well as in trypsin-digest broth or Lab Lemco media, and also in young cultures after incubation for 6–8 hr. Accordingly no swarming on 'swarm agar' could be achieved. Controls with motile strains of *Salm. paratyphi* B showed optimal motility on all these media.

It was observed early in the investigation that the growth of the non-motile H forms appeared to be tough and could not be removed with a loop from agar cultures as readily as this is accomplished with cultures of normal H forms or of the O forms. Washed off from agar slopes with physiological saline solution the growth came off in whole pieces. There was reason to believe that it would be difficult to obtain a homogeneous suspension. However, after thorough mixing by means of a pipette a homogeneous distribution of the bacilli was achieved. The suspensions remained quite stable even after 10 min. boiling in the water bath, and spontaneous agglutination did not take place in 6·8 % saline solution. The colonies on solid media could not be distinguished by their appearance from those of other strains. The growth in broth was homogeneous and did not show any granular deposit. Slimy growth was not observed. All the strains belonging to the three different variants produced a mucoid wall. None of these fermented *d*-tartrate.

The non-motile H forms of the first isolation have so far remained invariably non-motile, with full H agglutinability, for more than 15 months, those of the second isolation for more than 10 months.

In December 1945 and in May 1946 specimens of faeces of the patient were examined for the third and fourth time. Colonies of the three groups were obtained in large numbers, and the characteristics described above, in particular those of the second group, were confirmed anew.

In the patient's serum agglutinins were found only against the second (group) phase of the H antigen of *Salm. paratyphi* B in a titre of 1:1000.

DISCUSSION

The phenomenon described above is to be strictly distinguished from the absence of active motility resulting from poor development or from degeneration of the flagella themselves. The incapability of the non-motile H form to move actively is contrasted by an abundance of flagella which can be proved both microscopically and serologically. According to A. Fischer (1894), flagella may be fully developed and at the same time artificially immobilized on a medium containing 3–4 % ammonia. In contrast with this temporary paralysis, the non-motile H form has completely lost the motor function of the flagella, these being hereditarily paralysed and remaining so in all subcultures even under the most favourable external conditions. One might presume that this phenomenon was related to the tough appearance of the cultures which—in spite of their having preserved stability in saline—appeared to be in a state of partial roughness.

Kauffmann (1935), referring to the stability in saline of some rough forms of *Salm. paratyphi* B and *Salm. typhi*, suggested that this might be due to the presence of the Vi antigen. Variants of this kind are well known, e.g. the strain 'Ty. 6. S.' of Felix & Petrie (1938) and the strain 'Vi I' described by Bhatnagar, Speechly & Singh (1938). It may therefore be assumed that in the course of a partial degeneration of the somatic antigens the genetic factor responsible for the function or the development of the motor centre of the flagella has been affected.

The existence of such a centre in bacteria is still hypothetical. It might be conceivable that a comparative study of motile and non-motile H forms by more advanced staining and microscopic methods will throw light on this centre and its location.

Non-motile H forms might be encountered more frequently if search for them were made in a less

orthodox manner. For instance, Kauffmann (1939) found that the specific phase of *Salm. aberdeen*, which he employed to produce his *i*-serum, was non-motile, though strongly agglutinating with the *i*-serum. Kauffmann's comment was: 'Thus Salmonella cultures with well-developed H antigen need not always be motile.' This observation, however, was not mentioned at all when Kauffmann's (1939) paper was later incorporated in his book, *The Bacteriology of the Salmonella Group* (1941).

In view of the constancy with which the strain described above has retained its properties, there can be no doubt that this phenomenon constitutes a new form of bacterial loss-variation, that deserves attention both from a theoretical and from a practical-diagnostic point of view.

SUMMARY

From the faeces of a carrier a strain of *Salm. paratyphi* B was grown repeatedly which—even under conditions most favourable for bacterial motility—was completely non-motile despite its being richly flagellated and optimally H agglutinable. The strain has in all subcultures, so far, for more than 1 year retained its peculiarity, viz. absence of the motor function of the flagella which morphologically and serologically appears to be normally developed.

This new bacterial variant, the non-motile H form, is considered to be the result of a loss variation which—possibly related to a somatic degradation of the bacilli—affects the genetic factor responsible for the function or the development of the centre for the motility of the flagella.

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(*MS. received for publication* 16. XII. 46.—Ed.)