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A phage, $\phi\chi$, which attacks motile bacteria

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SUMMARY

A salmonella phage which attacks only flagellated bacteria (Sertic & Boulgakov, 1936*b*) has been studied. Tests with naturally occurring strains, and with artificial serotypes to which foreign H antigens had been transduced, have shown that sensitivity depends on the H antigen: bacteria with antigens of the *g*-complex are resistant, and with antigens *l*..., *e,h*, or Arizona *13*, are sensitive only to appropriate host-range mutants. Tests with non-motile and motile variants of the same strains showed that paralysed (non-motile H) as well as non-flagellated bacteria are resistant and thus that the flagella must be active as well as of correct antigenic type. Where resistance was due to absence of suitable flagella, it was associated with impaired adsorption of phage. Removal of the flagella from a sensitive strain led to diminished adsorption; a similar result was obtained when the bacteria were artificially paralysed in various different ways. No adsorption to detached flagella was detected, probably because they were inactive. Adsorption of the phage led to immobilization and agglutination of the bacteria, probably by a direct effect on the flagella. Electron micrographs showed phage particles attached to flagella, and infection could evidently follow adsorption to distal parts of a flagellum. The genome of the infecting particle may perhaps reach the bacterial body by being injected into an active flagellum at the point of initial attachment, and then travelling inside the flagellum.

INTRODUCTION

The susceptibility of a bacterium to phage infection primarily depends on whether or not the phage can adsorb to specific bacterial receptors (see Nicolle, Jude & Diverneau, 1953). Sensitivity is usually determined by structures on the surface of the bacterial body, which have sometimes been identified as somatic antigens (e.g. Burnet, 1930), or more superficial envelope antigens such as the Vi antigen (Craigie & Brandon, 1936; Sertic & Boulgakov, 1936*a*; Nicolle, Rita & Huet, 1951). However, a phage whose host-range appeared to depend on the presence of flagella was described by Sertic & Boulgakov (1936*b*). This phage, first named VIII-113, and later χ (Sertic & Boulgakov, 1936*c*), lysed a motile strain of *Salmonella typhi*, H 901, but not its non-flagellated variant, O 901 (Felix, 1930). This phage also selected resistant bacteria from H901 which were non-flagellated. Furthermore, it did not lyse H901 growing on agar containing 0·2 % (w/v) phenol, known to prevent the development of flagella (Braun, 1918), and also did not produce plaques on

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strains carrying abundant Vi antigen, known to interfere with motility. Flagella are structurally distinct from components of the cell proper, and the flagellar (H) antigen does not extend over the surface of the bacterial body (Craigie, 1931; Stocker & Campbell, 1959); thus, if the flagella are concerned in adsorption, it seems likely that the flagella themselves must be the structures to which the phage adsorbs.

The phage under discussion was further studied by Schiff & Bornstein (1940) who claimed that it specifically attacked salmonella species with H antigen *d*, characteristic of *Salmonella typhi*. Rakieten & Bornstein (1941) reported the isolation of five other phages whose host-ranges were associated with flagella. Apart from reports of the use of the χ phage for selecting non-flagellated mutants from motile strains (Stocker, Zinder & Lederberg, 1953), the only other description was given by London (1958), who tentatively concluded that its site of attachment was associated with the basal granules of the flagella. London's object was to identify the chemical groupings involved in adsorption of the phage by treating the bacteria in various ways and by altering the ionic composition and pH of the medium. In so far as our experiments have overlapped, our results have been broadly the same, with one notable exception: the present experiments have shown that paralysed (i.e. flagellated but non-motile strains; Edwards, Moran & Bruner, 1946; Hirsch, 1947; Friewer & Leifson, 1952), as well as non-flagellated strains, are resistant to the χ phage, and that it adsorbs very poorly to paralysed bacteria. London obtained good adsorption to bacteria washed and suspended in dilute salt solutions, a treatment which, in the present experiments, caused sufficient loss of motility for adsorption to be greatly decreased.

METHODS

Buffers. M/15 phosphate buffer (pH 7·0) containing 0·1 M-NaCl, 10^{-3} M-$MgSO_4$, and 10^{-4} M-$CaCl_2$ with gelatin 0·001 % (w/v). McIlvaine's citrate phosphate buffers pH 2·2, 2·6 and 3·0.

Media. Broth was that routinely made in this department from Tryptone (Oxoid), 10 g.; Marmite 5 g.; sodium glycerophosphate, 10 g.; potassium lactate 50 % (w/v) solution, 5 ml.; $MnSO_4.4H_2O$, 0·02 g.; $MgSO_4.7H_2O$, 0·2 g.; $FeSO_4.7H_2O_3$ 0·02 g.; with water to 1 litre. The pH was adjusted to 7·2 and sterile glucose solution was added to 0·2 % (w/v).

Nutrient agar consisted of broth solidified with 1·25 % (w/v) Davis N. Z. agar. In phage titrations by the overlay method, the bases and overlays contained 0·6 and 0·3 % (w/v) agar, respectively. Semi-solid motility medium (Edwards & Ewing, 1955) was dispensed in 8 ml. volumes in 2 in. diameter Petri dishes. All cultures were incubated at 37° without aeration.

Phage. The χ phage was originally obtained from Dr N. Boulgakov by Dr B. A. D. Stocker in 1952, and was usually grown and assayed on *Salmonella abortus-equi*, National Collection of Type Cultures (NCTC) 5727, a species not pathogenic for man. Before this strain had been found to be as good an indicator, Sertic & Boulgakov's usual propagating strain, *S. typhi* var Rough (now called SW 540) was used. Stocks were made by the overlay method (Swanstrom & Adams, 1951) with overlays and bases containing 0·3 and 1·25 % (w/v) agar, respectively. The homogenized overlays were clarified by centrifugation and the remaining bacteria were killed by heating at 56°–60° for 30 min. Titres of 5×10^{10}–2×10^{11} plaque-forming particles (p.f.p)/ml.

were readily obtained. In tests with a limited number of strains, the efficiencies of plating of stocks made on the two hosts, NCTC 5727 and SW 540, were similar.

Stocks of host-range variants of the χ phage. When the χ phage plated with low efficiency on a strain, one of the few plaques produced was diluted and replated with the strain to purify the phage, and a single plaque was then picked and grown with the strain concerned to make the variant stock. Stocks grown on NCTC 5727 were made from single plaques produced by the variant stocks on this strain. *Salmonella abortus-equi* strain NCTC 5727 was tested for possible lysogenicity with a phage whose presence in stocks might have been mistaken for a low efficiency of plating of the χ phage itself: supernatant fluids from cultures of NCTC 5727 treated in the same way as the χ phage stocks produced no plaques on any of the strains concerned. Nor did they produce plaques on strains of *Salmonella gallinarum*, a species sensitive to a wide range of salmonella phages (Dr E. S. Anderson, personal communication). To be sure that the phages with altered host-range were in fact variants of the χ phage, they were tested for neutralization by antiserum prepared against the wild-type phage: all were neutralized at the same rate as this was. Their activity against the strains which had selected them was neutralized to the same extent as their activity against NCTC 5727. They were also tested with motile and non-motile (O or paralysed) forms of a few different salmonella strains, and, like the wild-type phage, they attacked a strain only when it was motile.

Phage titrations. (1) Agar layer method. Satisfactory titrations giving consistent results were obtained by this method with *Salmonella abortus-equi*, NCTC 5727 or *S. typhi*, SW 540 as indicator strain, provided the volume of the overlay was not more than 2·5 ml. and the plates were kept strictly level. Salmonella strain NCTC 5727 tended to become less motile on subculture and the plaques then became hazy and small, but with passage of the indicator strain through semi-solid medium, the plaques again became larger and clearer.

(2) Surface method. Satisfactory plaques were usually obtained when drops of phage stocks were spotted on plates spread with indicator strain. Pipettes delivering drops of 0·02 ml. were used, and in general serial 10-fold dilutions were plated. With *Salmonella abortus-equi* strain NCTC 5727 as indicator, the same numbers of plaques were obtained by either the overlay or the surface method.

The agar layer method was suitable for use with strains of *Salmonella abortus-equi* or *S. typhi*; with other species, the number of plaques was greatly decreased when the concentration of agar in the overlay was high enough to give discrete plaques.

Where the titre of the phage is given, it refers to the number of plaque-forming particles (p.f.p.) on *Salmonella abortus-equi* strain NCTC 5727. The efficiency of plating (e.o.p.) on other strains was calculated using the titre on strain NCTC 5727 as denominator.

Total phage counts by fluorescence microscopy. Suspensions of phage were mixed with a bacterial suspension of known concentration. The mixture was examined by fluorescence microscopy (Anderson, Armstrong & Niven, 1959; Anderson, 1957) and the phage count estimated from the ratio of phage particles to bacteria.

Bacteria. The antigenic formulae for *Salmonella* are written according to the Kauffmann–White Scheme and no account has been taken of minor antigenic relationships or differences omitted from this scheme. For example, 1, 4, 5, 12 : *b* : *1,2*, the formula for *Salmonella paratyphi B*, indicates that the strain has somatic (O)

antigens 1, 4, 5, 12 and flagellar (H) antigens *b* and *1,2*, either of which may be present since they are subject to the rapid mutation and back-mutation known as phase variation (Andrewes, 1922, 1925). The formula for *S. typhi* is 9, 12 (Vi):*d*:–, and the formula for *S. abortus-equi* is 4, 12:–:*e,n,x*; these species are monophasic in phase 1 and phase 2, respectively.

The strains examined came from several sources which are indicated by the letters preceding their numbers: e.g. NCTC (National Collection of Type Cultures, Colindale Avenue, London, N.W. 9); SW, SL, SR, SY, or LT (Guinness–Lister Unit, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W. 1); some of these strains were described by Stocker *et al.* (1953) or by Lederberg & Edwards (1953); A (Dr E. S. Anderson, Central Enteric Reference Laboratory, Colindale Avenue, London, N.W. 9). Other strains were provided by Dr Joan Taylor (Salmonella Reference Laboratory, Colindale), and by Dr P. R. Edwards (Communicable Disease Center, Chamblee, Georgia, U.S.A.). One of the latter is mentioned individually preceded by the letter E.

Tests were made on as many non-motile strains as possible, some of which were non-flagellated while others were 'paralysed', i.e. they possessed inactive flagella of normal serological and morphological structure. Examination of stained preparations and electron microscopy of the paralysed strains used here revealed no differences from normal motile strains either in number or appearance of flagella.

Motile variants of some non-motile strains were sometimes provided; otherwise these were isolated when possible by selection of mutants in semi-solid medium or by transduction using phage P22 (Stocker *et al.* 1953) grown on a motile strain, either *Salmonella typhimurium* strain LT2 (1, 4, 5, 12:*i*:*1,2*), or *S. enteritidis* strain SL 431(1, 9, 12:*g,m*:–).

One strain, SJ 30, isolated as a spontaneous mutant from *Salmonella abortus-equi* NCTC 5727 by Dr Tetsua Iino, had 'curly' flagella (Leifson, 1951; Leifson & Hugh, 1953) which have the usual sinuous form in fixed preparations, but whose wavelength is less than normal (1·5 μ as compared with 2·6 μ in the case of SJ 30 and NCTC 5727; Dr Iino, personal communication). Strain SJ 30 showed no translational motility, but very vigorous jerking and rotation; it had also a very marked tendency to clump, as has been observed with other bacteria having flagella of this type. Motile back-mutants with 'normal' flagella lose this tendency to clump.

Synthetic strains with foreign H antigens introduced by transduction (Lederberg & Edwards, 1953; Lederberg & Iino, 1956). Most of these strains came from the Guinness–Lister Unit. The phase 1 antigens l,z_{13}, z_{10} and *z*, were introduced into *Salmonella typhi*, H 901, from *S. napoli* (1, 9, 12:l,z_{13}:*e,n,x*) NCTC 6853, *S. ituri* (1, 4, 12:z_{10}:*1,5*) NCTC 8275 and *S. shubra* (4, 5, 12:*z*:*1,2*) SL 652, respectively. The phase 2 antigen *1,7* was introduced into *S. abortus-equi* NCTC 5727 from *S. kaapstad* (4, 12:*e,h*:*1,7*) SL136. Phage P22 was used as transducing phage, and bacteria of the recipient strain with the new antigen were isolated by means of Edwards motility medium containing either anti-*d* or anti-*e,n,x* serum. In the course of unsuccessful attempts to introduce other phase 2 antigens into NCTC 5727, bacteria of this strain were isolated in phase 1, with antigen *a*, which is usually suppressed (Edwards & Bruner, 1939). Attempts to introduce other antigens (z_{29}; z_{38}; *k*; z_6; *1,6*; *y*) into these hosts failed, usually because no strains naturally carrying these antigens were found which were sensitive to phage P22.

Selection of Vi-negative variants of Salmonella typhi. A pool of Vi phages I–VII (kindly provided by Dr E. S. Anderson) was spotted on lawns of the Vi-positive strains of *Salmonella typhi*. Resistant bacteria selected by this phage pool have lost the Vi antigen (Dr E. S. Anderson, personal communication). Single colonies obtained by subcultivation from the area of lysis were isolated, and identified as Vi-negative when they were resistant to the phage pool.

Selection of monophasic populations from diphasic strains. Whenever possible, suitable colonies were identified by slide agglutination; when this failed the strains were passed through semi-solid medium containing H antiserum to the unwanted phase.

Bacterial counts. Viable counts were made by spreading 0·2 ml. samples from dilutions of culture on the surface of nutrient agar plates. Total counts were done in a Helber counting chamber.

The proportion of motile bacteria. This was estimated by dark-field microscopy of broth cultures, usually with an ordinary slide and coverslip. Repeated counts of the same suspension gave comparable results. When special accuracy was needed, a Helber chamber was used, the estimate being based on separate counts of the total number of bacteria and of the number of motile or non-motile organisms (whichever was in a minority). Motility was recorded either as 'translational' when the organisms progressed across the field, or as 'rotational' when they rotated or tumbled about one spot.

Sensitivity tests. A loopful of an overnight broth culture of the test strain was spread over 1/3 of a nutrient agar plate. Drops of 0·02 ml. of three dilutions of the χ phage were spotted on this lawn, the drops containing *c.* 5×10^8, *c.* 5×10^3 and *c.* 5×10^1 p.f.p., respectively. After overnight incubation, the degree of clearing by the most concentrated suspension was recorded as $+++$, $++$ or $+$, or $(+)$ where the clearing was so slight as to be seen only when the plate was viewed obliquely. The effects produced by the other dilutions were recorded as $+$, semi-confluent plaques, or as an approximate estimate of the number of plaques with a brief description of their appearance. *Salmonella abortus-equi* strain NCTC 5727 was always included as a control. The conditions for plaque formation appeared to be more critical with some strains than with others, and the tests had then to be repeated several times to obtain an unambiguous result. In a few tests with such strains with 0·6 % (w/v) agar, the strains did not appear to be more sensitive than on the higher concentration.

Almost all the motile strains were passed through motility medium before testing, and with all strains, the overnight cultures which provided the inocula for sensitivity tests were examined for motility: all the strains classified as motile showed more than 50 % of motile bacteria; almost all showed more than 80 %. The non-motile strains were tested for phage-sensitivity in parallel with their motile relatives. The naturally occurring motile strains were not routinely checked for antigenic structure in these tests; but the synthetic strains with foreign antigens and the motile derivatives of non-motile strains were always tested by slide agglutination with H antisera. The non-motile strains were also tested in this way to determine whether they were non-flagellated or paralysed.

