STUDIES ON A LYSOGENIC *BACILLUS* STRAIN

I. A BACTERIOPHAGE SPECIFIC FOR *BACILLUS ANTHRACIS*

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INTRODUCTION

Smith, Gordon & Clark (1946), in their monograph on the genus *Bacillus* which has brought order into that group, have reduced the named species to a reasonable number. Recognizing the capacity which members of this genus have for variation of observable characters, they have classified as variants of other species some strains which earlier workers have regarded as distinct species. In the case of most of the species which they suppress this seems justifiable, but in the case of *B. anthracis* they may have oversimplified the position. They state: '*B. anthracis* differed from *B. cereus* in being pathogenic and non-motile. Since both are mutable characters, *B. anthracis* has been placed as a variety of *B. cereus*.' Epidemiological considerations suggest that *B. anthracis* is a valid species despite the paucity of criteria for its differentiation from *B. cereus* in the laboratory; Chu (1950, and personal communication) has introduced some additional *in vitro* tests for the differentiation of typical *B. anthracis* from *B. cereus*, but since each one of the characters on which these tests are based has been shown to be liable to variation, the problem of differentiation of an atypical *B. anthracis* from a *B. cereus*, typical or atypical, still remains.

Many bacteriophages which attack exclusively strains of one taxonomic group, though not necessarily all strains of the group, have been described. In their monograph Smith *et al.* (1946) have described bacteriophages which they obtained from soil and which were active on various members of the genus *Bacillus*; some of these bacteriophages were strain-specific or attacked only a few strains of a particular species, others had a wider range. One which they describe attacked *B. cereus* and *B. anthracis* strains.

A bacteriophage active on *B. anthracis* has been described by Cowles (1931), who obtained it from sewage by serial passage of filtrates on a strain of *B. anthracis*. This bacteriophage attacked all the American Type Culture Collection strains of *B. anthracis* except two strains which Cowles found to be motile and which he therefore suspected were not *B. anthracis*; it also attacked a strain of *B. mesentericus*, a designation the significance of which is now uncertain. Cowles apparently only tested this bacteriophage on a small number of *Bacillus* strains.

A bacteriophage which is derived from an atypical *Bacillus* strain, called strain W has been found to attack all strains of *B. anthracis* which have been tested and to attack only two out of fifty-six strains of *B. cereus* tested; it attacks no strains of other species of *Bacillus* tested. The taxonomic position of strain W

is at present somewhat uncertain, but it would appear to be an atypical *B. cereus*. Filtrates of ageing broth cultures of this strain contain this bacteriophage, which has been called phage W. It attacks, and can be propagated on, strains of *B. anthracis*, and also strain W, the strain from which it is obtained. This unusual phenomenon, the lysis of a lysogenic strain produced by the application of the phage which it carries, is being further investigated, and will be the subject of a later paper.

METHODS AND MATERIALS

The sensitivities of the *Bacillus* strains to phage W were tested by spotting a drop of an undiluted filtrate on to a lawn of the particular strain.

The media used in the routine testing of the strains were Hartley's Tryptic Digest agar, and Lemco agar, each containing 1·2 % New Zealand powdered agar.

In the case of strains which were received on agar slopes, a quarter of an agar plate was inoculated directly with a loop from the slope of each strain. Those strains which were received in the dried state were grown overnight in broth, and the next day a loopful of the broth culture of each strain was spread over a quarter of an agar plate.

Two preparations of phage W were used in the routine tests. One of these was phage W propagated on the strain from which it was derived, strain W, the other was phage W propagated on another strain, *B. anthracis* strain Davis. The two preparations were made thus: several 5 ml. quantities of broth were inoculated with the bacterial strain, and incubated at 37° C.; about 2 hr. later a drop of an earlier filtrate was added to each tube. Eighteen hours later the broth cultures were pooled and filtered through sintered glass filters. The filtrates were stored in the cold room. Each filtrate was titrated, both immediately after filtration, and at intervals during the investigation, on *B. anthracis* strain Davis by a modified Miles and Misra technique, that is drops of 0·02 ml. of serial tenfold dilutions in broth of the filtrate were spotted on plates previously spread with a few drops of a broth culture of *B. anthracis* strain Davis, and dried at 37° C. with the lids off. During the whole investigation two preparations of the phage propagated on strain W, and two preparations of the phage propagated on strain Davis were used; each preparation gave a plaque count of approximately 5×10^8 per ml. on strain Davis, both immediately after filtration and after periods of storage in the cold room. A few experiments were made with phage W propagated on other strains with preparations made in the same way.

