A STUDY OF THE BACTERIOPHAGES OF PSEUDOMONAS AERUGINOSA

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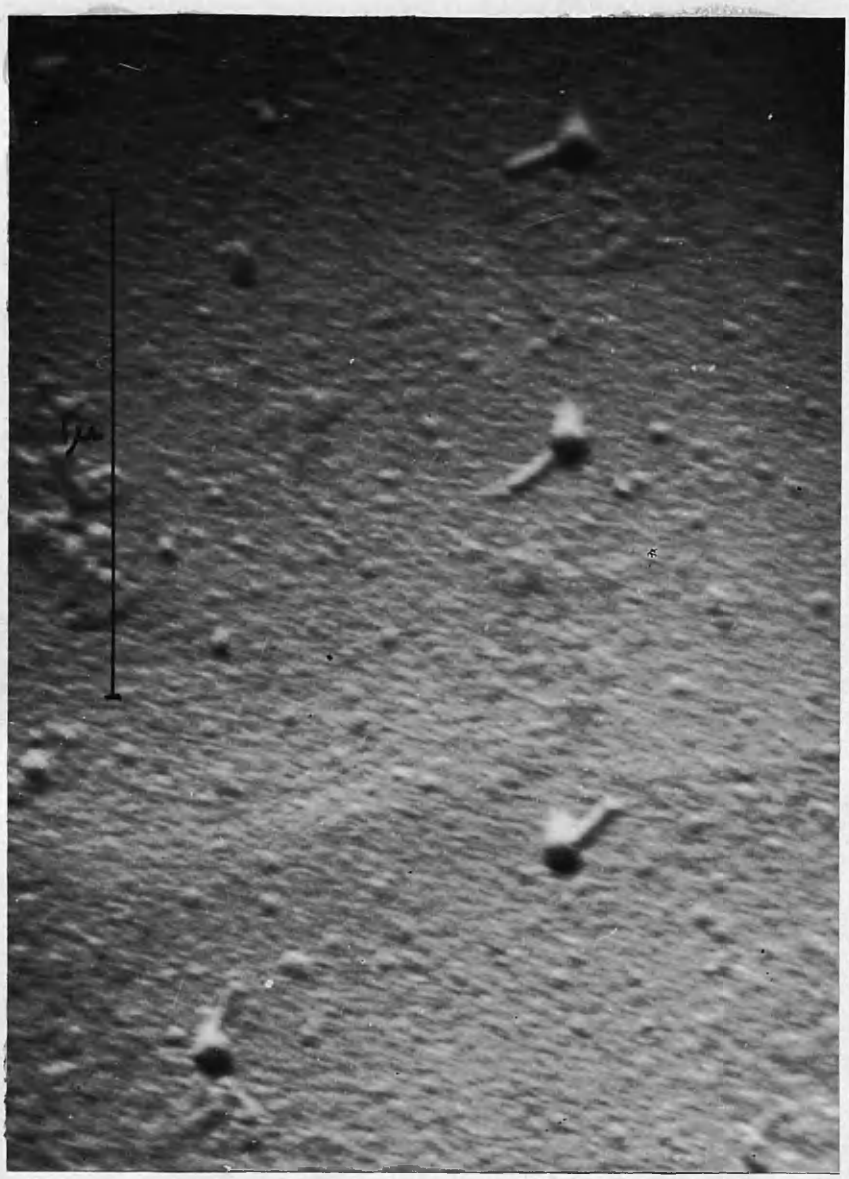

FRONTISPIECE. Electron micrograph of Ps. aeruginosa phage S2/4, shadow-cast with gold-palladium alloy. Note the "head" and "tail".

(The author gratefully acknowledges the expert assistance of Mr. J. W. Sharpe, M.A., F.Inst.P., Department of Natural Philosophy, Royal Technical College, in the preparation of this micrograph.)

PREFACE

This research was undertaken with the intention of investigating the effect of the application of type-specific phage preparations on burns infected by Pseudomonas aeruginosa (Ps. pyocyanea, B. pyocyaneus), an organism highly resistant to most wound disinfecting agents. Before such an investigation could be carried out it was necessary to isolate phages of Ps. aeruginosa and to use these phages for typing strains of this organism. This constitutes the major portion of the work embodied in this thesis.

The introduction briefly reviews the inadequacy of antibiotics and other therapeutic substances in the control of infection due to Ps. aeruginosa, discusses the possible use of phages for this purpose, and describes the conflicting opinions regarding phage activity in this species.

The main part of the investigation is divided into four sections:-

Section 1. This deals with the identification and characteristics of 203 strains of Ps. aeruginosa.

Section 2. This section describes the isolation and propagation of phages for Ps. aeruginosa by modifications of conventional phage technique.

Section 3. This section deals with the activity in vitro of the phages and their plaque morphology.

Section 4. This describes the grouping of strains

according to their phage sensitivity, these groups being compared with divisions based on other properties.

Two further sections deal with topics pursued as a result of observations made in the main study, namely, the relationship between Ps. aeruginosa and other fluorescin-producing species, and the isolation of phage from the laboratory atmosphere.

A discussion of the results is followed by a summary of the findings.

This work was carried out in the Departments of Pharmacy and Microbiology, School of Pharmacy, The Royal Technical College, Glasgow, under the supervision of Professor James P. Todd, Ph.D., Ph.C., F.R.I.C.

The author wishes to express his gratitude to Professor Todd for his helpful advice and encouragement throughout the work; to Mr. J.G.C. Campbell, B.Sc., Dip.Bact., Senior Lecturer in Microbiology, for much helpful criticism and for the photographs which appear in this thesis; to Mr. G. Rattray, Ph.C., F.R.M.S., for advice on the presentation of the work; to Miss D. Howatson, B.Sc., for her arduous task in freeze-drying, for further study, all the cultures used here; to the late Mr. A.R. Jamieson, B.Sc., F.R.I.C., Glasgow City Analyst, for arranging the supply of sewage samples; to the Governors of the Royal Technical College for providing

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INTRODUCTION

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Ps. aeruginosa in burns and other clinical conditions.

Ps. aeruginosa is widely distributed in nature, being found in polluted water and sewage (Bergey, 1948), and in soil (Coleman et al., 1943). It has been detected on normal skin in the regions of the axilla and perineum (Ruzicka, 1898).

The presence of Ps. aeruginosa in wounds is often indicated by pus ranging in colour from light green to dark blue-green. From such 'blue pus' the organism was first isolated by Gessard in 1882.

Infection of deep head wounds by Ps. aeruginosa may have fatal results (Cairns, 1944; Botterell and Magner, 1945), but to what extent the organism invades the tissues from infected surfaces is not known. Although it is apparently of low virulence in comparison with the staphylococci and haemolytic streptococci of wound infections, Colebrook et al. (1942-3) regard the bacillus as a true pathogen in burn wounds, since it is frequently accompanied by irregular fever and increasing anaemia. Bodenham (1943) found that Ps. aeruginosa could induce toxaemia and pyrexia when present alone in burns and surface wounds.

The degree to which this organism retards healing appears to depend on the extent of skin loss. Where

there is little loss of skin the influence on healing may be negligible (Pulvertaft, 1943). On the other hand, where wounds with extensive skin loss are heavily infected, no clinical improvement may occur even after removing penicillin-sensitive organisms (Barron and Mansfield,1944).

Bodenham (1943) indicated that Ps. aeruginosa has little adverse effect on the success of skin-grafting operations, but pointed out that the large amount of pus produced may float away the graft, and Gough et al.(1944) have suggested that the organism may exert toxic effects on the graft.

Ps. aeruginosa is commonly associated with suppurative lesions of the urinary and respiratory tracts and with middle-ear infections. It has been reported as the causative agent of meningitis by Moragues and Anderson (1943), Evans (1945), Paine et al. (1947), Vuylsteke (1947) and Lewin (1948), and has been involved in cases of infantile diarrhoea and gastro-enteritis (Sakula, 1943; Hunter and Ensign, 1947; Schaffer and Oppenheimer, 1948) and of ulceration of the cornea (Garretson and Cosgrove, 1927). Generalised infections due to the organism are occasionally encountered (Lilley and Bearup, 1928; Moragues and Anderson, 1943).

Therapy of Ps. aeruginosa infections.

It appears that no substance has yet been found which can be relied upon to eliminate Ps. aeruginosa from burns, wounds and other clinical conditions.

The organism is resistant to penicillin and may even inactivate it (Harper, 1943). Although streptomycin has been used successfully by Jones et al. (1944), Ryan et al. (1946), Florey et al. (1947), and Schaffer and Oppenheimer (1948), streptomycin-resistant strains develop readily (Robinson et al.,1944; Knop, 1946; Paine et al., 1947; Adcock and Plumb, 1947; Schwarz and Lazarus, 1947).

The sensitivity of Ps. aeruginosa to the sulphonamides and propamidine is variable (Cooper et al., 1939; Melton and Beck, 1939; Robson and Scott, 1941/42; Kohn et al., 1943; Morley and Bentley, 1943; Sakula, 1943; Vuylsteke, 1947). In view of this, the M.R.C. War Memorandum No.10 (1945) recommended the use of only the more active sulphonamides, the treatment to be discontinued if a response was not obtained within two or three days.

Ps. aeruginosa is resistant to C.T.A.B. (cetyltrimethylammonium bromide)(Williams et al., 1944), acriflavine and proflavine (Browning, 1943), and diflavine (Albert et al., 1938), while there appears to be some doubt as to the true merits of phenoxetol (ethyleneglycolmonophenyl ether), claimed to be effective by Berry (1944) and Gough

et al. (1944).

Alston (1944) found that boric acid in 4 per cent solution controlled, but did not eliminate, Ps.aeruginosa infection.

The available wound disinfectants, therefore, are of little value for dealing with Ps. aeruginosa.

Phage in the control of bacterial infections.

Phage may be used to control bacterial infections in two ways.

In the first, typing by means of phage can be employed to establish the true source of infection and thus assist its elimination. The value of phage-typing for strains of typhoid and paratyphoid bacilli and staphylococci is now established (Craigie and Yen, 1938; Felix and Callow, 1943; Wilson and Atkinson, 1945; Craigie and Felix, 1947). A phage-typing system for Ps. aeruginosa might prove equally valuable and enable the sources and mode of spread of the organism in hospital wards to be determined.

The second method consists of the use of phage preparations as a direct therapeutic measure. Reports on the use of phage in this way are not convincing (Krueger and Scribner, 1941). Some workers have found that blood, serum, pus and tissue debris can inhibit phage action (Zaytzeff-Jern and Meleny, 1936; Applebaum and Patterson,

1936), but others have claimed effective phage activity *in vivo* (d'Herelle, 1926; Larkum, 1926; Asheshov *et al.*, 1937; Dubos *et al.*, 1943). Much of the early work on phage-therapy is of little value since it was carried out before it was generally appreciated that a multiplicity of phage-type existed among strains of a single species, and it was seldom ascertained whether the phage used was, in fact, active against the organism to be treated (Behrens, 1949). Phage-typing is therefore an essential preliminary to phage-therapy.