Isolation of stable χ phage-resistant variants of sensitive strains. Twenty-six salmonella strains were chosen, and an attempt was made to isolate 6 independent

resistant variants from each by exposing 6 separate single colony isolates to the phage on agar. Resistant colonies from the patch of lysis were purified by 4 successive single colony isolations. Many isolates reverted to sensitivity during purification and were discarded. When none of 20 colonies from a lysed patch remained resistant, the original strain was grown in broth with the phage, but this was little more successful than culture on agar. Six resistant variants came from the collection of the Guinness–Lister Unit.

Determination of phage adsorption. (1) Measurement of unadsorbed phage. Cultures in the late exponential phase of growth were used when the viable count was about $2–5 \times 10^8$ organisms/ml. Except where the effect of multiplicity was specifically measured, the ratio of phage (p.f.p.) to bacteria was always less than 0·1. After 5, 10 or 20 min. at 37°, the mixtures or their dilutions were centrifuged and the supernatant fluids titrated for free phage. (2) Measurement of infected bacteria. In these experiments, the ratio of phage to bacteria was 0·05 or less. The mixtures were diluted into antiphage serum at a concentration which inactivated more than 99% of free phage in 2 min. After 5 min., they were further diluted and plated with *Salmonella abortus-equi* strain NCTC 5727.

Electron microscopy. (1) Fixed, platinum-iridium-shadowed preparations. The material consisted of phage bacterium mixtures containing 10–40 p.f.p./bacterium; after 0·5–15 min., formalin was added to 10 %(v/v). After standing overnight at room temperature, the mixtures were thoroughly washed in distilled water. It was calculated that, with the highest concentration of phage used, the washing was sufficient to leave less than 1 unadsorbed phage particle for every 15 bacteria. The specimens for electron microscopy were prepared and the electron microscopy performed by Mrs H. Ozeki at the Chester Beatty Research Institute. Phage stocks alone and a few bacterial strains without phage were also examined in this way. (2) Preparations negatively-stained with phosphotungstic acid (Brenner *et al.* 1959) were kindly made and examined by Dr E. H. Mercer and Dr M. Birbeck at the Chester Beatty Research Institute, using unfixed material consisting of phage alone or freshly made mixtures of phage and bacteria.

Blending. An M.S.E. Blendor (Measuring and Scientific Equipment Ltd., Spenser Street, London, S.W. 1) was used to stir 10–15 ml. volumes in 25 ml. 'Universal' screw-capped containers. With more than 1·5–2 min. blending of a bacterial suspension, no further decrease in the proportion of motile bacteria was observed. Blending for 2 min. did not alter the titre of a phage stock..

Preparation of detached flagella. Large crops of bacteria were grown on trays of nutrient agar incubated overnight at 37°. The growth was washed off in distilled water with minimal rubbing of the bacteria against the agar surface. The suspension was next treated in the M.S.E. Blendor in 10 ml. volumes followed by the removal of most of the bacterial bodies by centrifugation at 1100 *g*. The supernatant fluid was then centrifuged in a Spinco model L ultra-centrifuge at 20,000 *g* for 1 hr. to deposit the flagella. This deposit was resuspended in 30 ml. distilled water and centrifuged at 1100 *g* and the resulting supernatant fluid again centrifuged at 20,000 *g*. The deposit of flagella finally obtained was resuspended in about 2 ml. distilled water to give a grey opalescent suspension. When any bacterial bodies were seen microscopically in this suspension, they were removed by further low speed centrifugation.

Antiphage sera. Rabbits were injected intravenously with phage stocks grown on *Salmonella abortus-equi* strain NCTC 5727 and having titres of *c.* 2×10^{10} p.f.p./ml. The antisera were absorbed with the propagating strain before use.

Antibacterial sera. In most cases, slide agglutination alone was done to determine the flagellar antigens of a strain. Most of these tests were made with antisera prepared by the Standards Laboratory, Central Public Health Laboratory, Colindale. Antisera for inclusion in semi-solid motility medium were prepared here by inoculating rabbits intravenously with overnight broth cultures sterilized by heating for 1 hr. at 56°; where possible, monophasic strains were used.

RESULTS

General characteristics of the χ phage

The plaques of the χ phage on *Salmonella abortus-equi* NCTC 5727 and on *S. typhi* SW 540 with optimal plating conditions were *c.* 1 mm. in diameter, punched-out in appearance and containing only a little bacterial growth, usually in the form of a granular film. The conditions for plating were rather critical; small changes might cause the plaques to become either large, smeary and filled with growth, or so small as to be scarcely visible and greatly decreased in number. No plaque-type variants were obtained; differences in plaque appearance which were observed were accidental and were not inherited. Plaques similar to those produced in an agar layer, but usually rather smaller, were produced when the phage was spotted on full-strength (1·25% w/v) agar plates spread with strains NCTC 5727 or SW 540. There was no indication that the χ phage could lysogenize.

Almost clear plaques were also seen on other strains of *Salmonella abortus-equi* and on most sensitive strains of *S. typhi*, and the patch of lysis produced by drops of concentrated phage was similarly covered with only a thin film of bacterial growth. On the sensitive strains of other species the plaques and patches were usually much less thoroughly cleared and were often extremely shallow. Broth cultures were never cleared, in agreement with the findings of Dr Boulgakov (personal communication to Dr B. A. D. Stocker) and of Rakieten & Bornstein (1941) with their flagellar phages.

In one-step growth experiments, using *Salmonella abortus-equi* NCTC 5727 as host with a low multiplicity of infection, the minimum latent period was 55–60 min. and after the curve flattened *c.* 90 min. after infection, the average burst size was *c.* 200. With a multiplicity greater than one, the minimum latent period was about the same, but the burst size could not be estimated because of bacterial clumping, which will be described later.

Lysates with titres of $2–5 \times 10^{11}$ p.f.p./ml. were readily obtained. Filtrates through membrane filters were almost without activity; only $2{\cdot}5 \times 10^{-8} – 5 \times 10^{-7}$ of the original number of p.f.p. were obtained. Filtrates through Seitz filters were similarly inactive; less than 2×10^{-7} p.f.p. were present. With sintered glass filters, the filtrates contained *c.* 5% of the original number of p.f.p. The phage stocks which were used were freed from living bacteria by heating at 56°–60° for 30 min., although with 30 min. at 60°, the p.f.p. were decreased to 35–50%, and with 1 hr. to 12–20% of the original values.

The χ phage is about fifteen times as resistant to ultraviolet radiation as is phage T2 (Adams, 1959).

The χ phage was a good antigen; neutralizing sera with K values of 3500 were easily obtained.

Particles of the χ phage are tadpole-shaped. Electron micrographs of preparations negatively-stained with phosphotungstic acid showed a head about 675 Å in diameter and a long tail about 2300 Å in length and 125 Å in width (Pl. 1, figs. 1, 2). The tail showed fine transverse striations like those of phage T2 (Brenner *et al.* 1959), and in some particles the head appeared hexagonal in shape. A few particles with collapsed heads were seen in untreated phage stocks, but many more of these were seen in preparations which had been heated at 56°–60° for 30 min. When the phage had been deposited by high-speed centrifugation (16,000 *g* for 90 min.), 99·9 % of the original p.f.p. were inactivated and the heads of almost all the particles appeared to be collapsed.

The χ phage evidently multiplies intracellularly like other phages, for electron micrographs of sections of infected bacteria in plaques, kindly made by Dr E. H. Mercer using the technique he developed for phage T2 (Mercer, 1959), showed dense phage-like structures like those seen with phage T2 and thought to be the DNA of immature phage heads (Kellenberger, Séchaud & Ryter, 1959; Mercer, 1959). Also, the sequence of events after infection as observed by fluorescence microscopy was not qualitatively different from that seen with other phages (Anderson, Armstrong & Niven, 1959; Dr E. S. Anderson, personal communication). Thus, there is no reason to think that the χ phage is an agent active only against the flagella, as suggested by Rakieten & Bornstein (1941), a theory which would, moreover, presuppose that this phage constituted a completely new kind of bacterial parasite.

The susceptibility of highly motile strains of naturally occurring serotypes

The χ phage was tested against 524 naturally occurring salmonella strains of various serotypes to investigate the possible influence of antigenic structure on its host-range. The strains tested were highly motile on the assumption, taken from previous authors, that sensitivity was correlated with the presence of flagella. The identities of the strains are listed in the footnote to Table 1, which shows the results of the tests. The appearance of the plaques, i.e. size and degree of clearing, varied considerably from strain to strain, but with most strains on which any plaques could be seen, the efficiency of plating (e.o.p.) was over 0·05. Strains on which the phage had a much lower e.o.p. were:

(*a*) Nine strains of *Salmonella typhi*, (9, 12:*d*:–) comprising all of 8 strains of Vi-phage type 32 tested and the only strain of type B2; the former were completely resistant while Vi-positive. Plaques on Vi-negative derivatives of these strains were not regularly observed; when present, they were minute and the e.o.p. was *c.* 10^{-4}.

(*b*) all of 4 sensitive strains of *S. paratyphi A* (1, 2, 12:*a*:–), A. A203, A. BA528, NCTC 13, and NCTC 9452, the e.o.p. being 10^{-4}.

(*c*) one strain of *S. paratyphi B* (1, 4, 5, 12:*b*:–) SW 543 S (e.o.p. 10^{-4}), and 2 strains of *S. typhimurium* (1, 4, 5, 12:*i*:*1,2*), SW 964 S and SL 653 (e.o.p. 10^{-6}).

Table 1. *Host-range of the χ phage with highly motile naturally occurring* Salmonella *strains of various serotypes*

Five hundred and twenty four Salmonella strains were tested, which comprised 104 strains of *S. typhi* with Vi antigen, 13 strains of this species without Vi antigen, (37 of the Vi-positive strains were also tested in the Vi-negative state); 61 strains of other species in O Group D, 46 strains of *S. paratyphi* B, 2 of which were fixed in phase 1 with H antigen *b*, and 1 of which was also fixed in phase 1 although it had H antigen *1, 2*; 61 strains of *S. typhimurium*, 1 fixed in phase 1 and 3 fixed in phase 2; 74 strains of other species in O Group B; 13 strains of *S. paratyphi* A; 3 strains of *S. paratyphi* C; 33 strains of other species in O Group C1; 21 strains in O Group C2; 13 strains in O Group E1; 9 strains in O Group E2; 5 strains in O Group E3; 6 strains in O Group E4; 5 strains in O Group F; 8 strains in O Group G; 8 strains in O Group H; 6 strains in O Group I; and 35 strains in the further O Groups, one or a few from each Group.

The method of testing is described in the text. A strain was recorded as:

(1) *Fully sensitive* if (*a*) the undiluted phage stock produced clearing which was marked, or at least easily seen, and (*b*) the 10^{-5} dilution produced semi-confluent lysis, or at least 50–100 plaques, showing that the e.o.p. was not lower than 0·01.

(2) *Slightly sensitive* if the undiluted stock only produced slight thinning (recorded as (+)), and its dilutions had no effect.

(3) *Resistant* if the undiluted stock and its dilutions had no effect.

A few strains fell into two further categories: (4) those on which the undiluted stock produced marked clearing but the 10^{-5} dilution had no effect; more precise titrations showed that the phage had a low e.o.p. on these strains; and (5) strains on which the undiluted stock produced only discrete plaques. In the table, the denominator represents the number of strains of the particular serotype tested; in the numerator, the number outside brackets is the number of fully sensitive strains, the number in brackets is the number of slightly sensitive strains, the number preceded by C is the number cleared by the undiluted phage but showing no effect with the 10^{-5} dilution, and the number preceded by P is the number on which the undiluted phage, gave only discrete plaques. The difference between numerator and denominator gives the number of resistant strains. The diphasic strains, Sections 2 and 3, are represented twice, once under the H antigen of each phase.

Section 1. Monophasic strains with flagellar antigens

Somatic group	*a*	*b*	*c*	*d*	*i*	z_4z_{23}	z_4z_{24}	z_{36}	z_{29}	z_{38}	*g...*	*m, t*	*e, n, x*	*1, 2*	*1, 5*	*l, v*	Total strain	
A	C4(1)/13	.	.	.	.	.	.	.	.	.	.		.	.	.	.	C4(1)/13	A
B	.	C1/2	.	.	1/1	.	.	.	(1)/4	.	0/10	0/1	4/4	3/4	.	0/1	92C3(34)/181	B
C1	.	.	.	.	.	.		.	0/4	0/1	0/2	0/4	.	.	2(1)/5	.	8P1(7)/36	C1
C2				(1)/1	.	(1)/1	1/2	.	.	.	0/1		.	2/2	.		5(9)/21	C2
D				33C9(5)/50	.	0/1	.	.	(3)/3	(2)/4	0/19						42C9(14)/111	D
DVi+	.			82C1(7)/104		.	.	.	.	.	.		.	.	.	.	82C1(7)/104	DVi+
E1	.	.	.	.		.	.	1/1	.		0/1	.	.	.			2(2)/13	E1
E2	.	.	.	.		.	.	.				.	.	.	.		(2)/9	E2
E3	.	.	.	.	.	.	.	.	.	.	0/1	.	.	.	.	.	P2/5	E3
E4	.	.	.	.	.	.	.	.	.	.	0/3		.				(2)/6	E4
F	.	.	.	.		.	.	.			.	.	.	.	.		1(3)/5	F
G	.	(1)/1	.	.	.	.	.	.	0/1	.	0/1		.	.			(2)/8	G
H	.	.	.	.	.	1/1	0/1	.	.	.	0/1	.	.	.			2(1)/8	H
I	.	.	.	.		.	.	.			.	0/1	.	.			2P1(1)/6	I
17	.	.	.	.		.	.	.	.	.	0/1	.	.	.			(1)/2	17
18	.	.	.	.	.	1/2	1/1	.			.	.	.	.			3/4	18
21	.		.	.		.	.	.	.	.	.	.	.	.			0/1	21
28	.		.	.	.	.	.	.	.	.	.	.	.	.			(3)/3	28
30	.		.	.	.	.	0/1	.	.	.	0/1	.	.	.			1(2)/5	30
35	.		0/1	.	.	1/1	.	.	.	.	0/2	(1)/1	.	.			1(1)/5	35
38	.		.	.		.					.	.		.			1P1/3	38
39	.	.	.	.		.	.		.	.	.	.	.	.		.	0/1	39
40	.		.	.		.	.				.	.	.	.		.	1(1)/2	40
41	.		.	.	.	2/2	.	.	.	.	.	.	.	.	.	.	2/2	41
42	.		.	.	.		.	0/1	.	.	.	.	.	.		.	(1)/2	42
43	.		.	.			.	.			0/1	.	.	.	.	.	(1)/2	43
44	.		.	.	.	.	.	.	.	.	.	.	.	.	.	.	0/1	44
45	.		.	.		.	.	.			.	.	.	.	.	.	0/2	45
Total strains	C4(1)/13	C1(1)/8	0/1	100C9(6)/118*	1/1	5(1)/8	2/5	1/2	(4)/12	(2)/5	0/44	(1)/7	4/4	5/6	2(1)/5	0/1		

Section 2. Diphasic strains with flagellar antigens (also occur in monophasic strains)