Strain W produces an antibiotic which would appear to be active only on members of the genus *Bacillus*, resembling the colicines of Gratia and Fredericq, (Fredericq, 1948) which are produced by Enterobacteriaceae and are active only on members of that group, and the inter-strain antibiotic effect found by Francis & Rippon (1949) in *B. polymyxa*. This antibiotic inhibits the growth of many strains, not only of *B. anthracis*, but also of other species; it is not normally present in filtrates of broth cultures unless Tween 80 has been added before filtration, but very rarely it has been found in filtrates made in the absence of Tween 80. Each new filtrate of strain W was therefore tested to exclude the presence of the antibiotic by

spotting it on a strain of *B. cereus* known to be sensitive to the antibiotic but not to the phage; antibiotic activity was not found in any of the filtrates used for the phage-sensitivity tests.

For the routine phage-sensitivity tests a drop of each of the undiluted phage preparations was spotted on the quarter plate spread previously with the bacterial strain to be tested. The plates were incubated in air at 37° C. for 18 hr., and the results were recorded at the end of this time.

In all, 171 strains of *B. anthracis* were examined: 108 of these had been isolated at Bradford from infected hides, and their pathogenicity had been proven; 23 had been isolated at Wakefield, and their pathogenicity had been proven; 8 were isolated at Cardiff from human cases of anthrax, and their pathogenicity had been proven; 12 were strains from the National Collection of Type Cultures; 1 was a highly virulent stock culture of the London School of Hygiene and Tropical Medicine; 17 were stock cultures of the Bacteriology Department of Queen's University, Belfast. The characters of these last seventeen strains were fully investigated by the tests of Smith *et al.* (1946) and of Chu, and these strains were inoculated into mice. Fifteen of them were virulent, and the phage was tested on these both before and after they had been passed through mice. Two were avirulent; they had both been isolated from human cases of anthrax many years ago, and since then had been subjected to repeated sub-cultivation.

Two further avirulent strains, Weybridge and B_1, were also included in the investigation. Both give rise to non-mucoid rough growth on 20% serum agar in an atmosphere of high CO_2 content.

In all, 244 strains of other species of the genus *Bacillus* were examined. These consisted of:

B. cereus	29 strains from the National Collection of Type Cultures 13 strains from the Wellcome Bacterial Collection 14 strains from the Bacteriology Department of Queen's University, Belfast
B. mycoides	8 strains from the National Collection of Type Cultures
B. megatherium	17 strains from the National Collection of Type Cultures 13 strains from the Wellcome Bacterial Collection 1 strain from the Bacteriology Department of Queen's University, Belfast 2 strains (strain '899' and the 'mutilat 337' of den Dooren de Jong) from the Institut Pasteur, Paris
B. carotarum	3 strains from the National Collection of Type Cultures
B. subtilis	39 strains from the National Collection of Type Cultures 4 strains from the Bacteriology Department of Queen's University, Belfast
B. subtilis var. *niger*	3 strains from the National Collection of Type Cultures
B. licheniformis	22 strains from the National Collection of Type Cultures 2 strains from the Bacteriology Department of Queen's University, Belfast
B. pumilus	17 strains from the National Collection of Type Cultures
B. coagulans	5 strains from the National Collection of Type Cultures
B. lentus	1 strain from the National Collection of Type Cultures

B. polymyxa	6 strains from the National Collection of Type Cultures
B. macerans	5 strains from the National Collection of Type Cultures
B. circulans	9 strains from the National Collection of Type Cultures
B. laterosporus	4 strains from the National Collection of Type Cultures
B. alvei	6 strains from the National Collection of Type Cultures
B. brevis	8 strains from the National Collection of Type Cultures
B. sphaericus	11 strains from the National Collection of Type Cultures
B. sphaericus var. *loehnisii*	1 strain from the National Collection of Type Cultures
B. freudenreichii	1 strain from the National Collection of Type Cultures

RESULTS

All the strains of *B. anthracis* tested were found to be attacked to some degree by phage W. Except for two strains of *B. cereus*, none of the strains of other species of *Bacillus* were attacked. The two strains of *B. cereus* which were attacked, N.C.T.C. 1651 and N.C.T.C. 6222, were submitted to the tests of Smith *et al.* (1946) and to those of Chu, and they were found to have the characters of *B. cereus*: thus their growth on 20% serum agar in an atmosphere of high CO_2 content is rough and non-mucoid, they give a marked zone of turbidity on egg-yolk agar plates, and they are motile.