Ps. aeruginosa and phage action.

Phages for *Ps. aeruginosa* have not been studied as extensively as those for other species. Fastier (1945) reported the isolation of a phage for this organism and considered his publications to be the first on the subject, but, as will be seen, several workers reported phages for *Ps. aeruginosa* prior to Fastier's publications.

Considerable confusion surrounds phage activity on *Ps. aeruginosa*, due to peculiar phage-like areas which frequently develop on cultures of many strains of the organism. These areas appear after 10 to 48 hours incubation as silvery spots on the surface of growth and gradually increase in size to form pockets 1 to 4 mm. in diameter. The normal culture largely disappears from

these areas which, when viewed by transmitted light, appear transparent and thus resemble phage plaques. By reflected light, however, the areas present a striking metallic iridescence and in this they differ from normal phage plaques. The iridescent areas remain discrete while their number is limited but become confluent as the numbers increase until, in some cases, the normal culture completely disappears.

Opinions differ as to whether or not the iridescent areas are caused through phage action. The bulk of available evidence seems to point to an agent other than phage, but the iridescent areas sometimes resemble phage plaques so closely that their presence is frequently accepted as being indicative of phage activity. Dickinson (1948), for example, speaks of the "silvery sheen" as being "typical of phage activity in this organism".

The iridescent areas and typical phage plaques differ in that the former develop spontaneously, while the latter are only produced when a phage-containing filtrate is added to a sensitive culture. This is well illustrated in the work of Hadley (1924), who divided a culture of Ps. aeruginosa into two colony types "L" and "R".

The "L" type produced pyocyanin and fluorescin and was marked by one or several iridescent areas. Sub-cultures from these areas and also from the normal growth

surrounding them always produced iridescent areas, thereby indicating that the property of producing these areas was not confined to the areas themselves, as would be expected in the case of a culture infected with a typical phage, but was distributed throughout the culture.

Colonies of the "R" type, on the other hand, produced only fluorescin and were free from iridescent areas.

Filtrates from cultures of these types did not increase the number of iridescent areas when added to cultures of either type, but when added to cultures of certain other strains ("S" type) they produced typical phage plaques with no trace of metallic iridescence.

Hadley assumed that the iridescent areas and typical phage plaques were produced by the same agent, i.e. that both were due to phage action. In this he was supported by Burnet (1930). According to these authors the iridescent areas are produced through "slow-speed" phage action. Warner (1950b) has summarised the findings of other workers and, after presenting some additional evidence, concluded that the iridescent areas and phage activity are two distinct phenomena. Some of Warner's deductions are open to criticism, and the evidence does not, as he claimed, "suggest to the point of virtual proof" that the two phenomena are distinct. The question of the causative agent of the iridescent areas is discussed

later in this thesis.

For the present purpose it is necessary to consider whether or not the nature and occurrence of these areas have a bearing on the problem of phage-typing the organism.

If the areas are due to phage action they indicate a type of lysogenesis in which some cells of the 'lysogenic' culture are sensitive to the associated 'phage'. The remaining fraction of the culture, resistant to this 'phage', will almost certainly be sensitive to other phages and may therefore be typed in essentially the same way as a lysogenic strain of any other species.

Whether or not the areas are due to phage action, there is the obvious difficulty that, owing to their close resemblance to phage plaques, they may interfere with the detection of reactions produced by specific filtrates on sensitive cultures. This difficulty has been mentioned by Warner (1950a). As will be seen, the technique adopted here has overcome this factor.

Typical plaque-producing phages for Ps. aeruginosa have been reported by several workers, e.g. Okuda (1923), Hauduroy and Peyre (1923), Lisch (1924), Pesch and Sonnenschein (1925), Asheshov (1926), Rabinowitz (1934) and Warner (1950a,b).

Fastier (1945) reported the isolation of a phage for the organism from a strain obtained from the faeces of a

patient with acute colitis. Dickinson (1948) isolated phages from several lysogenic strains and used them as test viruses in studies on the chemotherapy of virus diseases. Schultz et al. (1948), by means of electron micrographs, found two phages active for Ps. aeruginosa to consist of sperm-shaped structures.

Although the phages reported by these workers have not been studied in such detail as those for other species, there is little doubt that they conform with general observations on phage behaviour.

Definition of terms.

In view of the close similarity between typical phage plaques and the iridescent areas, and the uncertainty surrounding the nature of the latter, it must be emphasised that the terms "phage" and "plaque", as applied throughout this work, do not refer in any way to the iridescent phenomenon.

These terms are applied only in relation to specific filtrates which, when applied to sensitive cultures, produce those effects which may be observed in a study of phage action with any other species.

For convenience, and in accordance with general usage, the term "phage" is used here in preference to "bacteriophage" except in the titles of sections.

SECTION I

CHARACTERISTICS OF STRAINS OF PS. AERUGINOSA

CHARACTERISTICS OF STRAINS OF PS. AERUGINOSA

In work involving phage relationships of a bacterial species, it is of the utmost importance to identify and characterise the strains of the species being used. Only then can reliance be placed on results obtained by the use of phages in investigating possible relationships of one species to another.

In identifying strains as Ps. aeruginosa, care was taken to exclude species which, because of their close similarity in pigment production, morphology and cultural appearances, have been included in many papers relating to this organism (Meader et al., 1925). At least one of these species, Ps. fluorescens, has been considered by some authors to be merely a form of Ps. aeruginosa which has become adapted to a saprophytic existence. The relationship of the two organisms will be dealt with in Section 5 of this thesis.

Source of strains.

During the course of this work, 203 strains of Ps. aeruginosa have been studied. These, except for laboratory cultures, were obtained directly from clinical sources or isolated in this laboratory from swabs supplied by these clinical sources. The strains were isolated in places as far apart as Glasgow, Edinburgh, Manchester, London,

Belgium, Switzerland, Malta and Melbourne, and from a variety of pathological conditions.

The collection consisted mostly of strains from urine, burns and wounds, ear infections, faeces, and various ulcers and abscesses. One strain had been isolated from sputum, four from empyemas, one from a gum swab, one from nasal pus, one from cerebrospinal fluid, one from a case of osteomyelitis and two from animal sources.

Criteria of identification.

Identification of strains as Ps. aeruginosa was based on human or animal source (for freshly isolated strains), temperature requirements, pigmenting activity, gelatin liquefaction, nitrate reduction, reaction in milk, and pathogenicity. These appear to be the most reliable of the characters given by Bergey (1948) for this organism.

In order to distinguish between Ps. aeruginosa and the saprophytic Ps. fluorescens, the organism most likely to be confused with Ps. aeruginosa, most importance was attached to temperature requirements. No strain was accepted as Ps. aeruginosa unless it had an optimum temperature of 37°C., and also showed growth at 42°C.(Topley and Wilson, 1946).

EXPERIMENTAL METHODS

Temperature requirements for the growth of each strain were determined by the incubation of inoculated media, both solid and liquid, at 25°C., 37°C., and 42°C.

Pigment production.

The bluish-green pigment produced by Ps. aeruginosa has attracted a great deal of attention. Early studies by Gessard (1890, 1891, 1892) and Jordan (1899) led to the general conclusion that the pigment consisted of two different substances:-

(a) Pyocyanin, which is nonfluorescent, bright blue in colour, and soluble in both water and chloroform; and

(b) Fluorescin, which is fluorescent, yellow-green in colour, and soluble in water but not in chloroform.

In later studies, Gessard (1917, 1925) reported the production of red and black pigments by strains of Ps. aeruginosa. Red pigment-producing strains (variete erythrogene) have also been reported by Mamelle (1918) and Meader et al. (1925). Strains producing the black pigment, melanin, have been described by Vuylsteke (1947).

Meader et al. (1925) were able to show that the red pigment, which they named "pyorubrin", was produced by all their strains when suitable methods of cultivation were employed. They also found that many of their

cultures turned black, but that this was always due to concentration of pyocyanin by a combination of abundant pigment production and desiccation. After diluting the medium and extracting with chloroform, no black pigment could be found.

Bergey (1948) describes Ps. aeruginosa as producing three pigments, pyocyanin, fluorescin and pyorubrin.

(1) Media.

For the detection of these pigments the following three media were used:-

(a) 2 per cent 'Lemco' agar;

(b) Medium 'F':

Magnesium sulphate.....0.05 per cent
Dipotassium hydrogen phosphate.....0.05 per cent
Asparagine.....0.30 per cent
in distilled water.

This is the medium suggested by Georgia and Poe (1931) as being most satisfactory for the production of fluorescin. Where the amount of fluorescent pigment produced was small, the cultures were observed under ultraviolet light.

(c) Medium 'P':

Peptone.....2 per cent
Glycerol.....5 per cent
Agar.....3 per cent
in distilled water and adjusted to pH 7.8.

The above medium was used by Meader et al. (1925) who showed that strains, which on isolation produced fluorescin only, could be made to produce pyocyanin by cultivation on

this medium.

(2) Examination of strains for pigmentation.

Strains being examined for chromogenesis were grown on the above media for 12 to 18 hours at optimum temperature (37°C.) and then left at room temperature for periods of up to 4 weeks, the cultures being examined daily.

Strains which showed no production of pyocyanin after a week at room temperature were subcultured on medium 'P', subculturing on this medium being continued until pyocyanin was produced or until 25 successive transfers had been effected.

(3) Identification of pigments.

The tests applied for the identification of the pigments were as follows:-

Fluorescin: production in medium 'F' of a yellow-green fluorescence which disappeared on the addition of acid;

Pyocyanin: a blue chloroform-soluble pigment, converted by acid into a red chloroform-insoluble compound, the latter being reconverted to pyocyanin by excess of alkali;

Pyorubrin: a red colour in the chloroform-insoluble fraction, unaffected by the addition of acid or alkali. Where this pigment was not detected within 7 days, cultures

were left for 3 to 4 weeks, when excess of alkali was added and the pyocyanin removed by extraction with chloroform. This was necessary in order to make certain that the presence of pyorubrin was not being masked by abundant production of pyocyanin. (Meader et al., 1925).

Pathogenicity was tested by injection of cultures into mice. The injections were made intramuscularly, giving 0.25 ml. of a 24-hour broth culture of the organism, and using two healthy male mice (approx. 25G.) for each culture, the test being repeated where necessary to obtain closely agreeing results, and autopsies being performed to re-isolate the organism from the heart-blood and spleen.

Iridescent areas.

The production of the iridescent phenomenon was noted on 24-hour agar slope cultures.

Indole.