Somatic group	*a*	*b*	*c*	*d*	*i*	z_4z_{23}	z_{29}	*g...*	*m, t*	*e, n, x*	*1, 2*	*1, 5*	*l, v*	Total strain	
A	.	.	.	.	.	.	.	.	.	.	.	.	.	C4(1)/13	A
B	.	28(12)/47	0/2	3/4	45C2(8)/60	.	.	.	.	(4)/10	76C2(25)/111	(2)/5	1(5)/10	92C3(34)/181	B
C1	1(1)/2	1(1)/3	4/4	(1)/3	.	(1)/1	.	.	.	(1)/1	.	4P1(1)/8	0/1	8P1(7)/36	C1
C2	.	(2)/2	1/1	.	(2)/3	.	.	.	.	1/1	(1)/5	(1)/1	(2)/2	5(9)/21	C2
D	1/1	1(1)/2	1(1)/3	1(1)/3	.	.	0/2	0/1	.	0/6	1/2	2/9	1(1)/8	42C9(14)/111	D
DVi+	.	.	.	.	.	.	.	.	.	.	.	.	.	82C1(7)/104	DVi+
E1	.	.	.	1/1	.	.				.	0/1	1(1)/5	0/1	2(2)/13	E1
E2	.	.	.	.	.	.	.	.		.	.	(1)/2	(1)/3	(2)/9	E2
E3	.	.	.	.	.	.	.	.		.	.	P1/3	.	P2/5	E3
E4	.	.	.	(1)/1	0/1	.	.	.		.	.	.	.	(2)/6	E4
F	1/1	.	.	0/1	(1)/1	.			.	0/1	(1)/1	1(2)/3	.	1(3)/5	F
G	.	.	.	(1)/1	.	.	0/1		0/1	0/1	.	0/1	.	(2)/8	G
H	.	.	.	.	.	.	.	.	.	1(1)/2	.	0/1	(1)/1	2(1)/8	H
I	1/1	0/1	.	(1)/1	.	.	.	.	.	0/1	.	1/1	P1/1	2P1(1)/6	I
17		(1)/1	.	.	.	.	.	.	.	.	(1)/1	.		(1)/2	17
18	.	.	.	.	.	.	.	.	.	.	.	1/1	.	3/4	18
21		0/1			.	.	.	.	.	0/1	.	.	.	0/1	21
28		.	.	.	.	.	.	.	.	(1)/1	.	(1)/1	.	(3)/3	28
30	.	(1)/1	.	.	(1)/1	.	.	.	.	(1)/1	.	1(1)/2	1/1	1(2)/5	30
35		.	.	.	.	.	.	.	.	.	.	.	.	1(1)/5	35
38		.	.	.	.	.	.	.	.	.	.	1P1/2		1P1/3	38
39		.	.	.	.	.	.	.	.	.	.	0/1		0/1	39
40	.	1(1)/2	.	.	.	.	.	.	.	.	.	1/1		1(1)/2	40
41		.	.	.	.	.	.	.	.	.	.	.	.	2/2	41
42		(1)/1	.	.	.	.	.	.	.	(1)/1	.	.		(1)/2	42
43	(1)/1	.		.	.	.	.	.	.	.	.	(1)/1	.	(1)/2	43
44	0/1	.												0/1	44
45	.	.	0/1	.		.	.	0/1	.	0/1	.	0/1		0/2	45
Total strains	4(2)/7	31(20)/61	6(1)/11	5(5)/15	45C2(12)/66	(1)/1	0/3	0/2	0/1	2(9)/28	81C2(28)/121	13P3(11)/49	3P1(10)/28		

Section 3. Diphasic strains with flagellar antigens (occur only in diphasic strains)

Somatic group	*l, w*	l, z_{13}	*k*	*r*	*y*	*e, h*	*z*	z_{10}	e, n, z_{15}	*l, 6*	*l, 7*	z_6	Total strain	
A													C4(1)/13	A
B	2(1)/10	0/1	1(1)/2	1(1)/4	(2)/3	(1)/9	2(1)/6	3(1)/4	2(3)/7	1(1)/2	(1)/6	3/5	92C3(34)/181	B
C1	(1)/4	P1/1	.	.	.	(2)/4	.	.	1(1)/5	(1)/1	(1)/1	1/1	8P1(7)/36	C1
C2	0/1	.	.	(1)/1	1/1	0/3	.	.	1/1	(1)/1	(1)/1	(3)/4	5(9)/21	C2
D	1/4	0/3	1/1	.	.	0/3	2/3	.	(1)/4	2(1)/3	1/3	3(2)/7	42C9(14)/111	D
DVi+													82C1(7)/104	DVi+
E1	0/1	0/1	(1)/1	.	(1)/2	0/3	0/1	0/1	.	0/1	0/2	(1)/1	2(2)/13	E1
E2	0/2	(1)/1	.	.	0/1	0/4	.	.	.	0/1	(1)/4	.	(2)/9	E2
E3			.	.	P1/1	.	.	P1/3	.	P1/1	.	.	P2/5	E3
E4			.	.	.	.	(1)/1	.	(1)/1	.	.	(1)/2	(2)/6	E4
F			.	(1)/1	(1)/1	.	.	.	.	.	.	.	1(3)/5	F
G	0/2	.	.	.	.	.	0/2	.	.	.	(1)/1	.	(2)/8	G
H			.	.	0/2	0/1	1/1	.	.	.	0/2	.	2(1)/8	H
I			1/1	.	.	.	.	.	1(1)/2	P1/1	.	.	2P1(1)/6	I
17			.	.	.	.	.	.	.	.	.	.	(1)/2	17
18			1/1	.	.	.	.	.	.	.	.	.	3/4	18
21			.	.	.	.	.	.	.	.	.	.	0/1	21
28			.	(2)/2	(1)/1	.	.	.	(1)/1	.	.	.	(3)/3	28
30			.	.	.	.	.	.	.	.	.	.	1(2)/5	30
35			.	.	.	.	.	.	.	.	.	.	1(1)/5	35
38			0/1	1/1	.	P1/1	.	.	.	0/1	.	.	1P1/3	38
39			0/1	.	.	.	.	.	.	.	.	.	0/1	39
40			.	.	.	.	.	.	(1)/1	.	.	.	1(1)/2	40
41			.	.	.	.	.	.	.	.	.	.	2/2	41
42			.	.	.	.	.	.	.	.	.	.	(1)/2	42
43			.	.	.	.	.	.	.	.	.	.	(1)/2	43
44	0/1	.	.	.	.	.	.	.	.	.	.	.	0/1	44
45			.	.	.	.	.	.	.	.	.	.	0/2	45
Total strains	3(2)/25	P1(1)/7	4(2)/8	2(5)/9	1P1(5)/12	P1(3)/28	5(2)/4	3P1/(1)/8	5(9)/22	3P2(4)/12	1(5)/20	7(7)/20		

* 37 strains of *S. typhi* were tested in both the Vi+ and Vi- state, see pp.261–262.

Strains on which the undiluted phage stock produced only discrete plaques were: *S. ness-ziona* (6, 7:*l*,z_{13}:*1,5*), NCTC 8717; *S. shanghai* (16:*l,v*:*1,6*), SL 651; *S. kasenyi* (38:*e,h*:*1,5*), NCTC 8278, (the plaques were on a background of slight clearing of degree (+), and dilutions of the phage sometimes also produced a much larger number of very indistinct plaques on this strain); *S. thomasville* (3, 15, 34:*y*:*1,5*), NCTC 9896; *S. harrisonburg* (3, 15, 34:z_{10}:*1,6*), NCTC 8258. A few of the strains which were classed as slightly sensitive may really have fallen into this category, as there was sometimes a suggestion of plaques in the partially cleared patch. These did not always appear on repeated testing and attempts to isolate phage from them which would give more definite plaques on the strain failed.

These host-range tests showed the following results:

(1) The H antigens could determine the sensitivity of a strain, for all strains which carried antigen *g* were resistant, regardless of whether this antigen was present as *g,m*, *g,p*, *g,s,t*, etc., (one slightly sensitive strain with antigen *m,t* constituted a weak exception to the resistance of strains with antigens of the '*g* complex'). Most strains with *g*-related antigens are monophasic, but there are a few diphasic serotypes (Kauffmann & Henning, 1952; Douglas, Taylor & McMath, 1951). Three such diphasic serotypes were tested and were found to be predominantly in the *g* phase and resistant when first tested; but by passing them through motility medium with antiserum it was possible to isolate bacteria in the alternative phase. When these strains were tested with the χ phage, one (with antigen *e,n,x*) was fully sensitive, and the other two (with antigens *e,n,x* and *1,5*, respectively) gave a (+) reaction.

The χ phage was also tested on 20 strains of the Arizona group (Edwards & Ewing, 1955; Edwards, Fife & Ramsey, 1959) carrying various O and H antigens. Eleven strains were sensitive. Some Arizona H antigens are shared with *Salmonella*; in particular, H antigen *13* cross-reacts with salmonella H antigen *g* (Edwards & Ewing, 1955; Edwards *et al.* 1959). Four of five Arizona strains carrying antigen *13* which were tested were χ phage-resistant, and the fifth, NCTC 7318, (O antigen 9:H antigen *13*, *15*) showed a few discrete plaques with the undiluted phage.

Strains carrying most of the other salmonella H antigens might be either sensitive or resistant, showing that susceptibility is not governed solely by H antigenic type.

(2) The somatic antigen probably did not determine sensitivity, for strains belonging to many different O Groups, as well as rough strains which had lost their O antigen (e.g. *Salmonella typhi* SW 540), could be sensitive. None of the strains belonging to O Groups E3, E4 or G which were tested were fully sensitive. Undiluted phage produced only discrete plaques on *S. harrisonburg* NCTC 8258 and *S. thomasville* NCTC 9896, belonging to Group E3. The results with some other somatic antigens do not carry much weight since so few strains were tested.

(3) The most sensitive species were *Salmonella typhi* and *S. abortus-equi*, both in the completeness of clearing in individual plaques and in patches produced by concentrated phage, and also in the proportion of strains found to be sensitive. One hundred and seventeen strains of *S. typhi* were tested, including 104 Vi-positive strains from 67 different Vi-phage types (Craigie & Yen, 1938), and 13 Vi-negative strains. Of 104 Vi-positive strains 82 fell into the fully sensitive category, but only 67 showed maximum clearing (+ + +), leaving 37 strains showing various degrees of resistance. Of these 37 strains, 21 owed their resistance either to the presence of the Vi antigen, as found by previous authors, or to poor motility. When the sensitivity

of the 117 strains was assessed, taking into account the reaction of these 37 strains either after loss of the Vi antigen or after passage through motility medium, only 17 of the 117 tested were not fully sensitive, with +++ clearing by undiluted phage.

Effect of Vi-phage type. The Vi-phage type of a strain was correlated with its reaction to the χ phage since: (*a*) 3/3 strains of Vi-phage type D4 were totally resistant; (*b*) the 5 strains giving a (+) reaction comprised 4/4 strains of type M (2 of type M1 and 1 each of types M2 and M3) and 1/1 strain of type 37; (*c*) the 9 strains showing plaques only irregularly and with low e.o.p. comprised 8/8 strains of type 32 and 1/1 strain of type B2.

Vi-phage typing depends on specific modifications in the host-range of Vi phage II (Anderson & Felix, 1953), but of the 67 types, only types D4 and M are resistant to all the *Salmonella typhi* Vi phages (I–VII) except phage III (Dr E. S. Anderson, personal communication). A correlation between χ phage resistance and Vi-phage type, especially if the type is rarely isolated and is restricted to a particular locality, might have no more significance than repeated tests on the same strain, but type D1 (4/4 strains), which is closely related to type D4, was fully sensitive to the χ phage. Clones of type D1 frequently arise in cultures of type D4, and such clones are sensitive to the χ phage while the parent culture remains resistant (Dr E. S. Anderson, personal communication). The change from type D4 to type D1 is not associated with loss of one of the recognized type-determining phages; types D4 and D1 carry the same type-determining phage, and type A (which is sensitive to all the adapted preparations of Vi phage II) remained fully sensitive to the χ phage after lysogenization with this phage isolated from either a type D1 strain or a type D4 strain. Thus the resistance of type D4 to the χ phage is not due to lysogenicity with a type-determining phage. One strain of type D4 absorbed the χ phage rapidly; therefore resistance in this strain at least was not due to failure of attachment.

The susceptibility of highly motile strains with artificial serotypes

The relation between χ phage sensitivity and H antigenic type was further examined by testing sets of artificial derivatives with differing H antigens prepared from single parental strains by transduction. The results in Table 2 allow the following conclusions.

(1) When a strain was originally sensitive, it kept its sensitivity with most of the foreign H antigens. The degree of clearing produced by the undiluted phage stock and the appearance of individual plaques were characteristic of the strain, and not of the H antigen it carried. Occasionally, a change of H antigen brought with it a small change in e.o.p. (*c.* five-fold) but without extensive tests it is impossible to be sure that this difference was greater than would have been found in tests on different clones of the same strain.

The low e.o.p. (*c.* 10^{-4}) of the phage on strains of *Salmonella paratyphi A* was characteristic of the bacterium and not of the H antigen *a*, for the phage still plated with an e.o.p. of 10^{-4} on derivatives of *S. paratyphi A*, strain A. 17689, in which antigens *i* or *r* had been substituted for *a*. SL 508 (*S. typhi* SY 79 with antigen *a* transduced from *S. abortus-equi* NCTC 5727) was fully sensitive, as was NCTC 5727 itself with antigen *a* in phase 1 which is normally suppressed (Edwards & Bruner, 1939).