N.C.T.C. 1651 is 'strain No. 3' which was received in the National Collection of Type Cultures from the Kral Collection through the Rothamsted Agricultural Experimental Station as *B. mycoides*. In 1938 it was examined by Gibson who classified it as a strain of *B. cereus*.

N.C.T.C. 6222 is No. 4342 of the American Type Culture Collection. It was isolated from milk in 1928 by Alban Stewart and called *B. lacticola*. In 1941 it was examined by Gibson who considered it to be *B. cereus*. Smith *et al.* (1946) classify it as *B. cereus*.

Relative activities of phage W propagated on strain W and on strain Davis

The preparation of phage W propagated on *B. anthracis* strain Davis was found to have a wider range of action on strains of *B. anthracis* than that propagated on strain W. It also had a more marked action on many strains which were attacked by both preparations. The results are shown in Table 1 where the figures refer to the number of strains of *B. anthracis* in each category.

The lesser virulence of W-propagated phage for some strains manifested itself in two ways. On some strains on which Davis-propagated phage produced an area completely devoid of growth, W-propagated phage produced only an area of thinned growth; on other such strains W-propagated phage produced only a few discrete clear plaques; and on others again it produced a few discrete clear plaques in an area of thinning. This could be interpreted to mean that where there was thinning a considerable proportion at least of the phage particles attacked the strain, though inefficiently, whereas the clear plaques were formed by rare mutant phage particles which were able to attack the strain efficiently. A preparation of phage from one of these plaques gave complete clearing of the strain on which

the plaque was formed, as did Davis-propagated phage. The action of Davis-propagated phage on strains which it attacked feebly was only manifested by a slight thinning of the growth in the area on which it had been spotted.

Table 1. *Action of phage W on strains of* Bacillus anthracis

Area where phage spotted	Phage W propagated on strain Davis	Phage W propagated on strain W
Completely clear Clear with a few discrete colonies	119	37
Thin growth	46	55
Just perceptible thinning of growth Isolated plaques	6	65
Appearance unaltered	0	14
Total number of strains	171	171

Effect of capsulation

Strains of *B. anthracis*, which in their normally rough state were strongly attacked by the phage, were not perceptibly attacked when they were in the mucoid state, that is when they were grown on 20 % serum agar in an atmosphere of high CO_2 content, unless the inoculum was very thin. It would seem, therefore, that capsulation protects an organism from attack by the phage. A proportion of the organisms in a culture may be attacked, but the amount of mucoid material formed by the others may be sufficient to mask the lysis of this proportion. Variants which gave rise to rough non-mucoid growth on serum agar in an atmosphere of high CO_2 content were attacked to an apparently equal degree on this medium and on nutrient agar without serum in air. A few of the strains which had been repeatedly subcultured gave, when grown on serum agar in an atmosphere of high CO_2 content, a lawn of confluent growth which was only slightly mucoid; the phage attacked these strains only slightly less markedly when they were in this state than when they were grown on nutrient agar in air, but on one or two of these the area where the phage had been spotted was marked by a circle of extremely mucoid growth, as though the phage were acting as a selective agent for the mucoid elements in the strains.

Effect of prolonged incubation

On examination of the plates on which the sensitivity tests were made after these plates had been incubated for 18 hr. at 37° C., and again after they had been kept at room temperature for a few days, several observations were made:

(1) On many strains the area where the phage had been spotted was surrounded by a mucoid ring which was sometimes apparent after the preliminary incubation of the plate, but more often not until the plate had been kept a further few days at room temperature.

(2) Several strains showed, either at 18 hr. or more often later, mucoid discrete colonies in the clear area where the phage had been spotted. Subculture from

these colonies almost always gave rise to normal rough growth sensitive to the phage.

Burnet (1929) describes mucoid colonies in areas of clearing produced by phage on strains of *Salmonella*. These mucoid colonies also showed normal phage-sensitivity on subculture.

(3) The secondary and tertiary colonies, which have been described in *Bacillus anthracis* by Preisz (1904), Hadley (1927) and others, did not appear on the area on which the phage had been spotted, even in the strains which were only feebly attacked.

(4) The action of the phage on strains which it attacks partially or feebly became much more obvious after the plates had been kept at room temperature for several days after their primary incubation for 18 hr. at 37° C. The growth in the area where the phage had been spotted did not merely appear thinner relative to the increasingly thick surrounding growth, but actually became thinner.