Indole was tested for in 24-hour peptone water cultures, using Kovac's reagent, viz. p-dimethylamino-benzaldehyde 5 G., in a mixture of 75 ml. of amyl alcohol and 25 ml. of concentrated hydrochloric acid.

The formation of indole by Ps. aeruginosa has been reported by various workers. Sherwood et al., (1926), reported that all 22 strains examined by them produced

indole, and Hadley (1924) observed that indole was produced by his "L" strain. On the other hand, Sandiford (1937) found 50 strains all negative for indole, and Pandalai (1941) obtained the same result with 85 strains. The latter authors have pointed out that previously reported positive results may have been due to the use of an acid-containing reagent, the red colour obtained being due, not to indole, but to the action of the acid on pyocyanin.

Kovac's reagent, however, cannot give a false reaction of this nature, since a positive result due to indole is denoted by a red colour in the amyl alcohol layer, and acid-pyocyanin is insoluble in amyl alcohol.

Haemolysis.

Haemolysis was noted on agar plates containing 5 per cent of oxalated horse blood.

Routine methods.

Routine methods were used for the following: catalase; methylene-blue reduction; nitrate reduction after 4 days at 37 °C.; gelatin liquefaction up to 4 weeks; reaction in milk, acid from glucose, and production of hydrogen sulphide, all up to 7 days. Bromocresol-purple (B.C.P.) was used as indicator in milk and glucose-peptone water.

RESULTS

Temperature requirements.

All strains grew at 25°C., had an optimum temperature of 37°C., and showed the ability to grow at 42°C.

Pigment production.

The number of strains producing the various pigments are shown in Table 1. Fluorescin was produced by most of the strains, both on agar and in medium 'F'; in only two strains was the pigment detected in the latter medium and not on agar. Medium 'F' is the better medium, however, since pyocyanin did not appear in this medium until at least two days after inoculation, whereas fluorescin was easily visible after overnight incubation. On agar, the presence of pyocyanin frequently masked the fluorescin produced and it was necessary to observe the culture under ultraviolet light to detect the latter pigment.

Of the strains which produced pyocyanin, about one-third did so only when cultivated on the particularly favourable medium 'P'. The number of transfers on this medium required before pyocyanin was produced varied from 1 to 7 (Table 2).

Pyorubrin production was so great in two strains that the production of the other pigments was completely masked. The total number of strains which actually

produce pyorubrin is probably greater than that shown in Table 1. Observations on the formation of this pigment were discontinued after four weeks, and it is likely that it would appear in even older cultures.

TABLE 1. - Production of pigments by strains of Ps. aeruginosa.

Pigments	Media	Strains	
		Number	Approximate percentage of total.
Fluorescin	agar or 'F'	196	96.63
	'F' only	2	0.99
Pyocyanin	agar or 'P'	130	64.10
	'P' only	64	31.55
Pyorubrin marked	agar or 'P'	2	0.99
Pyorubrin slight	agar or 'P'	44	21.69
Pyocyanin and fluorescin	agar, 'P' or 'F'	191	94.16
Pyocyanin but no fluorescin	agar, 'P' or 'F'	3	1.48
Fluorescin but no pyocyanin	agar, 'P' or 'F'	7	3.45
No pigment on isolation	agar, 'P' or 'F'	3	1.48
No pigment after optimum cultivation	agar, 'P' or 'F'	2	0.99
Total number of strains examined - 203			

TABLE 2. - Transfers on medium 'P' required for production of pyocyanin.

Number of transfers	1	2	3	4	5	6	7
Number of strains	18	14	12	4	8	7	1

Pathogenicity.

Results of pathogenicity tests indicated that varying degrees of virulence for mice existed among the 203 strains. About half this number were extremely virulent, producing a large abscess at the site of injection and proving fatal within 24 hours. A slightly lower proportion did not kill so quickly, the mice surviving for periods varying from about 36 hours to 7 days. In all these cases, the organism could be recovered from the fluid in the region of the abscess and from the heart-blood and spleen.

A small number of strains did not prove fatal within the period of 7 days, but caused only a local abscess. In these cases, cultures from the heart-blood and spleen were always negative, although the organism could be grown from the tissue fluid in the region of the abscess. A few strains proved entirely non-pathogenic, causing no abscess or other sign of pathogenicity.

A summary of these results is given in Table 3.

TABLE 3. - Pathogenicity of strains of Ps. aeruginosa for mice.

Degree of pathogenicity for mice (Intramuscular injection of 0.25 ml. of a 24-hour broth culture)	Strains	
	Number	Approximate percentage of total.
Fatal within 24 hours	102	50.29
Fatal within 7 days	79	38.95
Causing local abscess only	13	6.41
Total number of pathogenic strains.....194 Total number of strains examined.....203 Proportion of strains pathogenic for mice.....approx. 95.65 per cent		

Iridescent areas.

Almost half the total number of strains showed the phenomenon of iridescence in some degree. (Table 4).

TABLE 4. - Production of iridescence by strains of Ps. aeruginosa.

Degree of iridescence production on slope culture (24 hrs. at 37°C.)	Strains	
	Number	Approximate percentage of total.
Less than 5 small areas	14	6.90
Between 5 and 15 small areas	27	13.31
Large areas of confluent iridescence	21	10.35
Confluent iridescence almost covering culture	31	15.28

Total number of strains showing iridescence..... 93

Total number of strains examined.....203

Proportion of strains showing iridescence.....approx. 45.84 per cent

The similarity of the iridescent areas to phage plaques was most marked where the number of areas was below 15 on the slope culture, and the areas were accordingly discrete.

Reaction in milk.

All 203 strains gave an alkaline reaction in milk, this being the most constant of the biochemical tests.

With one exception, a soft coagulum was formed, and some degree of peptonisation took place in all, except two, strains; peptonisation was complete in the majority of cases by the end of 7 days, but was only partial in about 26 per cent at the end of this period. (Table 5).

TABLE 5. - Peptonisation in milk.

Degree of Peptonisation	Incubation at 37 °C. (days)	Strains	
		Number	Approximate percentage of total
Complete	2	6	2.96
Complete	3	10	4.93
Complete	4	31	15.28
Complete	5	18	8.87
Complete	6	22	10.85
Complete	7	61	30.07
Partial	7	53	26.13

Total number of strains giving peptonisation in milk.....201

Total number of strains examined.....203

Proportion of strains giving peptonisation in milk.....approx. 99 per cent

Liquefaction of gelatin.

The rate of gelatin liquefaction varied. Almost half the strains caused complete liquefaction after 24 hours at 37 °C., while in other cases liquefaction was delayed. The cultures were cooled in water before recording the result. (Table 6).

TABLE 6. - Liquefaction of gelatin.

Liquefaction of gelatin	Strains	
	Number	Approximate percentage of total.
Within 24 hours	94	46.34
Between 1 and 7 days	75	36.98
Between 7 and 28 days	30	14.79
Total number of strains liquefying gelatin within 28 days.....199		
Total number of strains examined.....203		
Proportion of strains liquefying gelatin within 28 days.....approx. 98.11 per cent		

It may be observed that Bergey (1948) describes a gelatin stab culture of Ps. aeruginosa as showing "rapid liquefaction". It would appear from these results that liquefaction does not always take place rapidly.

Nitrate reduction.

Nitrates were reduced by 196 strains, the majority producing nitrogen and a smaller proportion nitrite only. (Table 7).

TABLE 7. - Nitrate reduction.

Nitrate reduction (4 days at 37°C.)	Strains	
	Number	Approximate percentage of total
Reduction to nitrogen	161	79.37
Reduction to nitrite only	35	17.26
Total number of strains reducing nitrates..196 Total number of strains examined.....203 Proportion of strains reducing nitrates.....approx. 96.63 per cent		

Acid from glucose.

Acid was produced from glucose by over 60 per cent of the strains. In most cases an acid reaction was shown within the first two days of incubation, but in a small number of strains acid was detected only after 3, 4, 5 and even 6 days. (Table 8).

TABLE 8. - Production of acid from glucose.

Acid produced in days at 37°C.	Strains	
	Number	Approximate percentage of total
1	54	26.52
2	53	26.13
3	11	5.42
4	7	3.45
5	2	0.99
6	2	0.99

Total number of strains producing acid from glucose.....129

Total number of strains examined.....203

Proportion of strains producing acid from glucose.....approx. 63.5 per cent

Other properties.

Other properties of the strains are shown in Table 9. Of these properties, the production of hydrogen sulphide and indole are the most unusual; it was noted that these occurred together in three strains. Two of the four non-motile strains were freshly isolated, while two were laboratory cultures. Absence of motility in the former was, therefore, not due to prolonged laboratory cultivation.

TABLE 9. - Other properties of strains of Ps. aeruginosa.

Property		Strains	
		Number	Approximate percentage of total
Haemolysis on blood agar	strong	74	36.48
	weak	25	12.33
Methylene-blue reduction	complete	134	66.06
	partial	58	28.59
Indole and hydrogen sulphide		3	1.48
Hydrogen sulphide only		1	0.49
Indole only		4	1.97
Catalase		203	100
Gram-negative		203	100
Motility		199	98.10
Total number of strains examined.....203			

The properties described in the preceding pages are presented diagrammatically in Fig. 1, and a complete table of these properties for each strain is given in Appendix I. It will be observed that the PS numbers of the strains are not always consecutive. This is due to the fact that the cultures were numbered as received or isolated and before they were examined. When cultural properties indicated that any strain was not Ps. aeruginosa, it was omitted from the series but retained its number for study in Section 5 of this work.