Table 2. *Sensitivity of synthetic* Salmonella *strains with various flagellar antigens*

Strain	Antigen	Sensitivity	Donor of antigen	Sensitivity of donor*
S. typhi				
SY 79	*d* : –	+	.	.
Derivatives				
SW 520	*i* : –	+	*S. typhimurium* LT 7	+
SL 502	*b* : –	+	*S. abony* SW 803	–
SL 504	*r* : –	+	*S. heidelberg* SL 142	+
SL 505	*c* : –	+	*S. altendorf* SL 137	–
SL 508	*a* : –	+	*S. abortus-equi* NCTC 5727	+
SL 509	*1, 2* : –	+	*S. paratyphi B* SW 546†	+
SL 506	*e, h* : –	e.o.p. 10^{-6}‡	*S. chester* SL 139	–
SL 507	*e, h* : –	e.o.p. 10^{-6}‡	*S. kaapstad* SL 136	–
SL 503	*g, p* : –	–	*S. dublin* SW 553 (O)	–
SL 510	*g, m, s* : –	–	*S. hato* NCTC 9899	–
SL 511	*g, m* : –	–	*S. enteriditis* NCTC 4196	–
S. *typhi*				
SW 537 (H901)	*d* : –	+	.	.
Derivatives				
SW 569	*i* : –	+	*S. typhimurium* LT 2	+
SW 902	*c* : –	+	*S. altendorf* SW 825	–
SW 537.*z*	*z* : –	+	*S. shubra* SL 652	(+)
SW 537.z_{10}	z_{10} : –	+	*S. ituri* NCTC 8275	(+)
SW 668	*e, h* : –	e.o.p. 10^{-6}‡	*S. sandiego* SW 718	.
SW 537.*l*, z_{13}	*l*, z_{13} : –	e.o.p. 10^{-7}	*S. napoli* NCTC 6853	–
SW 667	*g, p* : –	–	*S. dublin* SW 553 (O)	–
S. abortus-equi				
NCTC 5727	– : *e, n, x*	+	.	.
Derivative				
NCTC 5727.*1, 7*	– : *1, 7*	+	*S. kaapstad* SL 136	–
S. paratyphi A				
A 17689	O strain	(–)	.	.
Derivatives				
SL 31	*i* : –	e.o.p. 10^{-4}	*S. typhimurium* LT 2	+
SL 37	*r* : –	e.o.p. 10^{-4}	*S. heidelberg* SL 28 (O)	+
S. paratyphi B (monophasic)				
SW 543	O strain	(–)	.	.
Derivatives				
SW 543 swarm	*b* : –	e.o.p. 10^{-4}	Self	.
SW 623	*i* : –	e.o.p. low	*S. typhimurium* LT 2	+
SW 940	*a* : –	e.o.p. low	*S. sendai* SW 771	.
SL 117	*r* : –	e.o.p. low	*S. heidelberg* SL 142	+
SW 633	*1, 2* : –	e.o.p. low	*S. paratyphi B* SW 546†	+
SL 163	*c* : –	e.o.p. low	*S. altendorf* SL 137	–
SL 159	*e, h* : –	–	*S. chester* SL 139	–
SL 165	*e, h* : –	–	*S. kaapstad* SL 136	–
SL 116	*g, p* : –	–	*S. dublin* SW 553 (O)	–
SW 679	*g, m* : –	–	*S. enteritidis* SW 764	–
S. typhimurium				
LT 2	*i* : *1, 2*	+	.	.
Derivatives				
SW 698	*i* : *e, n, x*	+	*S. abony* SW 803	–
SW 699	*b* : *1, 2*	+	*S. abony* SW 803	–
SL 141	*b* : *e, n, x*	+	*S. abony* SW 803 (two exposures)	–

Table 2 (*cont.*)

Strain	Antigen	Sensitivity	Donor of antigen	Sensitivity of donor*
S. typhimurium				
LL 22	*i* : *1, 2*	+	.	.
Derivatives				
SW 674	*g, p* : *1, 2*	.	*S. dublin* SW 553 (O)	−
Phase 1	*g, p*	−	.	.
Phase 2	*1, 2*	+	.	.
S. heidelberg				
SL 28	O strain	(−)	.	.
Derivatives				
SL 142	*r* : *1, 2*	+	Self	.
SL 118	*i* : *1, 2*	+	*S. typhimurium* SL 55	−
SL 121	*b* : *1, 2*	+	*S. paratyphi B* SW 609	(+)
SL 119	*g, p* : *1, 2*	.	*S. dublin* SW 553 (O)	−
Phase 1	*g, p*	−	.	.
Phase 2	*1, 2*	+	.	.
S. dublin				
SW 553	O strain	(−)	.	.
Derivatives				
SL 149	*g, p* : −	−	Self	.
SL 120	*b* : −	(+)	*S. paratyphi B* SW 543 (O)	e.o.p. 10^{-4}
SL 122	*r* : −	(+)	*S. heidelberg* SL 28 (O)	+
S. moscow				
NCTC 5768	*g, p* : −	−	.	.
NCTC 5768.*i*	*i* : −	+	*S. typhimurium* SL 375	+
S. essen				
NCTC 5723	*g, m* : −	−	.	.
NCTC 5723.*i*	*i* : −	+	*S. typhimurium* LT 2	+
S. hato				
NCTC 9899	*g, m, s* : −	−	.	.
SL 512.*i*	*i* : −	(+)	*S. typhimurium* SL 375	+
S. enteritidis				
SL 513	*g, m* : −	−	.	.
SL 513.*i*	*i* : −	(+)	*S. typhimurium* SL 375	+
S. enteritidis				
SL 433	*g, m* : −	−	.	.
SL 433.*i*	*i* : −	(+)	*S. typhimurium* LT 2	+
S. enteritidis				
SL 431	*g, m* : −	−	.	.
SL 431.*i*	*i* : −	−	*S. typhimurium* SL 375	+
S. enteritidis				
SL 432	*g, m* : −	−	.	.
SL 432.*i*	*i* : −	−	*S. typhimurium* SL 375	+

* Some donors were themselves nonmotile: this is indicated by (O) after the name of the strain and the sensitivity given is that of motile variants of the strain.

† SW 546 is a monophasic strain with *1, 2* as its phase 1 antigen (Lederberg & Edwards, 1953).

‡ Clear plaques (see text).

(2) The introduction of H antigen *g...* into a sensitive strain such as *Salmonella typhi*, SY 79 or SW 537, or the monophasic *S. paratyphi B*, SW 543, made the strain totally resistant. When antigen *g,p* replaced the natural phase 1 antigens *i* or *r* in the sensitive diphasic strains *S. typhimurium*, LT 22, or *S. heidelberg*, SL 28, respectively, the strains became resistant in this phase, while remaining sensitive in phase 2 (with antigen *1,2*). Six out of eight monophasic strains which naturally carried antigen *g...* and were resistant to the phage, became sensitive, or slightly sensitive, when another H antigen was substituted. Thus the resistance of some, although not all, natural strains carrying antigen *g...* is due to the presence of this antigen.

When antigen l,z_{13} was introduced into *Salmonella typhi* SW 537, concentrated phage produced only a small number of discrete plaques. When antigen *e,h* was introduced into *S. typhi* SY 79 or SW 537, the phage produced a small number of clear plaques with a much larger number of very indistinct plaques. The ratio of the former to the number of plaques produced on NCTC 5727 was about 10^{-6}, and that of the latter was about 10^{-3}–10^{-4}. When antigen *e,h* was introduced into the less sensitive *S. paratyphi B* SW 543, it made it totally resistant.

(3) A suitable H antigen was not the only requirement for sensitivity, for the reaction of the strain from which the antigen was transduced did not influence the reaction of the recipient, except where certain antigens, such as *g...*, l,z_{13}, or *e,h* were concerned.

Host-range variants

Several instances have been given where ordinary stocks of the χ phage had a very low e.o.p. on certain strains (e.g. 10^{-4}–10^{-7}) so that high concentrations of phage produced only relatively few plaques. The phage present in these plaques plated on the test strain concerned with almost maximum efficiency (e.o.p. 0·1–1). When the variants were again grown on *Salmonella abortus-equi* NCTC 5727, their behaviour divided them into two groups.

(1) The first group was that in which the e.o.p. on the test strain had again fallen to about the original value, so that the variant stocks had presumably shown only phenotypic variation and did not consist of mutants. The test strains concerned were:

Salmonella paratyphi A strains A 203; A. BA 528; NCTC 9542; SL 31 (with antigen *i* substituted for *a*); and SL 37 (with antigen *r* substituted for *a*); *S. paratyphi B* strain SW 543 S; *S. harrisonburg* strain NCTC 8258.

(2) The second group consisted of those variants which were evidently host-range mutants since their stocks grown on *Salmonella abortus-equi* NCTC 5727 retained a high e.o.p. on the test strain. The bacterial strains concerned here were:

SW 537.l, z_{13} (*S. typhi* H 901 in which H antigen l,z_{13} had been substituted for *d*);

NCTC 8717 (*S. ness-ziona* 6, 7 : l,z_{13} : *1,5*). This strain was found to have been in phase 1 (with antigen l,z_{13}) when it was tested and the phage mutant was selected;

SL 651 (*S. shanghai* 16 : *l,v* : *1,6*). This strain was found to have been in phase 1 (with antigen *l,v*) when it was tested and the phage mutant was selected;

SL 507	(*S. typhi* SY 79 in which H antigen *e,h* had been substituted for *d*);
NCTC 8278	(*S. kasenyi* 38 : *e,h* : *1,5*);
NCTC 7318	(Arizona O antigen 9 : H antigen *13, 15*);
NCTC 9896	(*S. thomasville* 3, 15, 34 : *y* : *1,5*);
SW 964S	(*S. typhimurium*, the motile variant of a strain, SW 964, which was received in the O state);
SL 653	(*S. typhimurium* C 56 of Boyd, which carries phage A2*d*).

In tests with a limited number of strains, the mutants showed no loss of activity for strains sensitive to the wild-type phage; thus their host-range was enlarged rather than diminished. Their plaques on *S. abortus-equi* NCTC 5727 and other strains sensitive to the wild-type χ phage were similar in appearance to the plaques of the wild type phage.

Table 3. *Activities of the host-range mutants against strains on which mutants were isolated*

		e.o.p. mutant of χ selected on strains					
Strain	e.o.p. χ	537. l,z_{13}	8717	651	507*	8278*	7318
SW 537.l,z_{13}	10^{-6}–10^{-8}	0·5–1	0·5–1	0·5–1	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$
NCTC 8717, phase with l,z_{13}	10^{-6}–10^{-7}	0·5–1	0·5–1	0·5–1	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$
SL 651, phase with *l,v*	10^{-6}–10^{-7}	0·5–1	0·5–1	0·5–1	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$
SL 507 (*e,h*) (or SL 506, or SW 668)	10^{-6}*	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$	0·5	0·5–1	$\leqslant\chi$
NCTC 8278, phase with *e,h*	10^{-6}–10^{-7}	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$	0·01	0·2–0·7	$\leqslant\chi$
NCTC 7318 (*13, 15*)	10^{-7}–10^{-8}	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$	$\leqslant\chi$	0·1–0·2

The phages were titrated by the modified Miles & Misra method, using preparations which had been grown on NCTC 5727 and whose titres on this strain were 10^{10}–10^{11}/ml. In some cases, a preparation grown on the strain which had selected the mutant was also tested, with similar results.

* Distinct, clear plaques (see text).

$\leqslant\chi$ the e.o.p. was no greater than that of wild-type χ phage.

Strains carrying H antigen l. The results of titrating the first six mutants on the bacterial strains by which each was isolated are summarized in Table 3. The results with the first three suggested that these strains had each selected a phage mutant which had gained the ability to attack bacteria carrying the H antigenic component *l*. Accordingly, strains with H antigen l,z_{13}, *l,v* or *l,w*, which had already been tested in the general host-range tests, were retested after separation into their two phases. Strains NCTC 8717 and SL 651, and all the strains which had appeared as fully sensitive were retested, together with most of the strains which had given a (+) reaction and a few of those which had appeared as resistant. Cultures in each phase were tested with the wild-type phage, and cultures, in the phase exhibiting antigen *l*... were tested with the mutant isolated from SW 537.l,z_{13} and grown on NCTC 5727; the results are shown in Table 4, Section A. A few strains were also tested with the mutants isolated from NCTC 8717 or SL 651, with similar results. The results supported the conclusion that the wild-type phage was unable to attack bacteria with H antigen *l*..., but could mutate to a form able to do so.

Strains carrying H antigen e,h. When the wild-type χ phage was plated with strains SL 506, SL 507 or SW 668 (*Salmonella typhi* in which antigen *e,h* had been substituted for *d*), very small indistinct plaques could occasionally be seen with the higher phage dilutions, the e.o.p. being about 10^{-3}. The lower dilutions produced partial clearing in which there were discrete clear plaques; the ratio of these to the total number of plaques which the preparation produced on NCTC 5727 (or on the same strains of *S. typhi* carrying antigen *d*) varied between 10^{-6} and 10^{-7}. The phage isolated from these plaques, or from plaques similarly produced on strain NCTC 8278 (*S. kasenyi* 38 : *e,h* : *1,5*) by the lower phage dilutions, gave clear plaques with high e.o.p. on the three *S. typhi* derivatives with antigen *e,h*, and also plated with high efficiency on strain NCTC 8278. Table 4, Section B shows the results when NCTC 8278 and 4 other strains with antigen *e,h* were tested in each phase with wild-type χ phage, and in the phase exhibiting *e,h* with the phage mutants isolated from SL 507 and NCTC 8278: these appear to be mutants which have gained activity for bacteria with H antigen *e,h*. The two mutants were not identical, for the one from NCTC 8278 plated with higher efficiency on these strains than the one from SL 507.

Table 4. *Sensitivity tests*

A. *Tests on naturally occurring strains carrying H antigens* l,v, l,w, *or* l,z_{13} *in one phase*

	No. of strains	Reaction to χ: Cultures in phase with antigen *l*...	Reaction to χ: Cultures in other phase	Reaction of cultures in phase with *l*... to mutant selected by SW 537.*l*,z_{13}
Strains with *l*,z_{13} as one antigen	1	Plaques	+	+
	1	−	(+)	+
	3	−	(+)	(+)
	2	−	−	−
Strains with *l,v* as one antigen	2	Plaques	+	+
	2	(+)	+	+
	4	−	+	+
	2	−	(+)	(+)
	3	−	−	−
Strains with *l,w* as one antigen	1	Plaques	+	+
	6	−	+	+
	2	−	−	−

B. *Tests on naturally occurring strains carrying H antigen* e,h *in one phase*

	No. of strains	Reaction to χ: Cultures in phase with antigen *e,h*	Reaction to χ: Cultures in other phase	Reaction of cultures in phase with *e,h* to mutants selected by NCTC 8278 or SL 507
Strains with *e,h* as one antigen	1	Plaques	+	+
	2	−	+	+
	2	−	(+)	(+)

Arizona strain NCTC 7318. Arizona NCTC 7318 has H antigen *13, 15*; since Arizona H antigen *13* closely resembles Salmonella H antigen *g* (Edwards & Ewing, 1955; Edwards *et al.* 1959), it seems likely that NCTC 7318 is resistant because it carries H antigen *13*, and that the mutant isolated on it is one which has gained activity for bacteria with this antigen. No other strains with antigen *13, 15* were available; but, on 2 of the 4 Arizona strains with antigen *13, 14* which were resistant to the wild-type phage, the mutant from NCTC 7318 had some activity in that concentrated phage gave some degree of clearing, although the dilutions produced no plaques. A number of salmonella strains with antigen *g*... were tested with the mutant from NCTC 7318 but all were resistant; these included the artificial strains SL 119 and SW 674 in the phase exhibiting antigen *g,p* (which were sensitive in the other phase), and strains NCTC 5723 and NCTC 5768 (which became sensitive when antigen *i* was substituted for *g*...). Thus, if the mutant isolated on NCTC 7318 is one which has gained activity for bacteria with Arizona antigen *13*, it can evidently distinguish between this antigen and the serologically related Salmonella antigen *g*. None of the other host-range mutants attacked bacteria with H antigen *g*.

Table 5. *Association between H antigenic type and reaction to the χ phage*

Antigen	Associated with	Evidence	
		Natural strains	Synthetic strains
a	Sensitivity	*S. abortus-equi* NCTC 5727 sensitive in phase 1 4 *S. paratyphi A* strains sensitive	*S. typhi* SL 508 sensitive
b	Sensitivity	3 strains *S. paratyphi B* sensitive in phase 1	*S. typhi* SL 502 sensitive *S. typhimurium* SW 699 sensitive in phase 1
c	Sensitivity	3 diphasic strains sensitive in phase 1	*S. typhi* SL 505 and SW 902 sensitive
d	Sensitivity	Most *S. typhi* strains sensitive	.
i	Sensitivity	*S. typhimurium* 1 monophasic strain and 2 diphasic strains sensitive in phase 1	*S. typhi* SW 520 and SW 569 sensitive
r	Sensitivity	2 diphasic strains sensitive in phase 1	*S. typhi* SL 504 sensitive
z	Sensitivity	2 diphasic strains sensitive in phase 1	*S. typhi* SW 537.*z* sensitive
z_{10}	Sensitivity	2 diphasic strains sensitive in phase 1	*S. typhi* SW 537.z_{10} sensitive
z_{36}	Sensitivity	1 monophasic strain sensitive	.
z_4, z_{23}	Sensitivity	5 monophasic strains sensitive	.
z_4, z_{24}	Sensitivity	2 monophasic strains sensitive	.
e,n,x	Sensitivity	4 *S. abortus-equi* strains sensitive	.