Degree of sensitivity to Davis-propagated phage

The degree of sensitivity of the strains to Davis-propagated phage divided them broadly into three categories:

(*a*) Those on which a drop of undiluted phage gave a completely clear area, or one in which there were only a few isolated discrete colonies, regardless of the size of the inoculum.

(*b*) Those on which a drop of undiluted phage preparation produced an area of thin growth when the inoculum was large, and a completely clear area when it was smaller.

In these two categories appropriate dilutions of the phage produced plaques on the strains.

(*c*) Those on which the undiluted phage preparation had an appreciable action only if the bacterial inoculum was large; in such strains the effect produced was a layer of growth only slightly thinner than that on the surrounding medium. If the inoculum was smaller the phage produced no detectable effect after 18 hr. incubation at 37° C., but it sometimes caused thinning after a further few days at room temperature.

The results of successive tests on individual strains were in most cases substantially the same. As a rule repeated subculture of a strain did not alter the degree to which it was attacked by the phage to such an extent as to change that strain from one category to another, though there were sometimes minor differences in the degree to which it was attacked; these differences might perhaps have been due to the fact that the bacterial inoculum was not standardized, and also to possible relevant differences in the medium and other conditions in each test. Repeated subculture of a strain has never been found to alter it in such a way as to render it less liable to attack by the phage (except when it resulted in the isolation of variants mucoid on nutrient agar incubated in air), but experiments which were performed with strains in category (*c*), and which are about to be described, showed that subculture of these strains under certain conditions gave rise to cultures which fell into categories (*a*) or (*b*).

The strains of group (*c*) comprise the strains which have been listed in Table 1 as just perceptibly attacked by Davis-propagated phage; they are resistant to W-propagated phage. They are:

Bradford 6401 Wakefield 435 L.S.H.T.M. Mouse 5 (Belfast) N.C.T.C. 5180	Typical virulent *B. anthracis* strains which give rise to mucoid growth on 20 % serum agar in an atmosphere of high CO_2 content
Weybridge	A non-pathogenic strain of *B. anthracis* which gives rise to non-mucoid rough growth on 20 % serum agar in an atmosphere of high CO_2 content

Some experiments were conducted on some of these feebly attacked strains in attempts to discover the reason for their comparative immunity to lysis by the phage.

A slightly more dilute preparation of the phage produced the same appearance as the undiluted phage when spotted on the strains, and below a certain titre it produced no action whatsoever, so there could be no question of a prozone.

None of the feebly attacked strains has been shown to be lysogenic.

It was argued that the low virulence of Davis-propagated phage for these strains was unlikely to be due to heterogeneity of the phage particles in a Davis-propagated preparation, for, if it were, the preparation would be more likely to produce a limited number of isolated plaques than an area of slight thinning. When the phage was propagated on several other strains of *B. anthracis*, and tested on all the feebly attacked strains and on some of those strains which were attacked rather more strongly, there was no obvious difference between the actions of these preparations and the action of Davis-propagated phage.

In some cases the strains may be protected from the action of the phage by a certain degree of capsulation of the organisms on nutrient agar incubated in air, since strains which are strongly attacked in their rough state are not attacked, or are attacked to a much less obvious extent when they are in a mucoid state. This, however, cannot be the explanation in every case, for *B. anthracis* strain Weybridge is a non-capsulated strain on serum agar in an atmosphere of high CO_2 content, and non-capsulated variants selected by the method of Sterne (1937) from *B. anthracis* strain L.S.H.T.M. were not attacked to any greater extent than was the parent form of this strain.

The low virulence of the phage for these strains may be due to inefficient adsorption, or to inefficient diversion by the phage of the metabolism of these hosts to phage synthesis. There is perhaps slight evidence for this second alternative in the fact that when the organisms have ceased multiplying with their initial rapidity the phage seems to be at an advantage and to produce a more marked lysis of them. In an attempt to find out if the action of the phage would be greater if these feebly attacked strains were growing on a poor medium the following experiment was carried out with strains N.C.T.C. 5180 and Weybridge.

Inocula, large and smaller, of each of the two strains were spread over quarter plates of a basal mineral salts medium with unwashed agar and glucose, to which

various additions had been made. The additions were Lemco broth, Lemco broth and calcium chloride, Lemco broth and sodium citrate, casein hydrolysate, casein hydrolysate and calcium chloride, casein hydrolysate and sodium citrate, various groups of amino-acids, all these amino-acids, all these amino-acids and aneurin, and aneurin alone. A control plate of Hartley's tryptic digest agar was put up in parallel. The results were read after the plates had been incubated at 37° C. for 18 hr., and again after they had been kept a further 24 hr. at room temperature. No obvious changes occurred in this second period except for the control nutrient agar plate on which both the bacterial growth became thicker and the phage action became more marked.