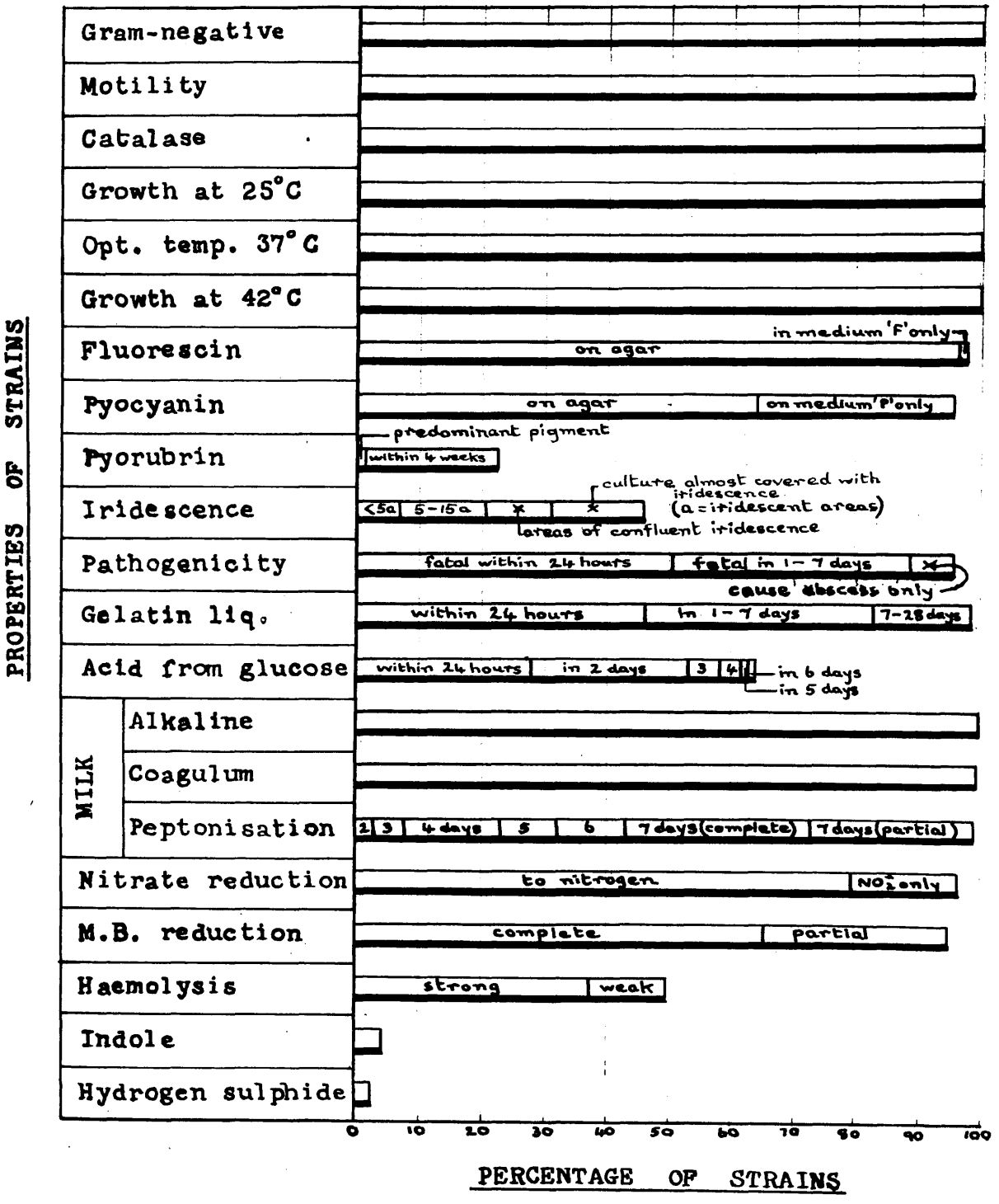


FIG. 1.- Diagrammatic summary of characteristics of strains of Ps. aeruginosa.

SECTION 2

THE ISOLATION AND PROPAGATION OF BACTERIOPHAGES FOR PS. AERUGINOSA.

THE ISOLATION AND PROPAGATION OF BACTERIOPHAGES FOR PS.AERUGINOSA

From the available literature it appears that the majority of phages for Ps. aeruginosa are strains of low virulence, causing only partial lysis of broth cultures, and readily allowing the production of resistant growth. Asheshov (1926) reported such a strain, while Fastier (1945) found that a culture of Ps. aeruginosa could be divided into resistant and non-resistant components by means of a single phage. Dickinson (1948) described Ps. aeruginosa as a "relatively resistant type of host" in relation to its phages.

In contrast to these reports, Schultz et al. (1948) reported their phage "238" as being more virulent than their other phage strains, being distinguished by its higher lytic titre, by a wider range of activity against strains of this organism, and by the lack of tendency on the part of cultures to acquire resistance to it. It seems, however, that the phage "238" was not a pure strain of phage but consisted of two distinct components, differing from each other in particle size and in the type of plaque produced. Observations on the development of resistance to this phage are, therefore, not comparable to reports concerning single strains of phage.

During this investigation, the methods adopted for the isolation and propagation of bacteriophages for Ps. aeruginosa have been designed to discourage any tendency towards the formation of excessive resistant growth, while allowing the lysis of sensitive bacteria and concurrent phage multiplication to proceed.

MATERIALS.

Media.

Throughout the investigation 'Lemco' broth (pH 7.5) was used as the liquid medium, while 'Lemco' agar was used for routine platings in a concentration of 1.5 per cent, and in other strengths for special purposes.

Indicator strains.

Twenty-four strains of Ps. aeruginosa (PS 1 - PS 24) were used as indicators for detecting the presence of phage in test filtrates and test cultures. Of these strains, five were isolated from infected burns, one from a surgical case, and five from urines. The remaining thirteen were laboratory cultures.

Fig. 2 shows, diagrammatically, that these strains were all typical Ps. aeruginosa. They all had an optimum temperature of 37°C., and showed growth at 42°C.; all liquified gelatin, reduced nitrates, gave an alkaline

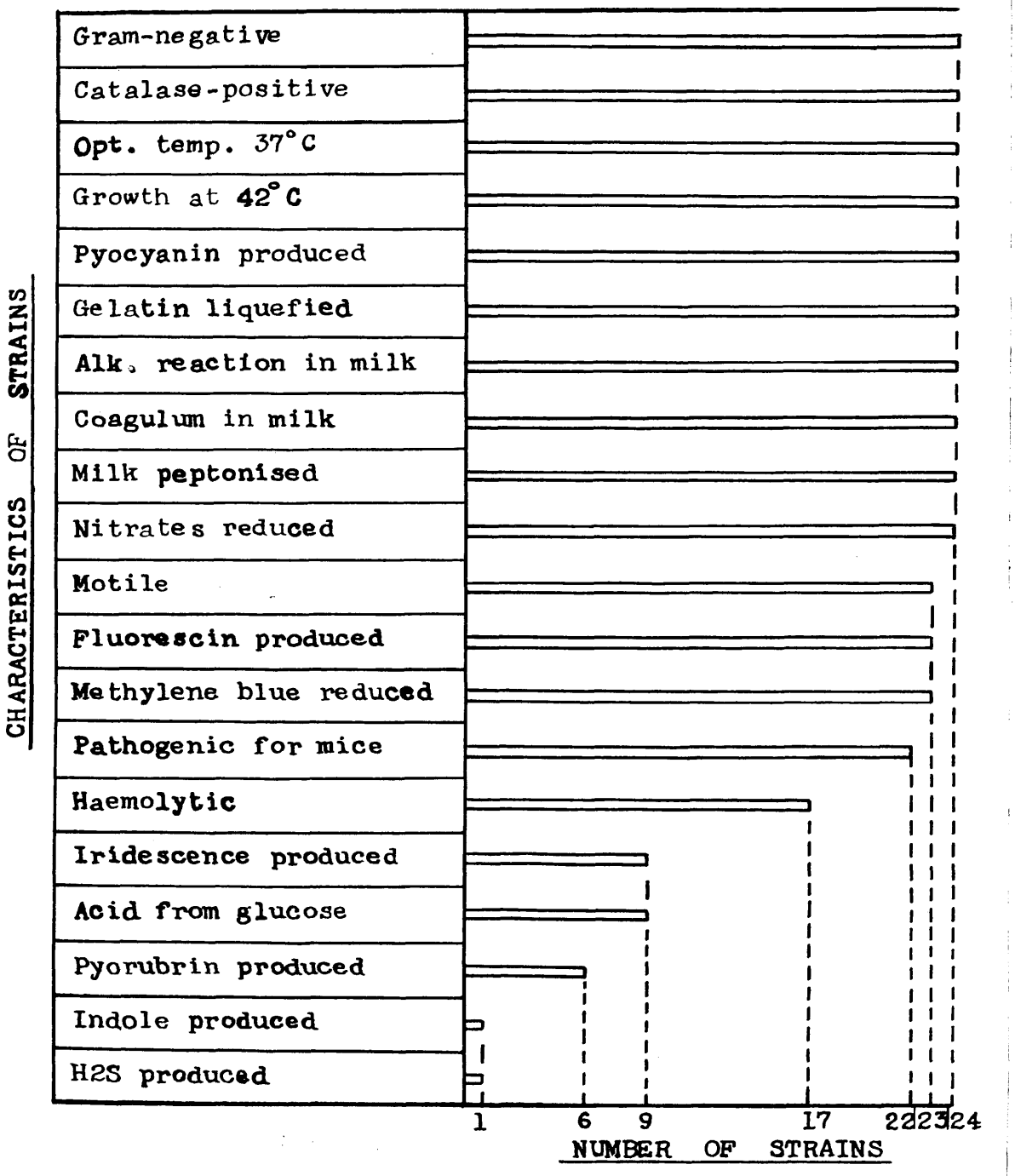


FIG. 2. - Diagrammatic summary of characteristics of indicator strains of Ps. aeruginosa.

reaction in milk accompanied by a coagulum and peptonisation, and produced the specific pigment pyocyanin, although only on the optimum medium 'P' in some cases.

Maintenance of strains.

All strains were maintained in duplicate on agar slopes and subcultured at monthly intervals. Reserve cultures were stored in the freeze-dried state.

Filtration.

Seitz filters were initially used for the filtration of cultures, but were abandoned at an early stage of the work in favour of 5/3 sintered-glass filters.

EXPERIMENTAL METHODS

Choice of method for phage detection.

Two methods are commonly employed to demonstrate the presence of phage for an organism. The first depends on the occurrence of visible lysis when a young broth culture of the organism is inoculated with phage, and was used originally by d'Herelle (1926) to demonstrate the presence of phage for the dysentery bacillus in a filtrate from faeces. Weak strains of phage, however, might not cause visible lysis in broth cultures and consequently would not be detected by this method.

The second method depends on signs of lysis occurring

when phage acts on a culture of the organism on a solid medium. Since this method would detect weak strains of phage it is more suited to general application, and has been used throughout this work.

Control of iridescence.

The proportion of strains of Ps. aeruginosa which produce iridescent areas is sufficiently high (93 out of the 203 strains studied) for the phenomenon to be regarded as quite a common feature of the organism. Since the function of the indicator strains in this study was to permit the isolation of phages active for as wide a range of strains of Ps. aeruginosa as possible, it was essential that the group of indicator strains should be a representative one and should include some strains which exhibited iridescence. The range of action of phages, isolated against non-iridescent strains only, might have presented serious deficiencies from the point of view of phage-typing.

Of the 24 strains chosen as indicators, 9 showed production of iridescence. A few preliminary experiments with these strains were sufficient to show that the presence of iridescent areas on a plate culture of an indicator strain could confuse or obscure reactions produced by typical phages. This was particularly the case where

confluent iridescence covered, or nearly covered, the culture and where the reaction due to typical phage consisted of a few isolated plaques. In order to use a plating method for the detection of phage, therefore, it was necessary to prevent or limit the production of the iridescent phenomenon.