Table 5 (*cont.*)

Antigen	Associated with	Evidence: Natural strains	Evidence: Synthetic strains
1,2	Sensitivity	5 monophasic strains sensitive	*S. typhi* SL 509 sensitive
1,5	Sensitivity	2 monophasic strains sensitive	.
1,7	Sensitivity	1 diphasic strain sensitive in phase 2	*S. abortus-equi* NCTC 5727. *1,7* sensitive
z_6	Probably sensitivity	4 diphasic strains sensitive in phase 2	.
e,n,z_{15}	Probably sensitivity	3 diphasic strains sensitive in phase 2	.
1,6	Probably sensitivity	2 diphasic strains sensitive in phase 2	.
k	Probably sensitivity	2 diphasic strains sensitive in phase 1	.
l,z_{13}	Sensitivity to specific *l...* variant	See Tables 3 and 4	.
l,v	Sensitivity to specific *l...* variant		
l,w	Sensitivity to specific *l...* variant		
e,h	Sensitivity to specific *e,h* variant		
y	Sensitivity to variant	3/4 diphasic strains sensitive to variant in each phase, but sensitive to wild-type χ only when not exhibiting *y*	.
z_{29}	.	2/9 monophasic strains (+) reaction	.
z_{38}	.	2/4 monophasic strains (+) reaction	.
g...	Resistance	0/50 monophasic strains sensitive. (1 slightly sensitive strain with *m, t*) 3 diphasic strains in *g...*phase while sensitive in alternative phase	*S. typhi* SL 503, SL 510, SL 511, SW 667 resistant. SW 674, SL 119 resistant in phase with *g...* and sensitive in alternative phase. When *i* was substituted for *g...* naturally present, 6/8 strains became sensitive

The implications of the results with the last three strains are not clear. In the case of NCTC 9896 (*Salmonella thomasville* 3, 15, 34 : *y* : *1,5*), the H antigen appeared to be concerned, for this strain reacted differently in each phase, and the host-range of a phage mutant which it selected included (among other bacteria not sensitive to the wild-type phage) bacteria with H antigen *y*. When four strains with antigen *y* were tested, none was sensitive to the wild-type phage in the phase which exhibited it, but three were fully sensitive in the other phase; these three strains in the phase with *y* were sensitive to the mutant selected by NCTC 9896.

Strains SL 653 and SW 964S carry H antigens *i* : *1,2* which are associated with

sensitivity to the wild-type phage in other strains; cultures in each phase reacted in the same way, and the phage mutant which each strain selected had gained activity only against that strain itself.

Table 5 summarizes the results of testing the different H antigens. The antigens which occur naturally only in diphasic serotypes (those of Section 3 of Table 1) were tested in so far as possible after transduction to fully sensitive strains of *Salmonella typhi* (monophasic in phase 1), or *S. abortus-equi* (monophasic in phase 2). They were also tested by selecting predominantly monophasic clones from diphasic strains.

Association of sensitivity and motility

The association of sensitivity to the χ phage with the presence of flagella was investigated with (1) naturally occurring non-motile strains and with (2) phage-resistant variants selected from sensitive populations by the χ phage.

Naturally occurring non-motile strains. These comprised 62 strains of various serotypes. Motile derivatives of 45 of them were available and consisted either of spontaneous mutants or of derivatives obtained by transduction. The results are given in Table 6. All the non-motile strains were phage resistant and most of the motile derivatives were sensitive, with the consistent exception of strains which turned out to have H antigen *g*.... It will be noticed that the non-flagellated strains were not the only resistant ones; all of 10 paralysed strains were also resistant. The motile derivatives of these paralysed strains were sensitive. Table 6 therefore reveals a further condition determining sensitivity to the χ phage, namely, that the flagella must be active. This is well illustrated with the O strain of *Salmonella typhimurium*, SW 573, where mutation leading to flagella production (SL 43) was not enough for sensitivity; this only appeared when with further mutation the strain became motile (SL 43 swarm).

SJ 30, the 'curly' mutant of NCTC 5727 with short wavelength flagella, showed as marked clearing and as many plaques as NCTC 5727 itself. This variant was not truly paralysed for, although it did not exhibit translational motility, it did show very active jerking movement.

Experiments with Salmonella abortus-equi. The number of flagella formed by freshly isolated strains of this species, and hence their motility, depend on the pH of the medium; many flagella are formed at pH 6·5 and few at pH 8 (Kato, 1954). Three freshly isolated strains obtained by Dr B. A. D. Stocker from Dr Kato were tested and this observation was confirmed. Also, the strains were found to be sensitive to the χ phage on agar of pH 6·5 and resistant on agar of pH 8·0. Variants of these strains obtained by passage through semi-solid motility medium of pH 7·4 were as sensitive at pH 8·0 as at the lower pH.

Salmonella typhi: *effect of the Vi antigen*

Previous authors noted that Vi-positive strains showed weaker motility and lower sensitivity to the χ phage than Vi-negative strains. Broth cultures of all the Vi-positive strains used here contained over 50% motile bacteria and thus none was carrying sufficient Vi antigen to interfere seriously with its motility in broth. Nevertheless, 37 of 104 Vi-positive strains did not show maximum sensitivity to the χ phage, and in 21 of these, loss of the Vi antigen resulted in an increase of sensitivity.

Table 6. *Action of the χ phage on non-motile* Salmonella *strains and their motile derivatives*

	Nonmotile strain		Motile derivative*		
Strain	O or paralysed	Sensitivity	Strain	Antigen	Sensitivity
S. typhi					
SL 100	O	–	SL 100 S	*d* : –	+
SL 101	O	–	.	.	.
SL 102	O	–	.	.	.
SL 77 (0901)	O	–	SL 77 S	*d* : –	+
SL 436	O	–	SL 436 T	*d* : –	+
A C 6 6225	O	–	.	.	.
A T 820	O	–	A T 820 T	*d* : –	+
SL 232	Par. *d*	–	SL 232 S	*d* : –	+
S. enteritidis					
SW 971	O	–	SW 971 T	*g, m* : –	–
NCTC 203	O	–	.	.	.
NCTC 3045	O	–	.	.	.
NCTC 6676	O	–	NCTC 6676 S	*g, m* : –	–
S. dublin					
SW 553	O	–	SL 146 (S)	*g, p* : –	–
SL 76	O	–	NCTC 5766 (S)	*g, p* : –	–
SL 435	O	–	SL 435 T	*g, p* : –	–
Group D, unknown sp.					
SW 970	O	–	.	.	.
SW 972	O	–	.	.	.
S. paratyphi B					
SW 543	O	–	SW 543S	*b* : –	low e.o.p.
SW 908	O	–	SW 908 S	*b* : *1, 2*	+
SW 966	O	–	SW 966T	*b* : *1, 2*	(+)
SL 51	O	–	SL 51 T	*b* : *1, 2*	+
A U 2	O	–	.	.	.
A U 15	O	–	A U 15 B (T)	*b* : *1, 2*	+
A U 26	O	–	A U 26 M (T)	*b* : *1, 2*	+
SR 107	O	–	SR 107 T	*b* : (*1, 2*)	(+)
SL 368	Par. *b* : *1, 2*	–	SL 368 T	*b* : (*1, 2*)	(+)
SW 906	Par. *b* : *1, 2*	–	SW 906 S	*b* : *1, 2*	+
S. typhimurium					
SW 541	O	–	SW 541 T	*i* : *1, 2*	–
SW 544	O	–	SW 544 S	*i* : *1, 2*	+
SW 545	O	–	SL 89 (S)	*i* : *1, 2*	+
SW 549	O	–	SW 594 (S)	*i* : *1, 2*	+
SW 964	O	–	SW 964 S	*i* : (*1, 2*)	+
SW 965	O	–	SW 965 S	*i* : (*1, 2*)	+
SL 15	O	–	SL 15 T	*i* : (*1, 2*)	+
A U 14	O	–	A U 14B (T)	*i* : *1, 2*	+
A U 20	O	–	A U 20M (T)	*i* : *1, 2*	+
SL 56	O	–	SL 61 (S)	*i* : *1, 2*	–
SW 573†	O	–	.	.	.
SL 43†	Paralysed from SW 573	–	SL 43 S	*i* : *1, 2*	+
SL 499	Par. *i* : *1, 2*	–	SL 499 S	*i* : *1, 2*	+
SW 1153	Par. *i* : *1, 2*	–	SW 1153 S	*i* : *1, 2*	+
SW 578	Par. *i* : *1, 2*	–	SW 582 (S)	*i* : *1, 2*	+
SW 580	Par. *i* : *1, 2*	–	SW 583 (S)	*i* : *1, 2*	+
SJ 60	Par. *i* : *1, 2*	–	SJ 60 S	*i* : *1, 2*	+

Table 6 (*cont.*)

Nonmotile strain			Motile derivative*		
Strain	O or paralysed	Sensitivity	Strain	Antigen	Sensitivity
S. heidelberg					
AU 1	O	–	AU 1 T	*r* : *1, 2*	(+)
AU 21	O	–	AU 21 M (T)	*r* : *1, 2*	–
SL 28	O	–	SL 142 (S)	*r* : *1, 2*	+
S. paratyphi A					
A 17689	O	–	.	.	.
SL 14	O	–	.	.	.
NCTC 13	O	–	NCTC 13 S	*a* : –	low e.o.p.
NCTC 8052	O	–	.	.	.
NCTC 8285	O	–	.	.	.
NCTC 8388	O	–	.	.	.
NCTC 8389	O	–	.	.	.
S. paratyphi C					
SL 236	O	–	SL 236 S	*c* : *1, 5*	+
SL 437	O	–	SL 437 S	*c* : *1, 5*	+
S. cholerae-suis					
NCTC 5735	O	–	NCTC 5735 S	*c* : *1, 5*	+
Group C1, unknown sp.					
AU 23	O	–	.	.	.
Group C2, unknown sp.					
AU 25	O	–	.	.	.
S. riogrande					
NCTC 7399.3	Par. *b* : *1, 5*	–	NCTC 7399.3	*b* : (*1, 5*)	+
S. milwaukee					
NCTC 9890	O	–	NCTC 9890 S	*f, g* : –	–
S. abortus-equi					
SJ 30	'Curly' No translational motility	+	SJ 30 S	– : *e, n, x*	+
SL 220‡	.	–	SL 223 (S)	– : *e, n, x*	+
SL 221‡	.	–	SL 224 (S)	– : *e, n, x*	+
SL 222‡	.	–	SL 225 (S)	– : *e, n, x*	+

* Motile derivatives are designated S or T depending on whether they were obtained as spontaneous mutants or by transduction.

Antigens given in brackets were probably present in the strain although not detected in the culture tested.

† SL 43 was isolated from the non-flagellated *S. typhimurium* strain SW 573 as a flagellated but non-motile (paralysed) mutant (Stocker, Zinder & Lederberg, 1953).

‡ SL 220, SL 221, and SL 222 were strains of *S. abortus-equi* from Dr Kato described in the text. They produced few flagella and only a small proportion of the bacteria were motile in media of pH 7·2.

Resistant variants selected by the χ phage. Most of the strains found to be sensitive in the host-range tests were grown overnight in broth with the χ phage and examined microscopically the next day to estimate the proportion of motile bacteria. The strains fell broadly into four classes as shown in Table 7: class 1, 0–0·1% motile; class 2, 5–10% motile; class 3, 30–50% motile (over 80% of bacteria in control cultures were motile) and class 4 showing no decrease in the percentage motile.

Most strains fell into class 1. The three species *Salmonella typhi, S. paratyphi B* and *S. typhimurium*, of which a large number of strains were tested, behaved

differently in that most of the *S. typhi* and *S. paratyphi B* strains were in class 1 while the *S. typhimurium* strains were more evenly distributed amongst classes 1–4. Three of the strains of *S. typhimurium* tested more than once gave different results on different occasions, while the strains of the other two species gave consistent results in repeated tests.

Table 7. *Reduction of motility in overnight broth cultures infected with the χ phage*

Species	Number of strains in motility class *			
	1	2	3	4
S. typhi	79	5	3	0
S. paratyphi B	37	0	1	0
S. typhimurium	12 (+3)	12	14 (+3)	9
S. paratyphi A	5	0	0	0
Others	35 (+2)	11	4 (+1)	6 (+1)

* See text.

It was difficult to isolate stable phage-resistant mutants from most strains. The present experiments confirmed the finding of Rakieten & Bornstein (1941) that cultures which remained non-motile so long as phage was present could give rise to subcultures which regained motility when freed from the phage. This could be explained if all the genotypically motile bacteria present did not actually exhibit motility, and if phenotypic nonmotility was enough to make them resistant to the phage. Genotypically homogeneous salmonella strains have been studied in which not every bacterium is motile, but in which the proportion of motile organisms is a genetic characteristic of the strain (Quadling & Stocker, 1957). The phage might eliminate the motile bacteria, leaving those which were nonmotile either in genotype (none or a small minority) or merely in phenotype (the majority). Those that were merely phenotypically non-motile would give motile descendents. Some of the nonmotile bacteria in the culture may have been paralysed by the χ phage itself, as will be described later; but such bacteria might not give rise to colonies.

Table 8 shows the stable resistant variants that were obtained after attempts to isolate six variants from each of 26 strains. Some of these were flagellated but paralysed, again showing that flagellar inactivity leads to resistance. The resistant variants appeared to be of three types: non-flagellated; paralysed; slightly motile, i.e. broth cultures contained 25% or less of motile organisms often showing slow or irregular movement. As might be expected, variants belonging to the third class were not completely resistant; the most concentrated phage suspension often produced slight (+) clearing. Variants of this kind were chiefly found in strains of *Salmonella abortus-equi* but this was not due to reversion to the state described by Kato (1954) for freshly isolated strains, since these variants were not more motile, or more χ phage sensitive, at pH 6·5 than at pH 8·0. Examination of films from these cultures stained by Leifson's (1951) method showed that the mean number of flagella/bacterium was much lower than in their motile, phage-sensitive derivatives.

All the motile mutants of the resistant variants regained phage sensitivity.

Table 8. *Stable resistant variants selected by the χ phage from sensitive strains*

Strain	Stable resistants obtained: Total	Nature: O	Nature: Paralysed	Nature: Slightly motile	Swarms obtained*
S. typhi					
A. A Cr.	4	4	0	0	O
A. C8 6608	4	3	1	0	1 (from O)
A. E4 5839	2	1	1	0	O
A D1 5434	0	.	.	.	.
A. L2 131	0	.	.	.	.
SW 537 (H901)	7	4	3	0	4 (from O) 3 (from par)
SW 540	5	5	0	0	2 (from O)
S. paratyphi B					
A. 1927	3	3	0	0	2 (from O)
A. 3a 1	1	1	0	0	O
A. 1815	0	.	.	.	.
A. 1249	0	.	.	.	.
A. 1910	0	.	.	.	.
A. Workshop	0	.	.	.	.
SW 546 (*1, 2* : –)†	1	1	0	0	1 (from O)
S. typhimurium					
SL 396	6	6	0	0	3 (from O)
LT 7	4	4	0	0	O
LT 2†	3	1	2	0	1 (from O) 1 (from par)
SW 593	6	6	0	0	5 (from O)
A. M4618	6	6	0	0	4 (from O)
A. U20M	6	6	0	0	3 (from O)
SL 394	0	.	.	.	.
SL 656	0	.	.	.	.
S. abortus-equi					
NCTC 5727	11	6	0	5	4 (from sl. mot.)
SL 224	6	4	0	2	2 (from sl. mot.)
S. stanley					
SW 536†	1	1	0	0	1 (from O)
S. cholerae-suis					
NCTC 5737	1	1	0	0	O
NCTC 5738	0	.	.	.	.
S. memphis					
NCTC 7402	4	3	0	1	2 (from O) 1 (from sl. mot.)
S. riogrande					
NCTC 7399	4	3	1	0	2 (from O) 1 (from par)

* All the swarms obtained from O, paralysed or slightly motile variants were highly motile and χ phage-sensitive.

