

A REPORT ON TWO PERMANENTLY NON-MOTILE SALMONELLA VARIANTS

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VARYING degrees of flagellar inagglutinability are frequently encountered in recently isolated Salmonella strains. Motility is usually rapidly regained following one or more subcultures in nutrient broth, especially when culture is performed at room temperature. When this procedure fails to restore *H*-agglutinability, selection of actively motile forms by passage through semi-solid (0.3 p.c.) agar is nearly always successful. Such strains showing temporary loss of motility are, as a rule, actively *H*-antigenic when injected into rabbits. Artificially induced non-motile variants of normally motile bacteria, resulting from such procedures as growing the strain on phenol-agar, tend to revert rapidly to the motile form and, although non-motile and inagglutinable by homologous *H*-antisera, usually possess the power of absorbing *H*-agglutinin and, in particular, of stimulating the production of *H*-antibody. Occasionally in Nature there occur and, in the laboratory there arise, true and apparently permanent non-motile variants of members of the Salmonella and other groups which, in their serological behaviour, simulate cultures in which the labile *H* component has been completely destroyed by physical or chemical means. Such variants have played an important part in elucidating the problems of *H* and *O*-type agglutination (Smith and Reagh, 1903; Beyer and Reagh, 1904) and of the role played by *H* and *O*-antigens and their respective antibodies in the causation of an immunity to disease (Schütze, 1930). They have also demonstrated their practical value in the preparation of *O*-antisera and of agglutinable suspensions for use in the qualitative Widal reaction; non-motile variants such as *Bact. typhosum* strain 9010, *Bact. typhimurium* strain Glasgow (Schütze) and *Bact. typhosum* strain Vi(1) (Bhatnagar, Speechly and Singh, 1938) now being used almost universally for this purpose. The isolation of true *O*-variants of Salmonellas from disease in man is rare and the complete absence of flagella may give rise to difficulties in bacteriological diagnosis.

The object of this paper is to record the isolation from human infection of permanently non-motile variants of *Bact. paratyphosum* C and *Bact. paratyphosum* A.

Bact. paratyphosum C, strain No. 628/43.

This strain was isolated from the blood of an adult Indian male and was sent to this laboratory for identification by Major M. T. Parker, R.A.M.C., to whom we are indebted for the following clinical description of the case:

5.12.43 : Admitted to hospital complaining of headache, fever and backache of four days' duration.

On Examination.—Temperature not raised ; pulse 70 ; respirations 20 ; spleen one finger enlarged and hard ; no other physical signs. In spite of the absence of fever, a blood film was said to have shown malaria parasites on the day of admission, although antimalarial treatment was not commenced until 7.12.43.

7.12.43 : Temperature rose to 102.2° F., falling to normal again the next day.

9.12.43 : Continuous fever developed, the temperature rising initially to 105° F., and lasted until 16.12.43, falling by lysis. During this time the patient's condition remained good and the pulse did not rise above 110. It does not appear that there was any intestinal disturbance, marked toxæmia or septic complications.

13.12.43 : *W.B.C.* 1,800 per c.mm. Blood culture in sodium taurocholate broth—the organism in question isolated.

Widal and Weil-Felix Reactions.

			<i>Titres v.</i>				
			<i>TO</i>	<i>AO</i>	<i>OX₁₉</i>	<i>OX₂</i>	<i>OX_K</i>
13.12.43	1/40	1/20	1/50	Nil	1/50
19.12.43	1/80	Nil	Nil	Nil	1/25
24.12.43	1/160	Nil	1/25	Nil	1/50

31.12.43 : Stool and urine culture negative.

20.1.44 : Discharged from hospital.

His illness was fairly trivial and, unfortunately, he was not exhaustively investigated. His serum was not tested for the presence of agglutinins against *Bact. paratyphosum CO* or *Bact. typhosum Vi* antigen.

Lieut.-Colonel Shone, I.A.M.C., in charge of the case, remarked : "... had not the man's blood culture been positive, his illness would probably have been called by one of the stock names reserved, according to fashion or ignorance, for such ailments."

Bacteriological Examination.—Strain No. 628/43 was found to possess the following properties :

Morphology : A Gram-negative, non-sporing, non-motile bacillus ; indistinguishable, in stained preparations, from other members of the genus *Bacterium*.

Cultural Characteristics : Colonies on nutrient agar were indistinguishable from those of other *Salmonellas*.

Biochemical Characteristics : Glucose, maltose, mannitol, dulcitol, arabinose, mannose and sorbitol were fermented with the production of acid and gas. Lactose, saccharose, rhamnose, raffinose, inulin and inositol were not fermented.

H₂S was detected by stab culture in lead acetate agar.

Indol was not produced.

Serological Reactions.—*Somatic Structure* : (1) Strain No. 628/43 was agglutinated to titre by an *O*-antiserum prepared against a stock strain of *Bact. paratyphosum C* but not at all by *Salmonella O*-subgroup A, B and D antisera.