Since, generally speaking, phages multiply on sensitive strains most rapidly during the first few hours of incubation, it was thought that it might be possible to arrange the period of incubation of cultures to favour bacterial growth and phage multiplication with its accompanying lysis rather than the production of iridescence.

To test this possibility, broth suspensions containing approximately 250 x 10^6 organisms per ml. were prepared from 12 - 18 hour agar cultures of the nine iridescent indicator strains. Five drops (0.1 ml.) of these suspensions were spread evenly over the surface of agar plates. When the inocula had been absorbed, the plates were incubated at 37°C., for different periods and then at room temperature (about 20°C.). The degree of growth and production of iridescent areas were noted when the plates were removed from the incubator and again 24 hours after inoculation.

The results (Table 10) showed that, after incubation for 6 hours at 37°C. followed by 18 hours at room temperature

iridescent areas did not become apparent, whereas bacterial growth was considered sufficiently dense for the detection of phage plaques. When the period of incubation at 37 °C. was increased to 8 hours, iridescent areas developed during the subsequent period at room temperature.

It was also considered that 6 hours at 37 °C. would be sufficient to allow phage action to take place. This has been confirmed by subsequent results.

In tests for phage activity made during this work, therefore, the iridescent phenomenon was prevented from interfering with observation of the results by restricting the period of incubation at 37 °C. to a maximum of 6 hours, and leaving the plates at room temperature for a further 12 to 18 hours. This procedure has proved entirely successful.

TABLE 10: The effect of various periods of incubation on the production of iridescent areas by 9 strains of Ps. aeruginosa.

Details of Incubation		Strains of Ps. aeruginosa																	
		PS 1		PS 2		PS 3		PS 5		PS 6		PS15		PS16		PS17		PS24	
Temperature	Hours	I	G	I	G	I	G	I	G	I	G	I	G	I	G	I	G	I	G
At 37 °C.	4	-	s	-	s	-	s	-	s	-	n	-	s	-	n	-	s	-	s
then at R.T.	20	-	m	-	m	-	m	-	m	-	s	-	m	-	s	-	m	-	m
At 37 °C.	5	-	s	-	s	-	s	-	s	-	s	-	s	-	s	-	s	-	s
then at R.T.	19	-	m	-	m	-	m	-	m	-	m	-	g	-	m	-	g	-	g
At 37 °C.	6	-	s	-	s	-	s	-	s	-	s	-	s	-	s	-	s	-	s
then at R.T.	18	-	g	-	g	-	g	-	g	-	g	-	g	-	g	-	g	-	g
At 37 °C.	8	-	m	-	m	-	m	-	m	-	m	-	m	-	m	-	m	-	m
then at R.T.	16	-	g	-	g	-	g	+	g	++	g	+	g	++	g	-	g	-	g
At 37 °C.	10	-	m	-	m	-	m	-	m	+	m	-	m	+	m	-	g	-	g
then at R.T.	14	+	g	-	g	+	g	+	g	++	g	++	g	LA	g	-	g	+	g
At 37 °C.	12	+	g	-	g	+	g	+	g	++	g	+	g	++	m	+	g	-	g
then at R.T.	12	+	g	+	g	+	g	++	g	LA	g	LA	g	LA	g	+	g	+	g
At 37 °C.	18	+	g	+	g	+	g	LA	g	C	g	LA	g	LA	g	+	g	++	g
then at R.T.	6	+	g	+	g	+	g	LA	g	C	g	C	g	C	g	++	g	LA	g
At 37 °C.	24	+	g	+	g	++	g	LA	g	C	g	C	g	C	g	++	g	LA	g

All plates spread evenly with 5 drops (0.1 ml.) of a broth suspension of the culture, containing approximately 250 million bacilli per mil.

G.....Bacterial growth
n.....no visible growth
s.....scanty growth
m.....moderate growth
g.....good growth

R.T.....room temperature

I.....Iridescence
-.....absent
+.....less than 20 small areas
++.....between 20-50 areas.
LA.....sufficiently numerous to give large areas of confluent iridescence
C.....culture almost completely covered with iridescence.

Sources of phage.

Phages were isolated from those indicator strains found to be lysogenic by the method described below and from samples of crude sewage.

Detection of lysogenic strains.

Tests for lysogenicity were confined to the 24 indicator strains, filtrates from each strain being spotted on plate cultures of all the others.

Each strain was grown in 10 ml. of broth for 18 to 24 hours at 37°C. The cultures were centrifuged and the supernatant liquid filtered free from bacteria. A plate containing about 15 ml. of agar and dried in the incubator was spread evenly with 5 drops (0.1 ml.) of a 12 - 18 hour broth culture of a strain being examined for phage-sensitivity. When the inoculum had been absorbed, drops of each of the filtrates were superimposed, and when these had been absorbed, the plates were incubated for 6 hours at 37°C. After standing overnight at room temperature, they were examined for evidence of phage action.

Detection of phage in sewage.

The method finally adopted for the detection of phage in sewage was as follows:-

The sample was pre-filtered through kieselguhr-coated filter paper on a small Buchner funnel, and then through

a 5/3 sintered-glass filter. Five ml. of this filtrate were added to 5 ml. of double-strength broth in a number of 6" x 1" test-tubes. Each tube was inoculated with a broth suspension of a 12 - 18 hour agar culture of one of the indicator strains, sufficient being added to produce a barely perceptible turbidity. The inoculated sewage-broth mixtures were incubated at 30°C. for 6 hours and left at room temperature overnight; incubation at 30°C. instead of 37°C. was used since it was considered that the slightly lower temperature would tend to favour phage multiplication rather than the development of resistant growth.

Next morning the resulting cultures (without filtration) were spotted on to a plate on which had been spread five drops of a 12 - 18 hour broth culture of the corresponding test organism. The plates were incubated for 6 hours at 37°C., and left at room temperature until the following morning when they were examined for evidence of phage action.

Restriction of the spotting test to the strain with which each sewage-broth mixture had been inoculated ensured that any phage detected must have been derived from the sewage sample under investigation, and not from possible lysogenicity of the inoculated strain.

The sewage-broth cultures in which phage was found to be present were clarified in the centrifuge and filtered, the filters being washed through with about 5 ml. of broth.

Propagation of phages.

The phage-containing filtrates (primary filtrates) from both of the above sources, were passaged on the susceptible organisms by the following method (shown diagrammatically in Fig. 3):-

Sufficient of a 12-hour agar culture of the organism was emulsified in broth to give a suspension of approximately 250 x 10^6 bacilli per ml. To 20 ml. of broth in a 6" x 1" text-tube were added 1 ml. of this suspension and 0.5 ml. of the primary filtrate. This was incubated at 30°C. for 6 hours, the tube being placed in a sloping position to give the greatest surface/volume ratio for aeration of the culture. After incubation, the culture was placed in the refrigerator until next morning when it was filtered.

Primary filtrates, drops of which produced confluent lysis on an agar culture of the sensitive organism, were passaged three times, and the remainder were passaged until confluent lysis was obtained, three passages being the minimum number given to any filtrate.

The purpose of storing the incubated culture in the refrigerator overnight before filtration was to restrict possible secondary growth while permitting the phage to continue its development and produce lysis. That lysis took place under these conditions was indicated by the fact that cultures showing a slight turbidity at the end of the incubation period were quite clear when removed from the refrigerator on the following morning. Control cultures, kept at room temperature overnight, showed increased turbidity. It was considered that the procedure would give a maximum yield of phage because of complete lysis, and would avoid loss of phage adsorbed on resistant growth which would be removed by filtration.

Purification of phages.

The crude filtrates obtained by the above process were purified by plating out in suitable dilution with the propagating strain, and subculturing from isolated plaques into dilute broth suspensions of the organism. The procedure was repeated three more times, or until the plaques produced were of a uniform character. When satisfactory purification was achieved, the phages were again passaged on the susceptible strains, as previously described, until they again produced confluent lysis on agar; they were then given two additional passages.

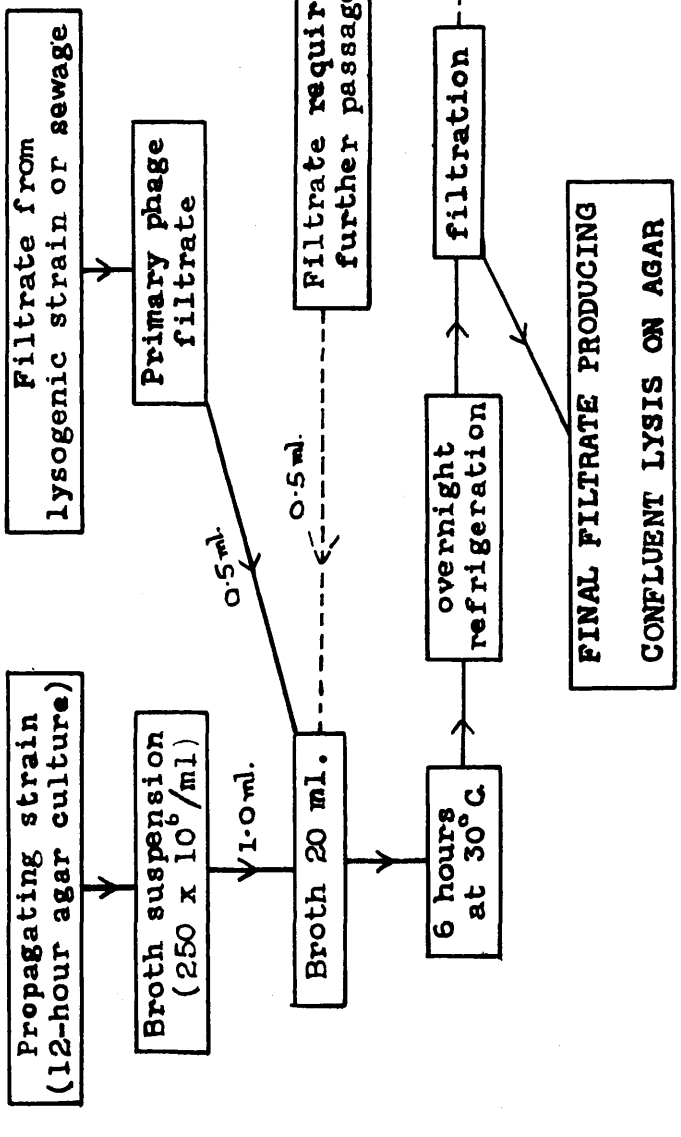


FIG. 3. - Outline of process for phage propagation.

Thus, as in the case of the crude filtrates, each pure phage was given a minimum of three serial passages.

The final filtrates were preserved as "stock phages", from which were prepared dilutions for typing purposes, i.e. "typing phages" or "typing preparations".

Storage of phage filtrates.