† Resistant variants obtained from the collection of the Guinness–Lister Unit.

Adsorption experiments

The χ phage adsorbed slowly to many strains that were sensitive, there being no significant decrease in titre of free phage after 10–20 min. contact with the bacteria at concentrations of $2–5 \times 10^8$ organisms/ml. (Table 9). Thus, it was difficult to attribute the resistance of any given resistant strain to failure of adsorption. In

Table 9. *Adsorption of the χ phage to naturally occurring motile strains*

Species	Sensitivity	Number of strains with which phage in supernatant was reduced to <20 %	reduced to 20–60 %	not detectably reduced
S. typhi	+	6	2	0
	+(shallow plaques)	1	1	0
	(+)	1	1	1
	−	1	0	1
S. abortus-equi	+	2	0	0
S. paratyphi B	+	2	0	0
	+(shallow plaques)	1	0	0
	(+)	0	1	0
S. typhimurium	+	0	2	0
	+(shallow plaques)	0	0	6
	(+)	0	0	1
	−	0	0	1
S. stanley	+	1	0	0
	−	1	0	0
S. paratyphi A	−	1	0	0
Others, with H antigens other than *g*...	+	1	0	0
	+(shallow plaques)	1	2	3
	(+)	2	4	1
	−	1	1	5
With antigen *g*...	−	0	0	2

examining non-motile strains (either O or paralysed) only the results with strains whose sensitive motile variants absorbed the phage rapidly were considered to have any significance (Tables 10, 11) and the same considerations applied when the effects of different flagellar antigens were compared (Table 12).

A marked contrast was seen between:

(1) adsorption to naturally occurring non-motile strains and to their motile derivatives (Table 10);

(2) adsorption to non-motile or poorly motile derivatives isolated by the χ phage from naturally motile strains, and to motile forms of these strains (Table 11);

(3) adsorption to derivatives of a single strain carrying either an H antigen which prevents infection and of an H antigen which allows infection (Table 12). One of these strains, SW 674, gave very striking results since it absorbed rapidly in phase 2, with antigen *1,2*, but not in phase 1, with antigen *g,p*. Only the host-range mutant to which the bacteria were sensitive, not the wild-type phage or other host-range mutants, adsorbed perceptibly to bacteria with H antigens *l*..., *e,h* or Arizona *13* (Table 12).

As shown in Tables 10 and 11, no detectable adsorption occurred to any of the non-flagellated strains, but two paralysed strains (SL 232 and SW 537.1*a*), and perhaps a third (SL 478) did produce a significant drop in the titre of free phage. With the paralysed strains of *Salmonella typhimurium* SL 43 and *S. riogrande* NCTC 7399.3, no antibody-resistant phage could be detected, and in an experiment with SL 232 in which 65 % of the phage was sedimented with the bacteria, only 0·08 % of the attached phage gave rise to infective centres after passage through antiserum. Thus, even if the phage does attach to a paralysed bacterium, infection is very unlikely to follow.

Table 10. *Adsorption to non-motile strains and their motile variants*

Strain	State of the organisms	Sensitivity	Phage remaining in supernatant
S. typhi			%
SL 77	Non-flagellated	−	NL
SL 77 swarm	90 % motile	+	10·5
SL 100	Non-flagellated	−	NL
SL 100 swarm	95 % motile	+	16·6
SL 232	Paralysed	−	63, 35
SL 232 swarm	60 % motile (slow)	+	30, 10
S. paratyphi B			
SW 906	Paralysed	−	NL
SW 906 swarm	95 % motile	+	16·5
S. typhimurium			
SW 573	Non-flagellated	−	NL
SI 43	Paralysed	−	NL
SL 43 swarm	90 % motile	+	40
LT 2	80 % motile	+	33
SL 499	Paralysed	−	NL
SL 499 swarm	90 % motile	+	48
SW 1153	Paralysed	−	NL
SW 1153 swarm	70 % motile	+	48
S. riogrande			
NCTC 7399	60 % motile	+	17·5
NCTC 7399.3	Paralysed	−	NL
NCTC 7399.3 swarm	90 % motile	+	12
S. abortus-equi			
SL 220	20 % motile, poorly flagellated	−	NL
SL 224	98 % motile	+	6
NCTC 5727	95 % motile	+	7·5
SJ 30	'Curly' flagella; rotating movement	+	NL

The adsorption mixtures were held at 37° for 10–20 min. before centrifugation.
NL = no detectable loss of phage from the supernatant.

Resistance of a strain was not in all cases due to failure of adsorption. Four strains which appeared resistant in the usual sensitivity tests on agar, nevertheless, rapidly absorbed the phage. Two of these strains, *Salmonella stanley* NCTC 92 and *S. typhi* A D4 T 107, were also tested in broth; the results showed that although 85–95:% of the phage had adsorbed, only 0·4% in the case of NCTC 92 and 3%in the case of A D4 T 107 gave plaques after passage through antiserum.

Association of flagella and adsorption

The effect of inhibiting flagella formation and of removal of flagella. *Salmonella abortus-equi* NCTC 5727, in common with other salmonellas, does not form flagella when grown at 44°. At 44° this strain grew more slowly than at 37°, reached a lower final concentration after overnight incubation and produced many short filaments.

Adsorption was measured at 44° and at 37° to cultures grown at each of these temperatures. No translational movement was seen in the culture grown at 44° although 1 % of the organisms were rotating, and there was no detectable adsorption

Table 11. *Adsorption to sensitive strains and χ-selected variants*

Strain	State of the organisms	Sensitivity	Phage remaining in supernatant (%)
Salm. typhi			
SW 537 (H901)	95 % motile	+	8·5
SW 537/χ	Non-flagellated	−	NL
SW 537/χ swarm	90 % motile	+	23
SW 537.1a	Paralysed	−	44
SW 537.1a swarm	90 % motile	+	12
S. riogrande			
NCTC 7399	95 % motile	+	17·5
NCTC 7399.6a	Non-flagellated	−	NL
S. memphis			
NCTC 7402	90 % motile	+	50
NCTC 7402.3a	Non-flagellated	−	NL
S. typhimurium			
LT 2	95 % motile	+	33
SL 478	Paralysed	−	70
S. abortus-equi			
NCTC 5727	95 % motile	+	7·5
3 isolates	Non-flagellated	−	NL
3 isolates	< 2 % motile	−	NL
3 isolates	*c.* 40 % motile	(+)	30–70
SL 224	90 % motile	+	6
SL 224.5b	1 % motile	−	NL
SL 224.5a	50 % motile	(+)	27

The adsorption mixtures were held at 37° for 10–20 min. before centrifugation.
NL = no detectable loss of phage from the supernatant.

after 15 min. at either 37° or 44°. On the other hand, there was good adsorption to the culture grown at 37°, in which 90 % of the organisms were normally motile, 86 % of the phage being absorbed at 37° and 91 % at 44°.

Flagella can be detached from bacteria without killing the organisms, either by short exposure to acid, by rubbing cultures over stiff agar, or by treatment in a Blendor (Stocker & Campbell, 1959). Any of these methods greatly reduced the adsorption to *Salmonella abortus-equi* NCTC 5727 (Table 13), the blendor being least effective, probably because the method leaves short stumps of flagella on the bacteria (Stocker & Campbell, 1959). The presence of residual flagellar fragments was indicated here by observing rotational movement in the blended suspension; the acid-treated and the rubbed suspensions contained virtually no motile bacteria.

Regeneration of flagella. Actively growing bacteria largely regenerate their flagella within one doubling time after treatment in the blendor (Stocker & Campbell, 1959). The rate of adsorption of the χ phage to *Salmonella abortus-equi* NCTC 5727 increased progressively, while the bacteria were incubated in broth after blending (Fig. 1), and electron microscopy showed that during this time the flagella increased from small stumps to their normal length and also became more numerous.

Table 12. *Adsorption of wild-type χ phage, and host-range variants to bacteria with different H antigens*

	Wild-type phage		Host-range mutants		
Strain	Sensitivity reaction	Phage remaining in supernatant (%)	Mutant selected by:	Sensitivity reaction	Phage remaining in supernatant (%)
NCTC 5723 (*g,m*)	−	NL	.	.	.
NCTC 5723.*i* (*i*)	+	35	.	.	.
SW 674 *g,p* phase	−	NL†	.	.	.
SW 674, *1,2* phase	+	4	.	.	.
SW 537	+	15	.	.	.
SW 569 (*i*)	+	15	.	.	.
SW 667 (*g,p*)	−	NL‡	.	.	.
SW 537.*l*,z_{13}(*l*,z_{13})	Low e.o.p.	NL	SW 537.*l*,z_{13}	+	18
			NCTC 8717	+	14
			SL 651	+	7
			NCTC 8278	$<\chi$	NL
			NCTC 7318	$<\chi$	NL
SW 668 (*e,h*)	Low e.o.p.	NL	NCTC 8278	+	50
		NL*	NCTC 8278	.	28*
			SW 537. *l*,z_{13}	$\leqslant\chi$	NL
			NCTC 7318	$\leqslant\chi$	NL
SL 651 *l,v* phase	Low e.o.p.	NL	SW 537.*l*,z_{13}	+	18
			SL 651	+	40
S. victoria (1, 9, 12 : *l,w* : *1,5*)					
E 504, *l,w* phase	−	NL	SW 537.*l*,z_{13}	+	12
E 504, *1,5* phase	+	30	.	.	.
NCTC 7318	Low e.o.p.	NL	NCTC 7318	+	36
		NL*	NCTC 7318	.	10*
			SW 537.*l*,z_{13}	$\leqslant\chi$	NL

The absorption mixtures were held at 37° for 10–20 min. before centrifugation.

* Adsorption mixture incubated for 2 hr. in the presence of chloramphenicol before centrifugation.
NL = no detectable loss of phage from the supernatant.
† 0·07 % was found to survive exposure to antiphage serum, which probably represents the proportion of bacteria in the opposite phase.
‡ About 0·005 % survived exposure to antiphage serum, which was no more than in a control suspension without bacteria.

Tests for adsorption of phage to detached flagella

The results described so far suggest that the primary site of adsorption may be the flagella themselves. However, suspensions of flagella from sensitive strains detached from bacterial bodies neither inactivated the phage nor protected it from neutralization by antiphage serum (Table 14); nor did they promote infection of the

non-flagellated strain of *Salmonella typhi* O 901, whose flagellated and motile derivative, H901, is fully sensitive. Moreover, the phage did not appear to adhere to free flagella so far as could be seen in experiments in which it was added to free flagella which were later removed from suspension by adding H antibody and formalinized bacteria (to which the phage no longer adsorbs) carrying flagella of the same antigenic type. On centrifugation the bacteria were deposited, presumably taking with them the free flagella; the titre of free phage remained unchanged.

Table 13. *Adsorption of the χ phage to bacteria whose flagella were removed by rubbing on stiff agar, by acid-treatment or by blending in an M.S.E. Blendor*

	Motility (%)	Viability (%)	Adsorbed phage (antibody-resistant) (%)	Unadsorbed phage (%)
Expt. 1				
Untreated	75	(*a*) '100'	90	14
		(*b*) '100'	91	18
		(*c*) '100'	87	15
Acid-treated (pH 2·6)	<0·1	(*a*) 25	0·4	85
		(*b*) 6·3	2·2	100
		(*c*) 12	.	95
Rubbed	<0·04	(*a*) 100	0·29	88
		(*b*) 100	0·1	96
		(*c*) 100	.	100
Blended	<0·05 translational (some rotators)	(*a*) 100	21	71
		(*b*) 100	38	60
		(*c*) 100	20	73
Expt. 2				
pH 2·2 ½ min.	<0·1	60	0·3	.
pH 2·6 ½ min.	<0·1	61	0·37	.
pH 3, 1 min.	<0·1	79	0·55	.
pH 7, 1 min.	80	'100'	75	.

S. abortus-equi NCTC 5727 was grown overnight on 1·25 % (w/v) agar plates. For the 'rubbed' preparation, bacteria were transferred by wire loop to a 4 % (w/v) agar plate and rubbed over it with a glass spreader for 3 min. They were then collected in distilled water and washed twice.

For the 'untreated', 'acid-treated' and 'blended' preparations, bacteria were gently soaked off in distilled water and washed once. The suspension was then divided into two parts; one part, which was to provide the untreated and acid-treated bacteria, was washed once again. The other part was blended for 3 min. and then washed once again. All the suspensions were adjusted to a total bacterial count of 5×10^{10}/ml., and dilutions in broth were examined for motility.

Acid treatment consisted in mixing 0·1 ml. of the previously untreated and well-washed suspension with 0·9 ml. of buffer pH 2·6 for 1 min. at 45°,* and then neutralizing with 9 ml. of either (*a*) buffer pH 7, (*b*) broth, or (*c*) broth containing 50 μg./ml. chloramphenicol. (Addition of either broth or buffer pH 7 raised the pH to about 6.) The neutralized suspensions were examined for motility. The 'untreated', 'rubbed' and 'blended' bacteria were similarly exposed to 45° for 1 min. using buffer pH 7; they were also tested after dilution into buffer pH 7, broth or broth with chloramphenicol.

Counts of viable bacteria were made, and adsorption of phage in each suspension was tested by mixing $7·5 \times 10^7$ phage particles with $6·5 \times 10^9$ bacteria, and counting: (1) the total number of plaques produced; and after 15 min. at 37° (2) the number of plaques after dilution through anti-phage serum (infected bacteria), and (3) the number of plaques produced by the supernatant after centrifugation.

In Expt. 2, the bacterial suspension, prepared as before, contained 3×10^{10} bacteria/ml. The acid was neutralized with buffer pH 7, and 3×10^9/ml. bacteria were present in the adsorption mixture.

* The rate of flagellar lysis is temperature-dependent (Weibull & Tiselius, 1945; Duncan, 1935).