The facts which emerged from this experiment were that the poorer the growth of both bacterial strains, the more marked was the action of the phage on them. The action of the phage was marked, and the bacterial growth was poor, on the media supplemented with amino-acids and amino-acids and aneurin; the action of the phage was rather less marked, and the bacterial growth was slightly more profuse, on the medium supplemented with casein hydrolysate. Calcium enhanced the action of the phage, and citrate reduced it, but these effects could not be dissociated from the increased action of the phage on poorly growing bacterial lawns, since calcium has an inhibitory effect on the growth of the bacteria, and, in a poor medium, citrate enhances their growth.

Action of the phage on variants of partially resistant strains

It was thought that it might be possible to obtain variants of these nearly resistant strains by such procedures as growth at 42° C., or incubation in large volumes of broth for prolonged periods, and that these variants might be more susceptible to attack by the phage than the parent strain. Experiment proved that this was so in the case of the only two strains tested, L.S.H.T.M. and Mouse 5; by both procedures it was possible to obtain cultures on lawns of which a drop of the phage gave complete clearing. It was not necessary to select single colonies, for the entire culture from a flask incubated for a few weeks at 37° C. and that from the last of a series of serial subcultures in broth incubated at 42° C. were highly sensitive.

Action of the phage on atypical variants of typical strains of Bacillus anthracis

Strains of *B. anthracis* N.C.T.C. 1607, N.C.T.C. 4991 and N.C.T.C. 7200 are attacked efficiently by the phage. To obtain variants of these strains each strain was inoculated into a flask containing 100 ml. of Lemco broth, and these flasks were incubated continuously at 37° C. At intervals of a few days a loopful from each flask was spread over the surface of a nutrient agar plate in such a way as to give single colonies. The plates were incubated in air at 37° C. for 18 hr. Colonies which differed in appearance from those of the parent strain were subcultured and tested for sensitivity to the phage. They were also tested for pathogenicity by mouse inoculation.

Colonial variants were obtained from each of the three strains; they were:

(1) *Mucoid colonies on nutrient agar incubated in air.* The phage only produced an area of clearing on these when the inoculum was small. They were non-virulent.

(2) *Phantom colonies Type* 1. These grew as phantom colonies (Nungester, 1929; Soule, 1928) on nutrient agar incubated in air, throwing off an occasional normal medusa colony. They produced mucoid growth on serum agar incubated in an atmosphere of high CO_2 content. They were attacked by the phage. They were mouse-virulent. The growth obtained from the heart-blood of the mouse was of normal thickness, and sensitive to the phage.

(3) *Phantom colonies Type II.* These grew as phantom colonies on both nutrient agar incubated in air and serum agar incubated in an atmosphere of high CO_2 content. They were attacked by the phage. They were non-virulent.

(4) *Mucoid colonies with thin rhizoid outgrowth on nutrient agar incubated in air.* This outgrowth on subculture gave rise to slowly growing rhizoid growth which was attacked by the phage. It was mouse-virulent. The growth obtained from the heart-blood of the mouse was of the same type, slowly growing thin rhizoid, with a few discrete medusa colonies of normal thickness. It was attacked by the phage.

(5) *White opaque smooth colonies.* These, on microscopic examination, were seen to consist of organisms many of which were shorter than normal, though they were not short enough to allow them to be called coccal variants. They were attacked by the phage. They were mouse-virulent. The growth obtained from the heart-blood of the mouse was of the same type, and was attacked by the phage.

(6) *Smooth.* These were attacked by the phage. They were non-virulent.

Chu has observed (personal communication) that some variants of *B. anthracis* may produce a quantity of lecithinase which equals that produced by *B. cereus* strains. This was confirmed by inoculation of the above variants of strains N.C.T.C. 1607, N.C.T.C. 4991 and N.C.T.C. 7200 on to egg-yolk agar plates, for it was found that the smooth variant of strain N.C.T.C. 4991 and the mucoid variant of N.C.T.C. 1607 produced zones of opacity on this medium very much wider than those produced by the parent strains.