Filtrates, without added antiseptic, were distributed in $\frac{1}{4}$ oz. and 1 oz. screw-capped bottles, small volumes being held in reserve in 1 ml. and 2 ml. ampoules, and stored in the refrigerator.

Preparation of typing phages.

Typing phages were prepared by the method adopted by Wilson and Atkinson (1945) for staphylococcal phages. A series of decimal dilutions of each stock filtrate was prepared in broth. These dilutions were tested against plate cultures of the homologous sensitive strain under the conditions of the typing test (page 40). The highest dilution which produced confluent lysis was used for the actual typing tests (Fig. 4).

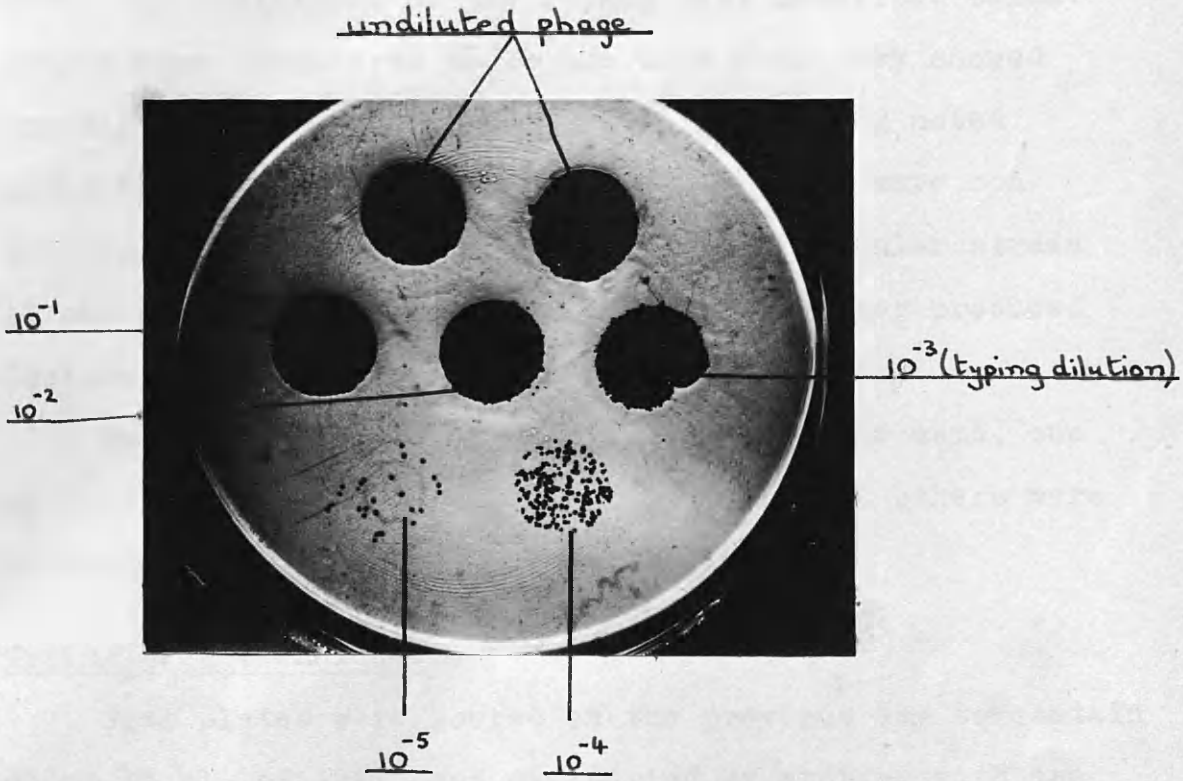


FIG. 4. - Determination of typing dilution.

Differentiation of typing phages.

The typing phages obtained were differentiated from each other by testing against 48 strains of Ps. aeruginosa under the conditions of the typing test described below. Phages were considered to be the same when they showed exactly the same host-range, the results being noted quantitatively. This means that two phages were considered to differ in their action on a particular strain if one produced confluent lysis while the other produced isolated plaques.

Where several phages were found to be the same, one of them was retained for typing tests and the others were discarded.

Typing-test technique.

Agar plates were poured on the previous day to contain about 15 ml. of agar, and over-dried by storing with the lids raised in the incubator overnight.

A broth suspension of the strain to be typed was prepared from a 12 - 15 hour agar culture to contain approximately 250 x 10^6 bacilli per ml. Ten drops (0.2 ml.) of this suspension were spread evenly over the surface of the plate. When the inoculum had been absorbed, drops of the typing phages were delivered on to appropriate areas (marked off on the base of the plate)

by means of standard dropping pipettes. When these drops had been absorbed, the plates were incubated for 6 hours at 37°C. and left at room temperature overnight. Results were recorded next morning.

Broth suspensions were used instead of cultures for spreading the plates, in order to standardise within reasonable limits the number of organisms plated. Cultures were not suitable for this purpose, since the density of growth in these varied very widely from strain to strain. Several cultures have been observed in which growth in broth was limited to the formation of a pellicle and sometimes a light sediment, the rest of the broth being clear or almost clear. This apparent lack of vigorous growth was not due to prolonged laboratory cultivation, but was seen in freshly isolated strains. The use of suspensions renders the process standard for all strains of the organism and, in addition, brings the technique of phage-typing more into line with serological typing.

The plates were overdried before use in order that the drops of phage would be absorbed quickly without undue spreading. All typing tests were carried out in duplicate.

RESULTS

Effect of filtration medium on the detection of lysogenic strains and on phage passage.

The tests for lysogenicity among the 24 indicator strains were carried out initially using Seitz filters. In all, 33 cross-reactions were obtained, the results being constant in three successive tests.

Attempts to propagate these 33 phages were successful in the majority of cases, successive filtrates showing increasing activity in spotting-tests on agar. With seven filtrates, however, no such increase was obtained. Five of these filtrates showed isolated plaques on agar at the commencement of propagation, and no advance beyond this was detectable even after 25 passages. In the two other cases the phage disappeared from the filtrate after the third or fourth passage. It was thought that these failures were due to any slight increase in titre during passage being cancelled out by adsorption of phage on the Seitz pad during the subsequent filtration. Adsorption of other phages on Seitz filter pads has been reported by several workers, e.g. Fisk (1942), Bernoulli (1943).

Attempts to propagate these phages were therefore repeated, the Seitz filters being replaced by sintered-glass (5/3) filters. The results were satisfactory.

The sintered-glass filters made no difference to the disappearance of phage from the two filtrates mentioned above, but enabled the other five phages to be propagated and purified successfully. The number of passages required before these five filtrates produced confluent lysis on agar varied from ten to eighteen.

Following these results, the original tests for lysogenicity among the 24 indicator strains were repeated, using sintered-glass filters instead of Seitz. This time 39 cross-reactions were obtained, the results again being constant in three successive tests. Repetition of the tests with Seitz filters gave the same 33 results as before and it was noted that in several cases where Seitz filtrates produced isolated plaques in the above tests, the corresponding sintered-glass filtrates produced confluent lysis.

These results seemed to indicate that adsorption of phage on sintered-glass filters did not take place to the same extent as on Seitz filter pads. Seitz filters, therefore, were discarded in favour of sintered-glass.

Indications of the presence of phage.

(1) Tests for lysogenicity.

In the tests for lysogenicity among the 24 indicator strains, the results indicating the presence of phage

fell into four groups:-

(a) Confluent lysis with no resistant growth;

(b) A number of isolated plaques in the area of spotting;

(c) Confluent lysis with an even layer of resistant growth;

(d) Confluent lysis consisting of a central disc of growth surrounded and separated from the rest of growth on the plate by a thin ring of lysis.

The first two of these groups were the appearances usually associated with the action of phage filtrates on plate cultures of sensitive organisms. Whether (a) or (b) was produced depended on the number of phage particles in the filtrate.

Result (c) was obtained where lysis of the sensitive culture by phage was followed by the development of secondary growth, while in the case of (d) the secondary growth had become lysogenic and, by causing lysis of the sensitive culture immediately surrounding it, had produced a thin, clear zone of lysis.

The term 'confluent lysis' is applied here to denote a uniform degree of lysis over the area covered by the drop of phage filtrate, and includes those cases in which a layer of resistant growth has developed after lysis of

sensitive organisms. 'Confluent lysis' is, therefore, not necessarily 'complete lysis' but includes the latter.

(2) Plating of sewage-broth cultures.

The process of plating out the test cultures without filtration resulted in any phage present and any resistant growth which had arisen being plated together on to the indicator strain. The result depended on the amount of phage present, the amount of resistant growth which had arisen, and whether or not the resistant growth had become lysogenic.

Three types of result were obtained, corresponding to those already described under (a), (b) and (d) in Tests for lysogenicity.

Where lysis had taken place in the test culture and had not been followed by the development of resistant growth, spotting-out was equivalent to spotting a sterile filtrate containing phage, and gave either an area of confluent lysis free from growth or a number of isolated plaques, depending on the amount of phage present.

Some of the cultures which gave isolated plaques on spotting showed a variable degree of turbidity before being plated. In such cases a limited amount of phage was obviously present in the test culture and the resistant growth causing the turbidity had not become lysogenic.

Where the resistant growth in the test culture had

become lysogenic, the result was a disc of resistant growth surrounded by a thin ring of lysis. The central discs of growth produced by plating the test cultures were usually thicker, and the ring of lysis consequently more definite, than those obtained by plating the sterile filtrates from lysogenic strains. This was to be expected, since plating of such test cultures was equivalent to plating a lysogenic strain on to a sensitive one, and the central disc of growth consisted of this lysogenic growth in addition to growth which had become lysogenic while the phage was acting on the plate culture. In the case of a sterile filtrate, however, the disc of growth was due to the latter fraction only.

No result obtained with the sewage-broth cultures showed confluent lysis with an even layer of resistant nonlysogenic growth. Where a layer of resistant growth was observed, it was always lysogenic.

Phage from sewage.

Examination of twelve samples of crude sewage obtained from sewage works in or around Glasgow gave the results shown in Table 11.

Phage was detected in ten of the twelve samples. One of the negative samples was not typical of the daily discharge, since it was collected in an afternoon

following a day of heavy rain when the flow was a million gallons a day above the average dry weather rate. In the ten positive samples a total of 46 phage reactions were noted.

TABLE 11. - Ps. aeruginosa phage from sewage.

Sample Number	Source	Phage detected for strains of Ps. aeruginosa.
S1	Dalmarnock	PS 1,2,3,6,15,17,19.
S2	Shieldhall	PS 1,2,3,4,7,10,12,14,15,16,17, 8p.
S3	Dalmuir	PS 2,3,4,6,8,14,15,16,17, 8p.
S4	Shieldhall	PS 16.
S5	Dalmuir	PS 1,2,3,6.
S6	Dalmarnock	PS 6,17.
S7	Hamilton	PS 15,17.
S8	Coatbridge	---
S9	Motherwell	PS 4,10,15,17.
S10	Airdrie	PS 2, 3.
S11	Johnstone	PS 8,17.
S12	Dumbarton	---

It appears from Table 11 that the phage content of samples from the same source but taken on different occasions is not of a constant nature, as determined by the method described. Sample S2, for example, showed phage action on 12 strains, while S4, from the same source but taken at a later date, showed only one. A longer

series of tests would, however, be necessary to arrive at any definite conclusions on this point.