(2) An antiserum prepared by the immunization of a rabbit with strain No. 628/43 agglutinated an alcoholized suspension of the stock strain of *Bact. paratyphosum C* to its homologous titre. Strain No. 628/43 was agglutinated in granules only by this serum, no floccule formation being observed.

(3) Absorption experiments showed that strain No. 628/43 was capable of removing all *O*-agglutinin from *Bact. paratyphosum O*-antiserum. Similarly,

Bact. paratyphosum C exhausted all agglutinin from strain No. 628/43 antiserum.

The somatic structure of strain No. 628/43 is, therefore, identical with that of *Bact. paratyphosum* C and contains the antigens VI, VII.

Presence of Vi antigen in the strain.—The serum of a rabbit immunized with strain No. 628/43 developed a titre of 1/5,000 against *Bact. paratyphosum* CO and of 1/80 against *Bact. typhosum* strain Vi(1). Prior to injection the rabbit's serum did not agglutinate suspensions of either organism in dilutions of 1/5 or over. Results of simple agglutination and absorption tests between strain No. 628/43 and *Bact. typhosum* strain Vi(1) and their homologous and heterologous antisera are given in the table.

TABLE.

Serum	Absorbing suspension	*Titre v. suspension of $\frac{1}{2}$			
		Strain 628/43		Vi (1)	
		Untreated	Boiled 1 min.	Untreated	Boiled 1 min.
†Vi (pure) ..	Nil	160	0	160	0
Vi (pure) ..	Vi (1)	0	—	10	—
Vi (pure) ..	628/43	5	—	10	—
628/43 ..	Nil	5,000	5,000	80	5

*Titre expressed as the reciprocal of the highest dilution of serum showing agglutination after two hours at 37° C. and 18 to 24 hours at room temperature.

†Produced by immunization of a rabbit with strain Vi (1), followed by complete absorption with *Bact. typhosum* strain 901H.

These findings, which will be discussed later, show that strain 628/43 shortly after isolation possessed at least a considerable part of the Vi antigen of *Bact. typhosum*. Soon after these investigations were carried out, however, Vi antigen became no longer detectable in suspensions of twenty-four-hour agar cultures. Thirty months later the strain was retested. Suspensions of overnight agar cultures proved completely inagglutinable by pure Vi antiserum both on a slide and in tubes and, in a concentration of 60×10^9 organisms per ml., failed to remove significant agglutinin from an equal volume of this serum. Logarithmic phase cultures, however, were agglutinated on a slide by an equal volume of pure Vi serum (titre = 1/160) but were not clumped by a 1/5 dilution when tested in tubes (see Hayes and Freeman, 1945).

Evidence of the Complete Absence of Flagella from the Strain.—Formolized broth cultures of strain 628/43 had shown no trace of H-type agglutination with either *Bact. paratyphosum* CH (specific) or *Bact. cholerae-suis* var. kunzendorf H antisera. The latter organism, now known as monophasic *Bact. cholerae-suis*, is identical with the flagellar group phase of *Bact. paratyphosum* C save for the absence from it of Vi antigen. An attempt was therefore made to produce a motile variant. The strain was passed through thirty subcultures in broth at 37° C. and each day the previous day's culture was examined for motility and seeded to one limb of a "U"-tube containing semi-solid (0.3 per cent) agar. The same procedure was repeated at room temperature. No motility was observed and the strain consistently failed to pass through semi-solid agar. After about twelve subcultures in broth a formolized broth culture was prepared and used to immunize a rabbit.

Rabbit No. 1 died after a single intravenous injection of 100 million organisms. Rabbit No. 2 received 100, 100, 200, 400, 400, and 400 million organisms in one ml. volumes at intervals of five days. Five days after the last injection the animal was bled. This serum agglutinated a formalized broth culture of strain No. 628/43 and a suspension of *Bact. paratyphosum* CO in granules to a titre of 1/5,000. No *H*-type agglutination was observed with the homologous culture. It failed, in a dilution of 1/25 or over, to agglutinate *H*-suspensions of any of the following organisms: *Bact. thompson* (k); *Bact. virchow* (r); *Bact. potsdam* (lv); *Bact. bareilly* (y); *Bact. enteritidis* (gm); *Bact. paratyphosum* A (a); *Bact. typhosum* (d); *Bact. paratyphosum* C (c); *Bact. newport* (eh); *Bact. abortus equi* (enx); *Bact. typhimurium* (i); *Bact. typhimurium* var. binns (123); *Bact. cholerae-suis* var. kuzendorf (5). These organisms cover between them the entire range of *H* antigens found in the *Salmonella O*-subgroups C₁ and C₂ (Bornstein, 1943).

Finally, antisera against *Bact. paratyphosum* CH (specific) and *Bact. cholerae-suis* var. kuzendorf *H* were heavily absorbed with suspensions of strain No. 628/43 without reducing at all their homologous titres.

Bact. paratyphosum A, strain 113/44.