Effect on adsorption of artificially paralysing Salmonella abortus-equi *NCTC 5727*

It is clear from the preceding experiments that genetically paralysed bacteria are resistant to the χ phage and that this is associated with impaired adsorption of the phage. This observation raises the question as to whether a phage-sensitive motile strain would become resistant to the phage and absorb poorly if it were immobilized without loss of flagella. This can be done in several ways, for example, by thoroughly washing the bacteria (Stocker & Campbell, 1959). Experiments with *Salmonella abortus-equi* NCTC 5727 showed that bacteria grown in broth were still motile when resuspended in 10^{-4}M-phosphate buffer containing 10^{-3}M-$CaCl_2$ which

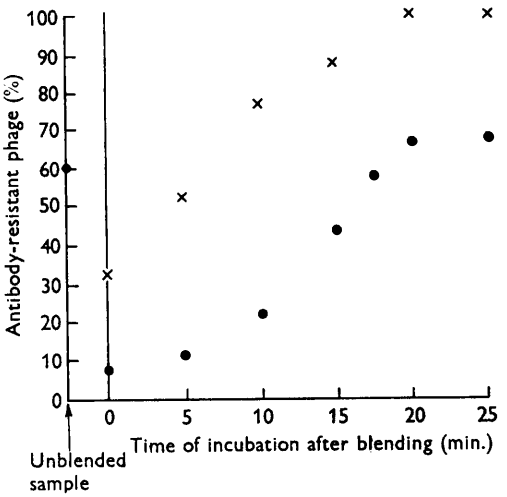

Fig. 1. Regeneration of flagella and adsorption. An actively growing culture of *Salmonella abortus-equi* NCTC 5727 was blended for 3 min. to detach the flagella and then incubated at 37° in a shallow layer of broth in a flask. Immediately after blending, and at 5, 10, 15, 17½, 20 and 25 min., samples were transferred to tubes containing 50 μg./ml. chloramphenicol (which arrests flagellar synthesis; Kerridge, 1959) and held at room temperature. The samples were examined for motility and total bacterial counts were made on the first and the last. *Sample taken immediately after blending*: total count, $2{\cdot}7 \times 10^8$/ml.; motility, 0·93 % transitional movement, 22% rotational movement.

Sample taken at 25 min.: total count, $4{\cdot}6 \times 10^8$/ml.; motility, 82 % translational movement. Samples taken at intermediate times showed progressively increasing proportions of motile bacteria; at first rotating bacteria predominated; at 15 min. 33 % of the bacteria showed translational movement which was rather slower than normal; and at 20 min. 60 % appeared to be fully motile.

When all the samples had been collected, phage was added to each to a concentration of $7{\cdot}5 \times 10^6$ p.f.p./ml. The numbers of infected bacteria (●) 2 min. and (×) 20 min. later were measured by plating the mixtures after exposure to antiphage serum.

must have contained only about 1 % (v/v) broth, and the phage adsorbed normally. However, after washing 3–4 times in buffer, no bacteria showed translational motility and less than 5 % were rotating. Phage adsorption to such suspensions was greatly decreased (Table 15); it increased again when the bacteria regained motility, which they did immediately after either broth or histidine were added to the buffer suspension (Stocker & Campbell, 1959).

Similar results were obtained with bacteria immobilized by several unrelated agents such as chloral hydrate or Merthiolate (Eli Lilley & Co) (Robertis & Peluffo, 1951), 2,4-dinitrophenol, aureomycin or erythromycin (Table 14). 2,4-Dinitrophenol at $2{\cdot}5 \times 10^{-3}$M or more was needed; and its action was immediately

Table 14. *Tests for adsorption of the χ phage to detached flagella*

Mixtures		Total p.f.p.	Proportion of antibody-resistant phage (%)	Phage in supernatant
Expt. 1.				
Flagella – : *1,2*. Phage	15 min., 37°	$7{\cdot}1 \times 10^8$	$<0{\cdot}05$	.
Flagella *i* : *1,2*. Phage	15 min., 37°	$7{\cdot}1 \times 10^8$	$<0{\cdot}05$	.
Broth. Phage	15 min., 37°	$6{\cdot}7 \times 10^8$	$<0{\cdot}05$	.
Expt. 2.				
Flagella – : *1,2*. Phage	15 min., 37°	$1{\cdot}2 \times 10^9$	$<0{\cdot}09$	.
Buffer. Phage	15 min., 37°	$1{\cdot}1 \times 10^9$	$<0{\cdot}09$	.
Expt. 3.				
Flagella – : *1,2*; O 901 $2{\cdot}5 \times 10^8$/ml. Phage	20 min., 37°	.	$<0{\cdot}04$	.
Flagella *i* : *1,2*, O 901 $2{\cdot}5 \times 10^8$/ml. Phage	20 min., 37°	.	$<0{\cdot}04$	.
O 901 $2{\cdot}5 \times 10^8$/ml. Phage	20 min., 37°	.	$<0{\cdot}04$	.
Broth. Phage	20 min., 37°	$7{\cdot}9 \times 10^8$	$<0{\cdot}04$	.
Expt. 4.				
Flagella – : *1,2*. Phage	5 min., 37°	$7{\cdot}6 \times 10^7$	$<0{\cdot}013$	7×10^7
After 5 min. formalinized. SW 1061 added		.	.	.
Flagella – : *1,2*. Phage Formalinized SW 1061	10 min., 37°	$8{\cdot}7 \times 10^7$	$<0{\cdot}01$	$5{\cdot}1 \times 10^7$
Formalinized SW 1061. Phage	10 min., 37°	$6{\cdot}2 \times 10^7$	$<0{\cdot}01$	$6{\cdot}3 \times 10^7$
Broth. Phage	10 min., 37°	$6{\cdot}6 \times 10^7$	$<0{\cdot}01$	$5{\cdot}7 \times 10^7$*

In each individual experiment, the mixtures contained the same amount of phage, and after the time stated were assayed for total number of plaque-forming particles (p.f.p.) and by dilution through antiphage serum for antibody-resistant phage. Expt. 3 was made to see if the presence of flagella would promote infection of the non-flagellated strain of *S. typhi*, O 901. In expt. 4, a test was also directly made for physical adherence to flagella: formalinized bacteria of *S. typhimurium* SW 1061 (– : *1,2*) were added to the mixtures either at once or after 5 min., and H antibody *1,2* was subsequently added to agglutinate the free flagella (Craigie, 1931) and, it is presumed, to cause their attachment to bacteria whose flagella were of the same antigenic type. After centrifugation, the supernatant was assayed for unattached phage.

* Here there were no bacteria or flagella present, and the mixture was not centrifuged: the figure given is the plaque count after addition of anti-*1,2* serum, which did not affect the phage.

The flagellar suspension in Expt. 4 was used at a final dilution of 1/20, and a dilution of 1/2 in the first mixture before the addition of the formalinized bacteria. In Expts. 1–3 the suspensions were used at a dilution of 1/10, at which they showed a faint opalescence.

annulled on dilution to below 3×10^{-4}M. Chloral hydrate and Merthiolate have been reported to immobilize *Proteus vulgaris* reversibly, but in the present case chloral hydrate, and to a lesser extent, Merthiolate, at concentrations sufficient to immobilize, killed a considerable proportion of the bacteria. Aureomycin was more effective in immobilizing than erythromycin, but a high concentration of each was needed and both took about 30–40 min. at room temperature to produce their full

Table 15. *Adsorption to artificially paralysed* NCTC 5727

Bacteria treated with:	Motility	Phage in supernatant (%)	Motility restored by	Phage in supernatant (%)
Washing	0 % translational 1–5 % rotating	70–80	1/5–1/25 broth (motility 90–100 %)	6–15
			1/75 broth (motility 5 % slow)	50
			0·01 M histidine (motility 40 % slow + 10 % rotating)	34–47
			0·05 M histidine (50 % slow)	40
Merthiolate				
9×10^{-3} M-3×10^{-3} M	1–10 %	NL	.	.
$9{\cdot}8 \times 10^{-4}$ M	40 %	31	.	.
$3{\cdot}4 \times 10^{-5}$ M	95 %	12	.	.
Chloral hydrate				
0·12 M	0	75	.	.
2,4-dinitrophenol				
$2{\cdot}5 \times 10^{-3}$ M	0	80	Removal of drug (60	22
$3{\cdot}3 \times 10^{-3}$ M	2 % rotating	76	80 % motility)	14
Aureomycin				
0·5 mg./ml.	(*a*) 40 min., <1 % translational, 5 % rotating	60–100	.	.
	(*b*) 2 min., 90–98 % motile	10–20	.	.
Erythromycin				
0·5 mg./ml.	40 min., 2 % translational 5–50 % rotating	60	.	.
Terramycin				
0·5 mg./ml.	98%	7	.	.
Streptomycin				
0·5 mg./ml.	98%	6	.	.
Chloramphenicol				
0·5 mg./ml.	95–98%	4–9	.	.

Adsorption was measured after 10 min. to late log or stationary phase bacteria at about 5×10^8/ml., with a phage multiplicity of less than 1. In a few experiments with bacteria treated by washing, dinitrophenol or aureomycin, the adsorption mixtures were also assayed after passing through antiphage serum to measure the proportion of infected bacteria. This agreed in all cases with the estimates obtained from assay of the supernatant. In each experiment, control bacteria which had not been treated left 10 % or less of the phage in the supernatant.

Bactericidal effect of the drugs; survival after 15 *min. exposure:*

Merthiolate	9×10^{-3} M	10 %	Aureomycin	1 mg./ml.	50 %
	$1{\cdot}7 \times 10^{-3}$ M	50 %	Erythromycin	1 mg./ml.	30 %
Chloral hydrate	0·12 M	<0·1 %	Terramycin	1 mg./ml.	90 %
2,4-dinitrophenol	$2{\cdot}5 \times 10^{-3}$ M	85 %	Streptomycin	1 mg./ml.	46 %

Merthiolate, 3×10^{-3} M for 15 min., inactivated 40 % of the phage. The phage was stable in the other drugs over the period of the experiments.

effect. Before it had affected the motility of the bacteria, aureomycin did not affect adsorption of the phage. The effect of aureomycin on motility may not have been reversible, but electron micrographs showed that the flagella were not destroyed. Terramycin, streptomycin and chloramphenicol did not affect motility or phage adsorption. It may be concluded from these experiments that bacteria which are merely phenotypically non-motile adsorb the χ phage more slowly, just as do genotypically paralysed strains.

Motility was irreversibly abolished by formaldehyde or by heating at 56° for 30 min.; no detectable adsorption occurred to cultures treated in either way. Neither treatment is supposed to alter the H antigen, but formaldehyde evidently denatures the flagellar protein (Astbury, Beighton & Weibull, 1955).

Electron microscopy

Electron micrographs of formalin-fixed, air-dried platinum+iridium-shadowed mixtures of *Salmonella abortus-equi* NCTC 5727 and the χ phage, which had been washed to remove the free phage, showed phage particles on the flagella, apparently attached by the tips of their tails (Pl. 2, figs. 3 and 4). The tails of some particles were curved as if they were genuinely attached in this way and had been bent while the specimens were prepared (Pl. 2, fig. 5). The bacteria could evidently absorb only a limited number of particles, as shown by estimates of the proportions of unadsorbed phage in an experiment where bacteria were mixed with varying concentrations of phage. The capacity appeared to be about 6 p.f.p.; if only about 1/5 of the total particles were plaque-formers, as the particle counts by fluorescence microscopy suggested, this would represent about 30 particles/bacterium, which was about the maximum number seen on electron microscopy.

It was impossible to say that there were no phage particles attached to the bodies of the bacteria, for phage particles were often seen near the body; but, as there was always a network of flagella in the region, they might as well have been attached to a flagellum as to the body itself. Control preparations of *Salmonella abortus-equi* NCTC 5727 with phage P22 (Pl. 2, fig. 6) showed many particles of this phage along the bodies of the bacteria, and none on the flagella, which strongly suggested that particles of the χ phage seen on the flagella were specifically attached. Attachment appeared to occur anywhere along the length of a flagellum, but groups of two, three, or more particles were commonly seen attached at or around a single point (Pl. 2, fig. 7). In contrast, phage stocks did not contain clumped particles except rarely, and then these were centred round a fragment of detached flagellum. The presence of these clusters suggests either that some portions of a flagellum favour phage attachment, or that the attachment of one particle encourages the attachment of others.

When the bacteria had been deflagellated in the blendor before mixing with the χ phage, an occasional phage particle was seen on the remaining flagellar stumps; none was seen on the bodies of the bacteria. When the bacteria were blended after mixing with the phage, fewer phage particles were seen than with bacteria which had been blended before exposure to the phage; this suggests that the distal parts of flagella which are removed by blending can compete for adsorption of the phage with the proximal parts which survive blending.

The synthetic strain SW 674 (*g,p*: *1,2*) was examined in both the phase

exhibiting *1,2*, which was phage sensitive, and the phase with *g,p*, which was resistant. The flagella of the two phases did not differ in appearance, but large numbers of particles were seen on the flagella exhibiting antigen *1,2* while none was seen with those exhibiting antigen *g,p*. Analogous observations were made on the following strains in either their paralysed or their normally motile forms: *Salmonella typhimurium* SW 1153, a paralysed variant of strain LT2; *S. riogrande* NCTC 7399.3 (40 : *b* : *1,5*); *S. typhi* SW 537.1*a*. The latter is the only one of these three paralysed strains to which any adsorption of the phage was detected and here electron microscopy showed an occasional particle attached to the flagella. Individual flagella appeared normal in bacteria immobilized by aureomycin although the flagella tended to form skeins. The numbers of particles seen on the flagella decreased only when the motility had fallen, 30–40 min. after exposure to the drug.

Immobilization and clumping

There are few published observations on the effect of phage infection on motility, but infection by most phages seems to leave motility unaffected until the latent period is advanced, or even until lysis (Murphy, 1957). *Salmonella abortus-equi* NCTC 5727 is certainly not immobilized for some time after adsorption of a clear-plaque mutant of phage P22, and the same is probably true of a motile strain of *Escherichia coli* B (Furness & Rowley, 1955) and phage T2. (Bacteria of this latter organism, unlike salmonellas, tend to stop swimming in preparations held between slide and coverslip; however, no difference could be observed between bacteria which had absorbed phage T2 and control preparations of uninfected bacteria.) On the other hand, immediately after mixing salmonella NCTC 5727 with the χ phage at sufficiently high multiplicity, the bacteria became immobile and formed clumps of 10–20 bacteria. The effect was most striking, and could be readily observed when a drop of high titre phage stock was placed on a slide at one edge of a coverslip, a drop of highly motile culture placed at the opposite edge and the two allowed to mix gradually. Where they met, rapidly swimming bacteria came to an abrupt halt and then often joined other already immobilized bacteria to form clumps. Agglutination of infected bacteria was reported by Beardsley (1960), but only began when new phage started to be released; a loss of motility which this author also mentioned evidently did not immediately follow on infection.

Immobilization of *Salmonella abortus-equi* NCTC 5727 by the χ phage could be prevented either by treating the phage stock with antiphage serum or by removing the phage particles by centrifugation. However, phage inactivated by ultraviolet radiation or by over-centrifugation appeared to immobilize as efficiently as infective phage, suggesting that the effect was due to adsorption of the phage, which need not be followed by multiplication.

When the phage was diluted, progressively fewer bacteria were immobilized. The process went rapidly to completion with *Salmonella abortus-equi* NCTC 5727; with a concentration of phage which left some motile bacteria, the proportion of these did not alter perceptibly after the first few minutes. Serial dilutions of several different phage preparations were tested for their immoblizing activity by estimating the proportions of bacteria which still showed translational motility. In each experiment the immobilizing activity decreased more rapidly with dilution of the phage than would be expected if one phage particle per bacterium were sufficient. The

possibility is not excluded that an individual flagellum might be inactivated by adsorption of a single particle.