An interesting point arose during these tests. An additional indicator strain PS8p was included in the series. This strain was derived from PS8 through accidental phage contamination, and has remained lysogenic for PS8 throughout this study. Sewage sample S2 gave a strong phage reaction on PS8p but no reaction on the parent strain PS8. The phage resulting from the propagation of a filtrate from S2 on PS8p, however, did react on PS8 in its typing dilution. It would seem that either the method used for detecting phage in sewage cannot be guaranteed to detect every possible case, or that the possession of the contaminating phage by PS8p actually assisted in the adsorption of the new phage.

The first alternative is likely to be the correct one but the second, although not supported by general experience, is not impossible. It is not unlikely that there are symbiotic relationships between phages as exist among different bacterial species.

In contrast to sample S2, sample S3 gave a reaction on both PS8 and PS8p; this is what would be expected.

Phage-typing preparations.

In all, 85 cross-reactions were obtained, viz. 39

from tests for lysogenicity and 46 from sewage samples. Of these 85 potential strains of phage, 77 were propagated successfully, the remainder dying out during passage. It is known that strains may vary in their suitability for phage propagation (Asheshov et al., 1933), and it is to this fact that the failures were probably due.

When diluted to typing strength and differentiated as described, the 77 phages were resolved into 38 preparations, each of which differed from the others in host range as determined on the first 48 strains of the series. In some cases the differences were slight, but proved to be constant in successive tests.

The typing preparations are listed in Table 12, which shows the dilution of the concentrated phage which the typing preparation represents; in a few cases, the concentrated phage is the typing strength.

The phages have been numbered according to their source and the strain on which they were propagated, e.g. S3/3 is the phage isolated from sewage sample S3 and propagated on strain PS3; phage L1/5 was obtained from lysogenic strain PS1 and propagated on strain PS5.

Several of the phages were isolated from more than one source; the source indicated by the designation of phages as shown in Table 12 is that from which the phage was first isolated and from which the filtrate used was

actually taken.

The host-range of the 38 phage-typing preparations over 48 strains of Ps. aeruginosa is shown in Table 13. The arrangement of phages in this Table is such that those with a wide range of activity are placed first and are followed by those which have a more restricted range.

TABLE 12. - Typing dilutions of phage preparations.

Phage	Typing dilution		Phage	Typing dilution
S3/3	10^{-1}		S10/2	10^{-1}
S6/6	10^{-1}		S1/3	undiluted
L1/17	10^{-3}		S3/2	10^{-1}
S1/6	10^{-3}		S5/1	10^{-2}
S1/17	undiluted		L1/5	10^{-2}
S2/4	10^{-2}		L2/8	10^{-2}
S2/15	10^{-2}		L2/17	10^{-2}
S2/17	10^{-2}		L1/8	10^{-2}
S2/10	10^{-2}		L5/8	10^{-1}
S2/14	10^{-1}		L5/17	10^{-1}
S9/15	10^{-3}		L12/8	10^{-2}
S2/7	undiluted		L19/15	10^{-2}
S3/4	10^{-2}		L19/6	10^{-1}
S2/8p	10^{-3}		L19/8	10^{-1}
S3/15	10^{-2}		L3/15	undiluted
S3/14	10^{-3}		L11/14	10^{-3}
S2/1	10^{-3}		L11/9	undiluted
S1/1	10^{-2}		S5/6	10^{-2}
S5/3	undiluted		L14/6	10^{-2}

TABLE 13. - Range of action of 38 phage-typing preparations over 48 strains of Ps. aeruginosa.

TP.....typing-preparation.

TP	Strains of Ps. aeruginosa: PS nos. 1-24.																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
S3/3	C	C	C	R	R	R	-	R	-	R	-	R	R	C	R	R	R	R	-	R	R	-	C	R
S6/6	-	-	-	C	C	C	-	-	P	C	-	-	-	R	C	R	C	-	-	-	-	-	-	-
L1/17	-	-	-	C	C	C	P	-	P	C	-	P	P	R	C	R	C	-	-	-	-	-	-	-
S1/6	-	-	-	P	C	C	-	R	-	P	-	-	C	-	C	P	C	-	-	-	-	-	P	-
S1/17	-	-	-	C	C	C	P	-	P	C	-	-	P	R	C	R	C	-	-	-	-	-	-	-
S2/4	-	-	-	C	C	C	R	-	P	C	-	R	R	R	C	C	C	-	-	-	-	-	R	-
S2/15	-	-	-	C	C	C	R	-	R	C	-	R	R	C	C	C	C	-	-	-	-	-	R	-
S2/17	-	-	-	C	C	C	R	-	P	C	-	R	P	C	C	C	C	-	-	-	-	-	R	-
S2/10	-	-	-	C	C	C	R	-	-	C	-	P	P	P	C	C	C	-	-	-	-	-	P	-
S2/14	-	-	-	C	C	C	R	R	R	C	-	C	C	C	C	C	C	-	-	-	-	-	R	-
S9/15	P	P	P	C	C	C	-	P	P	C	-	-	P	R	C	P	C	-	-	-	-	-	-	-
S2/7	-	-	-	C	C	C	C	P	P	C	-	C	P	R	C	C	C	-	-	-	-	-	P	-
S3/4	-	-	-	C	C	C	-	C	-	C	-	-	C	-	C	C	C	-	-	-	-	-	R	-
S2/8p	C	C	C	C	C	C	P	C	-	C	-	-	C	-	C	C	P	-	-	-	-	P	-	-
S3/15	-	-	-	-	C	C	-	-	-	-	-	-	R	-	C	-	-	-	-	-	-	-	R	-
S3/14	C	C	C	-	R	C	-	R	-	R	-	R	-	R	-	R	R	P	-	P	-	-	R	P
S2/1	R	R	R	-	R	R	-	R	-	-	-	R	-	R	-	-	-	-	-	-	-	-	R	P
S1/1	C	C	C	-	R	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5/3	C	C	C	-	-	R	R	-	-	-	-	-	-	-	P	R	P	-	-	-	-	-	-	-
S10/2	C	C	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S1/3	C	C	C	-	R	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3/2	C	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-
S5/1	C	C	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L1/5	-	-	-	-	C	-	-	P	-	-	-	-	-	-	-	P	P	-	-	-	-	-	-	-
L2/8	-	-	-	-	R	-	-	C	-	-	-	-	-	-	-	P	C	-	-	-	-	-	-	-
L2/17	-	-	-	-	-	P	-	C	-	-	-	-	-	-	P	-	C	-	-	-	-	-	-	-
L1/8	-	-	-	-	-	P	-	C	-	-	-	-	-	-	P	R	C	-	-	-	-	-	-	-
L5/8	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	R	C	-	-	-	-	-	-	-
L5/17	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	P	C	-	-	-	-	-	-	-
L12/8	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-
L19/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-
L19/6	-	-	-	-	-	R	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-
L19/8	-	-	-	-	R	-	-	R	-	-	-	-	-	-	R	R	R	-	-	-	-	-	-	-
L3/15	-	-	-	-	R	-	-	-	-	-	-	-	-	-	R	P	-	-	-	-	-	-	-	-
L11/14	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-
L11/9	-	-	-	-	R	R	-	-	C	-	-	-	-	-	-	R	R	-	-	-	-	-	R	-
S5/6	-	-	-	-	-	R	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-
L14/6	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C...confluent lysis with no resistant growth.
P...isolated plaques.
R...confluent lysis with resistant growth.
-...no reaction.

TABLE 13. - Range of action of 38 phage-typing preparations over 48 strains of Ps. aeruginosa (contd.).

TP.....typing-preparation

TP	Strains of Ps. aeruginosa: PS nos.25-48																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
S3/3	R	R	R	R	-	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S6/6	P	-	-	-	P	-	-	-	P	R	-	-	-	-	-	-	-	R	R	-	-	-	-	-
L1/17	-	-	-	-	P	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S1/6	-	-	-	-	-	-	P	P	-	P	-	-	-	-	-	-	-	P	P	-	-	-	-	-
S1/17	-	-	-	-	-	-	-	-	R	R	-	-	-	-	-	-	-	R	R	-	-	-	-	-
S2/4	P	-	-	-	P	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S2/15	P	-	-	-	R	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	R	-	-	R
S2/17	P	-	-	-	P	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S2/10	P	-	-	-	P	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S2/14	R	-	-	-	C	-	-	-	C	C	R	-	-	R	-	R	R	C	C	-	R	-	R	R
S9/15	-	-	-	-	-	-	-	-	R	P	-	-	-	-	-	-	-	P	P	-	-	-	-	P
S2/7	-	-	-	-	P	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S3/4	-	-	-	-	-	-	R	R	-	P	-	-	-	-	-	-	-	P	P	-	-	-	-	-
S2/8p	-	-	-	-	P	-	-	-	-	C	-	-	-	-	-	-	-	C	C	-	-	-	-	C
S3/15	R	-	-	-	-	-	-	-	R	C	-	-	-	-	-	-	-	C	C	-	-	-	-	-
S3/14	-	-	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S2/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
S1/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5/3	-	-	-	-	-	-	-	-	R	R	-	-	-	-	-	-	-	R	R	-	-	-	-	R
S10/2	-	-	-	-	-	-	-	-	R	R	-	-	-	-	-	-	-	R	R	-	-	-	-	-
S1/3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S3/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5/1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L1/5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2/8	-	-	-	-	-	-	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2/17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L1/8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L5/8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L5/17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L12/8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L19/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L19/6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L19/8	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L3/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L11/14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L11/9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S5/6	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L14/6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C...confluent lysis with no resistant growth.
P...isolated plaques.
R...confluent lysis with resistant growth.
-...no reaction.

SECTION 3

CHARACTERISTICS OF BACTERIOPHAGES FOR PS. AERUGINOSA

CHARACTERISTICS OF BACTERIOPHAGES FOR PS. AERUGINOSA

Two properties of the phages isolated were investigated, namely, their ability to produce visible lysis in broth cultures, and the nature of the plaques produced on agar cultures, of their propagating strains. The first property was intended to give a preliminary indication of the possible effectiveness of the phages as wound disinfecting agents; the second was to be used as a basis for arranging the typing phages in groups, as an alternative to a serological grouping. Similarity in plaque morphology is usually associated with similarity in serological character (Burnet, 1933).

MACROSCOPIC LYSIS IN BROTH CULTURES

Method.