DISCUSSION

Most of the characters of *B. anthracis* which serve to distinguish it from *B. cereus* are observed to be subject to frequent variation in the laboratory; they are pathogenicity, ability to form the mucoid polypeptide capsule under appropriate conditions, limited production of diffusible lecithinase, limited proteolytic activity, and non-motility. Even motile variants of *B. anthracis* have been described (Haag, 1927; Bartley, 1930; Tomcsik, 1950) and non-motile variants of *B. cereus* are not rare. The studies of Knight & Proom (1950) have shown that the minimal nutritional requirements of *Bacillus* strains are of taxonomic value in the differentiation of other species of the genus, but reports on the nutritional requirements of *B. anthracis* (Gladstone, 1939, 1949; Brewer, McCullough, Mills, Roessler, Herbst & Howe 1946; Chu, 1950 and personal communication), and a limited number of investigations of some of the strains mentioned in this paper, suggest that the nutritional requirements of *B. anthracis* vary from strain to strain to such an

extent that tests based on these are not of value in distinguishing between *B. anthracis* and *B. cereus*.

Study of the serology of spores by Chu (1950 and personal communication) has shown that though the majority of strains of *B. anthracis* may be distinguished from the majority of strains of *B. cereus* by the use of an anti-spore serum prepared against a suitable strain of the one species and absorbed with the spores of a suitable strain of the other, there are a few strains of *B. anthracis* the spore antigens of which are very closely related to those of a few strains of *B. cereus*.

The results of the investigations with phage W described in this paper suggest that sensitivity to this phage is a further characteristic feature of *B. anthracis*.

A species may be regarded phylogenetically as all the descendants of some common ancestor, forming a giant clone, or it may be regarded empirically as a group, the members of which possess all or many of a set of characters by which they may be distinguished from other such groups, the members of which lack all or most of these characters. Notions of phylogenetic relationship are based on observations of groups which possess distinctive characters showing a high degree of correlation, and so species conceived on either a phylogenetic or an empirical basis are likely to agree.

It would seem expedient, and, in comparison with other groups of bacteria which are given specific rank, justifiable from a biological point of view, to consider *B. anthracis* as a species distinct from *B. cereus* but closely related to it. It seems probable that *B. anthracis* and *B. cereus* derived from a common ancestor; typical *B. anthracis* possesses a collection of characters which enable it to invade animal tissues and cause disease, and unless the series of mutations which gave rise to *B. anthracis* occurred more than once, strains of *B. anthracis* are likely to be in their serological and phage reactions more closely related to each other than to most strains of *B. cereus*.

It is likely that in nature *B. anthracis* perpetuates itself only as a pathogen, in general only as a lethal pathogen. A number of observations (unpublished) suggest that its failure to multiply in soil may be due to its lesser production of, and greater sensitivity to, antibiotics produced by *Bacillus* strains and active within the genus *Bacillus*. In pure culture in the laboratory, however, an organism which is deficient in one or more of the characters on which pathogenicity depends is able to survive and multiply, for it is released from the selective influence of *in vivo* conditions. Unless conditions in laboratory culture are such as to exert a selective influence on basic characters such as the spore antigen and phage-sensitivity, these are likely to remain stable, so that a variant of *B. anthracis* atypical in its other characters remains distinguishable from *B. cereus*.

Sensitivity to phage W has a high correlation with the other recognized characters of *B. anthracis*, and, moreover, since all of an admittedly limited number of variants of *B. anthracis* strains tested were sensitive to the phage, it seems that sensitivity to phage W is a fairly stable character. Phage-sensitivity tests might therefore detect atypical variants of *B. anthracis* and might provide a method of discovering if *B. anthracis* throws off avirulent variants which survive in nature. The above considerations suggest that such variants do not survive.

There is no reason to suppose that the two phage-sensitive strains in this investigation listed as *B. cereus* are avirulent *B. anthracis* for they conform in all other characters, including motility, to *B. cereus*, and the fact that these two strains are attacked is therefore probably merely evidence of the close relationship between the two species.

Thus tests with phage W tend to support the concept of *B. anthracis* as a valid species, closely related to, but distinct from, *B. cereus*.

SUMMARY

A bacteriophage is described which attacks almost exclusively strains of *Bacillus anthracis*, and attacks all the strains of this species which have been tested.

As well as attacking typical strains of this species it attacks atypical variants.

Strains of *B. anthracis* are not attacked by this phage when they are in a mucoid state.

A few strains of *B. anthracis* are only feebly attacked by this phage. Some experiments, conducted in an attempt to discover the reason for this, are described.

The results are discussed and arguments in favour of regarding *B. anthracis* as a valid species are put forward.

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