Other sensitive strains besides *Salmonella abortus-equi* NCTC 5727 were also immobilized by the χ phage, but generally the process was slower, and there was less clumping of the immobilized bacteria. Among the strains that were tested was SW 674 which was rapidly immobilized in the phase with H antigen *1,2*, but showed no loss of motility in the phase with *g,p*. Strains SW 537.l,z_{13}, NCTC 8717 exhibiting l,z_{13}, and SL 651 exhibiting *l,v*, were immobilized by the phage mutants which they had selected, but not by the wild-type phage or the other host-range mutants. Strain E 504 (*l,w* : *1,2*), in the phase with *1,2* was immobilized by either wild-type phage or the mutant selected by SW 537.l,z_{13}, but in the phase with *l,w* it was immobilized only by the latter. Analogous results were obtained with strains SL 507 (*S. typhi* carrying *e,h*) and NCTC 8278 (*e,h* : *1,2*) which was tested in each phase. Similarly, Arizona NCTC 7318 was slowly immobilized by the mutant which it had selected, but not by the wild-type phage or other mutants. Each of the host-range mutants rapidly immobilized *S. abortus-equi* NCTC 5727. Experiments were made with NCTC 5727 whose flagella had been removed by the blendor. Here, since the bacteria were necessarily immobile, only clumping could have been observed. None occurred. Thus it appears that the clumping is a sequel to attachment of the phage to the flagella. Immobilization and clumping following phage adsorption was not accompanied by morphological changes in the flagella so far as could be seen by electron microscopy.

DISCUSSION

Previous authors reported that the χ phage attacked only flagellated bacteria, but it is now clear that flagella alone do not lead to susceptibility; these must be both active and of a correct antigenic type. They must also be present in suitable bacteria, for not all motile strains with the correct H antigens were sensitive. Resistance in the latter case might be due to inability of the phage to multiply in the bacteria (particularly with the few strains to which the phage readily adsorbed); but when resistance was associated with absence of suitable flagella, it was evidently due to failure of adsorption. The importance of the H antigen in the control of susceptibility immediately suggests that the phage adsorbs to the flagella themselves, since the H antigen is present only on the flagella and does not extend over the surface of the bacterial body (Craigie, 1931; Stocker & Campbell, 1959). Removal of the flagella from sensitive bacteria impaired adsorption. Direct evidence for attachment of phage particles to active flagella of correct antigenic type was provided by electron micrographs.

The importance of the bacterial strain itself in the control of sensitivity was most clearly seen when two strains carrying the same H antigen differed in their reactions, although the H antigenic determinant of the one had been received from the other by transduction, when the antigenic complex is known to be transferred unaltered (Lederberg & Edwards, 1953). Presumably, either the phage could not multiply in the resistant strain or the flagella of the two strains differed significantly in characteristics, other than the H antigen, which affected adsorption or initiation of infection. Transduction of the H antigenic determinant is unlikely to change all the

genes concerned with flagella (Stocker *et al.* 1953); hence, in a hybrid resulting from transduction of an H antigen, the other flagellar characters would probably be those of the recipient and not those of the donor strain.

The phage presumably failed to adsorb to isolated flagella for the same reason as it failed to adsorb to paralysed strains, namely, because the flagella were not functioning. Flagella such as are carried by salmonellas are helical in shape (Leifson, Carhart & Fulton, 1955); in living preparations of moving bacteria, they appear as rotating spirals (Reichert, 1909; Pijper, 1938; Weibull, 1950, 1951), and in fixed preparations they are flattened into a sinuous form. It is currently thought (Astbury *et al.* 1955) that motility as well as the helical shape of flagella result from the passage of a spiral wave of contraction down the flagellum, the helical line of contraction which is continuously moving along being due to the transmission of contractile pulses in subfibrils of which the flagellum is probably composed. The reflexions seen in X-ray analysis of flagellar preparations have been interpreted to indicate the presence of polypeptide chains in two different states of folding, and it is thought that the undulations leading to active flagellar movement are brought about by a rhythmical change of length between one configuration and the other. The two polypeptide chain configurations are always present simultaneously in preparations of flagella detached from the bacterial body, and these, in addition, retain their wave shape; thus it is supposed that flagella detached in the course of transmitting the wave of contraction remain fixed exactly as they were at the moment they were broken off (Astbury *et al.* 1955). Paralysed and motile bacteria are morphologically and serologically similar. The flagella of paralysed bacteria, although not moving, show no difference in wave form from the flagella of motile ones, and no difference in X-ray diffraction pattern (Beighton, Porter & Stocker, 1958). In particular, flagella isolated from paralysed bacteria also possess the features which indicate that the protein is present in two different configurations. Thus motile and paralysed flagella may both have similar helical lines of contraction, but in a paralysed flagellum this is static and not transmitted as a wave. In other words, isolated flagella and flagella of paralysed bacteria are thought to have the same array of features as active flagella, which consequently do not possess any unique structures that could be tentatively identified with the phage receptor. Two possible explanations for the need for active flagella are either that the phage receptor becomes unmasked during active movement, or that functioning of the flagella is required *per se* for adsorption either to occur, or to be irreversible and to lead to infection. The slight adsorption which was detected with a few paralysed strains, which was not followed by infection, may mean either that attachment can occur with decreased efficiency, perhaps transiently, to inactive flagella, or that the flagella of these particular strains had perhaps some slight activity short of that required for motility. The degree of activity associated with motility is evidently necessary for initiation of infection, possibly for the injection of the phage DNA (Hershey & Chase, 1952).

It was suggested by London (1958) that the site of adsorption of the χ phage to the bacterium may be at the basal granule of the flagellum. However, the present electron micrographs show phage particles attached along the length of the flagella, distally as well as proximally. In addition, comparisons of untreated and blended bacteria have shown that phage particles adsorbed distally can certainly infect the bacterium:

(1) When the flagella have been reduced to short stumps by blending, not only is the rate at which the phage particles attach to the bacteria decreased, but so is the rate at which the bacteria become infected (as measured by antibody-resistant infective centres) and this returns progressively to normal as the flagella regenerate (Fig. 1). The alternative explanation for this finding, namely, that the motility of the flagellated bacteria increases the chances of collision between the phage and the proximal part of the flagella, seems intuitively unlikely to account for the magnitude of the difference.

(2) If phage particles which have attached to distal parts of a flagellum fail to infect, then a phage preparation should show a higher titre when titrated by adsorption to blended bacteria than to bacteria with long flagella, provided a sufficiently long time is allowed for adsorption; no difference in titre is observed.

If, therefore, the primary site of phage adsorption can be at any point along the flagellum, the phage genome might reach the bacterial body by any of the following routes:

(*a*) by passage of the entire phage particle up the outside of the flagellum until it reaches the junction with the body and there injects its DNA. Although no precise estimates have been made, electron micrographs of samples of phage-bacterium mixtures, which had been fixed at different times after mixing, gave no indication of a drift of phage particles up the flagella towards the bacterial bodies;

(*b*) by adsorption of the phage particles to the bacterial body in the usual way, following some kind of 'activation' by contact with a flagellum. If this were so, then a phage particle after attachment to a flagellum belonging to one bacterium might be able to infect a different bacterium, but there was no indication that this could occur;

(*c*) by being injected into the flagellum at the point of initial attachment, and then travelling in the flagellum into the bacterial body. The pattern common to larger flagella and cilia is evidently a cylindrical arrangement of 9 subfibrils enclosing 2 more, and theoretically this allows the latter to roll round in the cylinder (Astbury *et al.* 1955). If bacterial flagella are structurally similar, the χ phage DNA might pass along the channels or potential channels that this arrangement might provide. Electron microscopy has sometimes suggested that bacterial flagella are made up of subfibrils (Starr & Williams, 1952; Labaw & Mosley, 1955) although in most electron micrographs, including the present ones, no fine structure can be seen.

With fully motile bacteria, the phage genome evidently reaches the bacterial body not long after adsorption, for the stages could not be separated by removing the flagella: when bacteria were mixed with phage for 1 min. (when 50 % of the phage had adsorbed) and then blended for 1·5 min., there was no decrease in the total number of plaque-forming entities (i.e. infected bacteria + free phage) nor in the number of infected bacteria alone (measured by the number of antibody-resistant infective centres sedimented by centrifugation at 1100 *g*.) Nor was there any increase in number of non-sedimentable p.f.p. as might occur if phage in a state able to infect new bacteria were detached with the flagella.

The sudden loss of active flagellar movement upon adsorption of phage seems to be an effect peculiar to the χ phage. In general, phage infection interferes only with bacterial syntheses, not with energy-yielding processes (Cohen, 1949) which, together with the fact that more than one particle of the χ phage per bacterium is needed, may imply that the immobilization observed here is due to a direct effect on the flagella.

This might be either an effect on the energy supply or on the structure of the flagella. Attachment of coliphage T2 results in activation and liberation of phage enzymes, notably the phosphatase (Dukes & Kozloff, 1959; Kozloff & Lute, 1959), and the cell-wall lytic enzyme (Weidel & Primosigh, 1958) which can cause gross morphological changes in the bacterium. For example, adsorption of coliphage T2 causes isolated bacterial cell walls to shrivel (Williams & Fraser, 1956). Thus, by analogy, adsorption of the χ phage to the flagellum might well cause gross changes in flagellar structure and immediate loss of activity. Such changes could also account for the agglutination that follows phage adsorption, since flagella (Scholtens, 1938) and their H antigens (Ogonuki, 1940) are known to influence the stability of flagellated organisms in suspension. Agglutination is not due to immobilization *per se* since genetically and artificially paralysed bacteria do not clump. It is interesting to note that two strains with abnormal 'curly' flagella, *Salmonella abortus-equi* SJ 30 and a similar variant of a *S. typhimurium* strain (Dr Iino, personal communication), agglutinate spontaneously whereas their derivatives with normal flagella are stable.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Particles of the χ phage negatively stained with phosphotungstic acid. $\times 163{,}000$.

Fig. 2. Particles of the χ phage negatively stained with phosphotungstic acid. Some collapsed heads can be seen. $\times 130{,}000$.

PLATE 2

Fig. 3. Particles of the χ phage on the flagella of NCTC 5727: fixed, shadowed preparation. $\times 24{,}300$.

Fig. 4. Particles of the χ phage on the flagella of NCTC 5727: fixed, shadowed preparation. $\times 7800$.

Fig. 5. Particles of the χ phage on a flagellum of NCTC 5727: the tail of one is bent (see text). Preparation negatively stained with phosphotungstic acid. $\times 270{,}000$.

Fig. 6. Particles of phage P22 surrounding the bacterial bodies of NCTC 5727: fixed, shadowed preparation. $\times 8000$.

Fig. 7. Cluster of particles of the χ phage (see text). $\times 8000$.

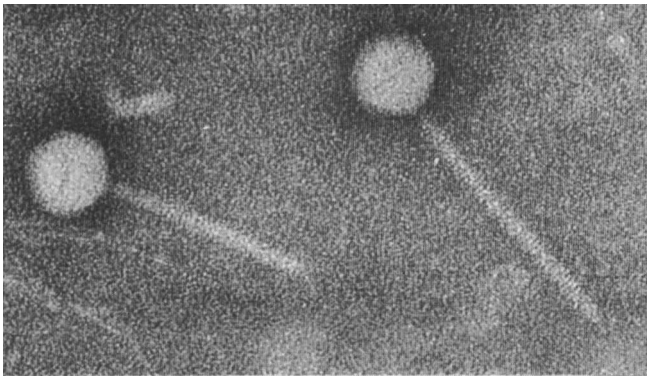

Fig. 1

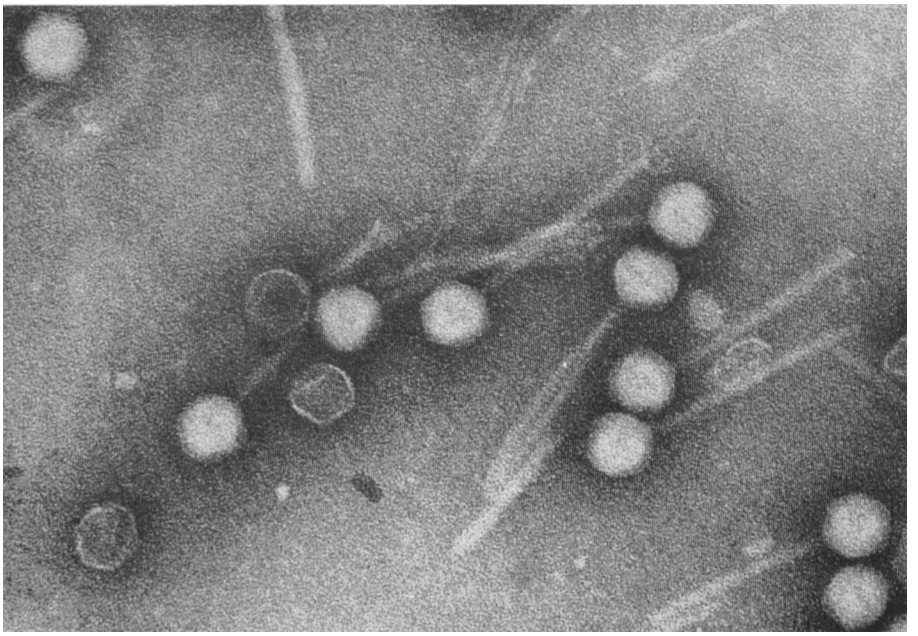

Fig. 2

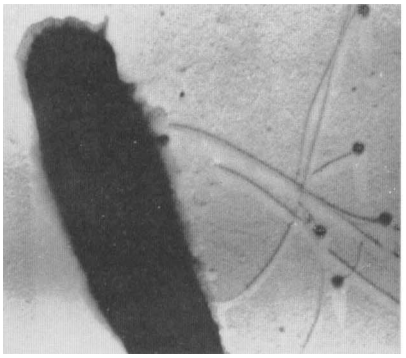

Fig. 3

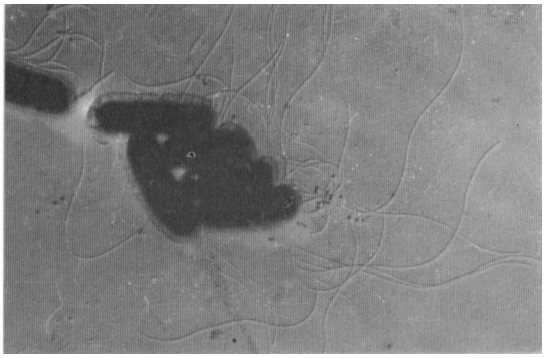

Fig. 4

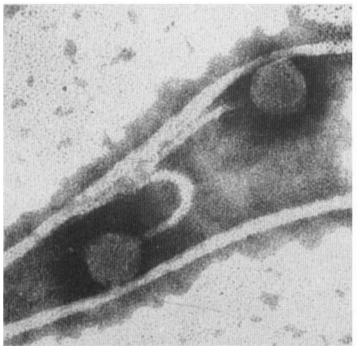

Fig. 5

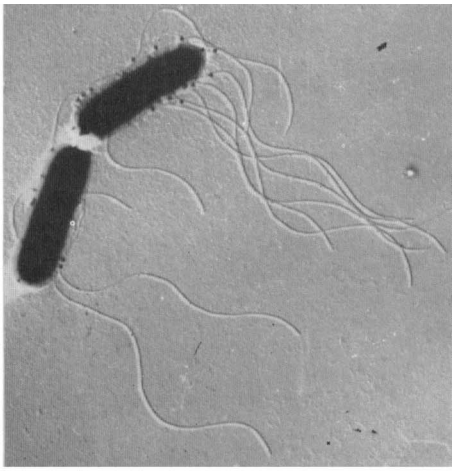

Fig. 6

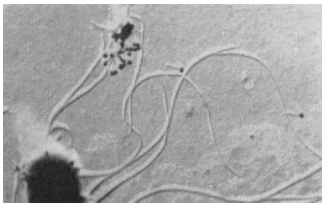

Fig. 7