This strain was isolated from the blood of a British sergeant and was sent to this laboratory for confirmation of identity by Captain J. Nicholas, I.A.M.C., to whom we are indebted for the following clinical account of the case:

The patient reported sick in February, 1944, and was admitted to hospital. He gave a history of an evening rise of temperature for four days prior to admission. He was also suffering from gonorrhoea and was under treatment with M & B 693. While in hospital he ran a continuous temperature, varying from 100 to 100.8° F., for fourteen days. Slight jaundice appeared about the thirteenth day but this gradually cleared. The patient had no complaints except of an occasional headache. He was discharged from hospital on 19.4.44.

Stool and urine culture negative.

Bacteriological Examination.—Strain No. 113/44 was found to possess the following properties:

Morphology: A Gram-negative, non-sporing, non-motile bacillus; indistinguishable, in stained preparations, from other members of the genus *Bacterium*.

Cultural Characteristics: Colonies on nutrient agar were slightly smaller than but otherwise indistinguishable from those of other *Salmonellas*.

Biochemical Characteristics: Glucose, mannitol, dulcitol, sorbitol and mannose were fermented with the production of acid and gas. Maltose, arabinose and rhamnose were fermented with the production of acid only. Lactose, saccharose, inulin, inositol and raffinose were not fermented.

Indol was not produced.

Serological Reactions.—*Somatic Structure:* Cross absorption tests demonstrated the identity of the somatic antigen of strain No. 113/44 with that of *Bact. paratyphosum* A. The strain therefore contains the somatic antigens I, II, XII₁, XII₃. Recent experiments have failed to demonstrate somatic phase variation in factor I of the strain, this antigen being highly agglutinable

by a *Bact. seftenberg* (I, III, XIX) antiserum in suspensions prepared from every colony picked (Kauffmann, 1941).

Evidence of the complete absence of flagella from the strain.—This strain was investigated along similar lines to strain No. 628/43. Motility could not be induced by repeated subculture in broth both at room temperature and at 37° C., nor by attempted passage through semi-solid agar. Antisera produced against a formolized broth culture of the strain agglutinated the immunizing suspension and suspensions of *Bact. paratyphosum* AH in granules only. The organism was completely inagglutinable by *Bact. paratyphosum* AH antiserum and failed to absorb any agglutinin from it.

DISCUSSION.

The strain of *Bact. paratyphosum* C and of *Bact. paratyphosum* A described above fulfil all the criteria necessary for their designation as true non-motile variants of the type strain.

(1) They are completely non-motile, have failed to show any evidence of motility after repeated subculture in broth and attempted passage through semi-solid agar.

(2) They have shown no trace of floccular *H*-type agglutination when mixed either with homologous antiserum or antisera against the flagellar antigens of motile strains of homologous type.

(3) They have failed to absorb *H*-agglutinin from antisera against motile strains of homologous type.

(4) On injection into a rabbit they fail to produce any *H* antibody either against themselves or against the flagella of any type within their homologous *O*-subgroups.

The diagnosis of *Bact. paratyphosum* strain No. 113/44 presented no difficulty since the somatic antigen of this type is unique in possessing the antigen II. Such, however, was not the case with *Bact. paratyphosum* C strain No. 628/43 since fifteen other *Salmonella* types belong to the *O*-subgroup C₁ and share with it a common somatic antigen. The identification of the strain depended upon the demonstration in it of the Vi antigen of *Bact. typhosum*. The only other *Salmonella* strains described as possessing this antigen are *Bact. ballerup* whose somatic complex has nothing in common with that of *Bact. paratyphosum* C, and *Bact. newport* whose *O* antigen contains the factor VIII not possessed by strain No. 628 (Bornstein, 1943). In this connexion the results of absorption tests tend to suggest that the Vi antigen in this strain is deficient in some minor component possessed by that of *Bact. typhosum*. Although both strain No. 628 and *Bact. typhosum* strain Vi(1) showed the same sensitiveness to agglutination by pure Vi antiserum, strain No. 628 removed this antibody to a greater extent against itself than against strain Vi(1), while incomplete absorption of the serum with strain Vi(1) removed all agglutinin against strain No. 628 but left a residual titre of 1/10 against the absorbing suspension. Unfortunately, rapid loss of Vi antigen by strain No. 628 prevented repetition of the experiment.

Both of these strains have now been in constant use for over two years for the production of *O*-antisera and agglutinable suspensions for issue to military laboratories in India, and have proved satisfactory in every respect. The sensitivity to agglutination of strain No. 628/43 is similar to that of *Bact. paratyphosum* CO suspension issued by the Emergency-Vaccine Laboratory, Everleigh. The sensitivity of strain No. 113/44 is similar to that of the *Bact. paratyphosum* AO suspension issued by the Standards Laboratory, Oxford.

It is unfortunate that the serum of neither of the cases from whom these strains were isolated was tested for the presence of homologous *H*-agglutinin.

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