The production of visible lysis in broth cultures by each phage was tested for under the following conditions:

The phages were tested against their own propagating strains, which were grown on agar slopes for 12 hours at 37°C. The growth was emulsified in broth and a suspension prepared in 5 ml. of broth to contain about 100 million bacilli per ml. as judged by turbidity. This suspension was inoculated with 0.5 ml. of the typing dilution of the particular phage, and the culture was incubated at 37°C.,

being examined hourly up to 12 hours, then at 18 hours and at 24 hours. The same size of tube was used for each phage and the tubes were incubated in the vertical position to ensure a uniform degree of aeration between different cultures.

Control tubes without phage were included for purposes of comparison.

Results.

Under these conditions, 25 of the 38 phages produced clear cultures in periods ranging from 3 to 7 hours, lysis being preceded by a slight increase in turbidity. The remaining 13 phages caused a partial degree of lysis, which, in some cases, was barely detectable by comparison with the control.

None of the phages produced permanent lysis, resistant growth developing in periods of from 6 to 18 hours after the commencement of the test. Where partial lysis only had taken place the turbidity increased most rapidly, but in all cases there was little or no detectable difference in turbidity after 24 hours between the cultures which had been lysed and the control tubes. These results are given in Table 14.

From the point of view of phage-therapy these results are not encouraging, since they indicate that resistant

growth develops comparatively readily. It may be possible, however, to enhance the virulence of the phages by prolonged serial passage. The amount of resistant growth may be reduced also by the use of various combinations of phages instead of single preparations.

TABLE 14. - Lysis in broth cultures at 37°C., by Ps. aeruginosa phages.

Phage	Time in hours for			Resistant growth after 24 hours
	Complete lysis	Partial lysis	Detection of resistant growth	
S3/3	6	-	11	Heavy
s6/6	5	-	18	Moderate
L1/17	6	-	12	Moderate
S1/6	3	-	18	Moderate
S1/17	4	-	7	Moderate
S2/4	4	-	11	Moderate
S2/15	4	-	18	Moderate
S2/17	4	-	18	Moderate
S2/10	5	-	12	Moderate
S2/14	6	-	9	Heavy
S9/15	4	-	6	Heavy
S2/7	-	6	6	Heavy
S3/4	4	-	11	Moderate
S2/8p	3	-	18	Moderate
S3/15	-	6	6	Heavy
S3/14	5	-	9	Moderate
S2/1	4	-	10	Moderate
S1/1	7	-	9	Heavy
S5/3	-	5	5	Heavy
S10/2	-	6	6	Heavy
S1/3	7	-	10	Moderate
S3/2	-	6	6	Heavy
S5/1	5	-	7	Heavy
L1/5	6	-	10	Heavy
L2/8	5	-	8	Heavy
L2/17	5	-	10	Moderate
L1/8	6	-	12	Moderate
L5/8	4	-	10	Heavy
L5/17	4	-	10	Heavy
L12/8	-	3	3	Heavy
L19/15	-	5	5	Heavy
L19/6	-	6	6	Moderate
L19/8	-	6	6	Heavy
L3/15	-	4	4	Heavy
L11/14	4	-	8	Moderate
L11/9	-	4	4	Heavy
S5/6	-	5	5	Moderate
L14/6	-	4	4	Moderate

PLAQUE MORPHOLOGY

It is generally accepted that the concentration of agar in the medium and the density of the bacterial spreading affect the size of plaque produced by any strain of phage. In studying the plaques of these phages for Ps. aeruginosa it has been found that such factors alter the nature of the plaques produced to such an extent that it is necessary to specify the conditions under which they were examined, in order to be able to attach any significance to the results.

Method.

The effect of various factors on the plaques produced was studied by a plating method similar to that used by Cherry and Watson (1949) for assay purposes.

Plates to be used were poured on the previous day with 15 mls. of 1.5 per cent agar, and dried with lids raised in the incubator at 37°C. for four hours. A suspension of the indicator strain was prepared in broth from a 12-hour agar culture. A small amount (0.25 ml.) of this suspension and a drop (0.02 ml.) of a suitable dilution of the phage being examined were added to 3 ml. of melted agar at 50°C. and mixed by rotation of the tube. The mixture was poured on to the surface of an agar plate and allowed to spread and set in a thin layer; the plates

were then incubated.

The effect of varying,

(a) the concentration of bacteria,

(b) the strength of agar in the plating mixture, and

(c) the time of incubation of the plates,

was studied.

Results.

The results are shown in Tables 15, 16, 17, and the accompanying illustrations.

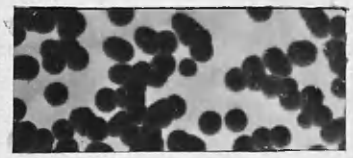
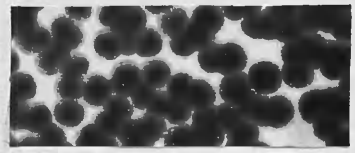
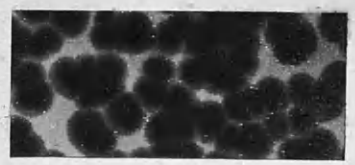
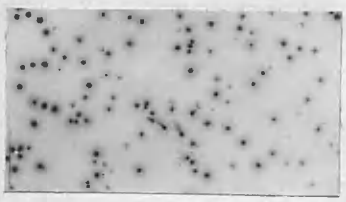
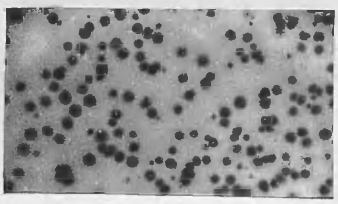
The greater the concentration of bacteria in the plating mixture the smaller were the plaques produced; the halos of partially lysed growth surrounding some of the plaques were wider with low concentrations of bacteria (Table 15). Similar results as regards plaque size were obtained by varying the strength of the agar; the effect on halo formation was not pronounced (Table 16).

The greatest single factor controlling the formation of halos round the plaques was found to be the period of incubation of the plates. As the latter was increased formation of halos became more apparent. Thus while phage S1/6 showed no halos after 6 hours at 37°C., and 15 hours at room temperature, cultures incubated for 24 hours at 37°C., showed halos about 2 mm. wide (Table 17).

The halos produced by this phage appear to consist of two distinct bands, the inner one consisting of growth

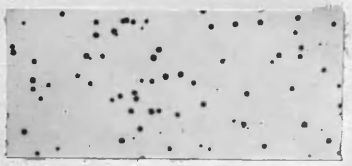
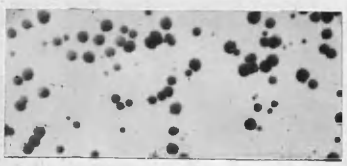
arising within the previously clear central portion, and the other one of growth only partially lysed. The two bands may be seen quite clearly in the illustration in Table 17.

TABLE 15. - Effect of concentration of bacteria on plaque size and halo formation.

Phage	I.S.	P.M.	Plaques	Illustrations
S1/6	3,200 x 10^6/ml.	245 x 10^6/ml.	Clear; 2.5-3mm.diameter; No halo	
S1/6	320 x 10^6/ml.	24.5 x 10^6/ml.	Clear; 3.5-5mm.diameter; Some show a faint halo,0.5-1mm.wide.	
S1/6	160 x 10^6/ml.	12.25x 10^6/ml.	Clear; 3.5-5mm.diameter; Halos, approx. 1 mm. wide.	
L11/14	8,700 x 10^6/ml.	665 x 10^6/ml.	Clear; Approx.0.5 mm. diameter; Surrounded by halos, approx. 0.5 mm. wide	
L11/14	870 x 10^6/ml.	66.5 x 10^6/ml.	Clear; Approx.1 mm. diameter; Halos, up to 2mm. wide,but less marked than above.	

I.S.:- Cell count per ml. of indicator suspension (plate count)
P.M.:- Cell concentration per ml. of plating mixture.
Plating Mixture: Agar, 0.5 per cent..... 3 ml.
Bacterial suspension,of stated density.....0.25ml.
Phage filtrate.....0.02ml.
Plates incubated for 6 hours at 37°C., then 15 hours at room temperature.

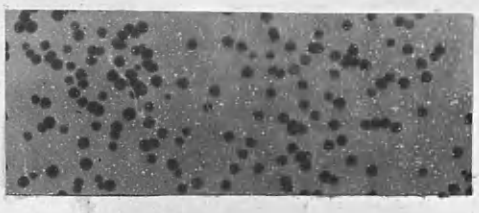
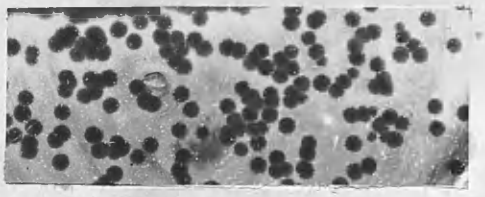
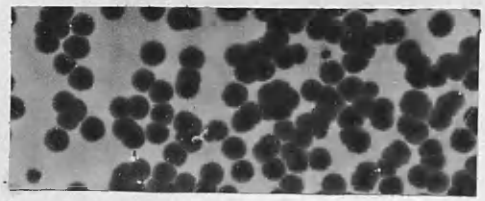
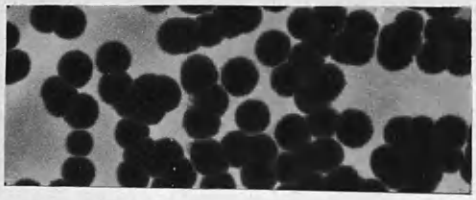
TABLE 15. - Effect of concentration of bacteria on plaque size and halo formation (contd.).

Phage	I.S.	P.M.	Plaques	Illustrations
S2/10	9,100 x 10^6/ml.	695 x 10^6/ml.	Clear; Approx.0.5mm. diameter; No halo.	
S2/10	910 x 10^6/ml.	69.5 x 10^6/ml.	Clear; 1-1.5mm.diameter. No halo	

I.S.:- Cell count per ml. of indicator suspension (plate count).
P.M.:- Cell concentration per ml. of plating mixture.
Plating Mixture: Agar, 0.5 per cent..... 3 ml.
Bacterial suspension, of stated density.....0.25ml.
Phage filtrate.....0.02ml.
Plates incubated for 6 hours at 37°C., then 15 hours at room temperature.

TABLE 16. - Effect of agar concentration on plaque size.

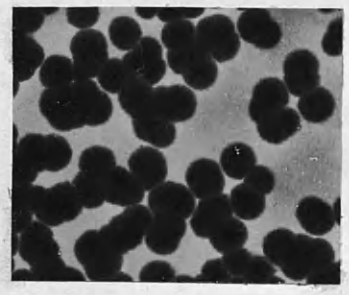
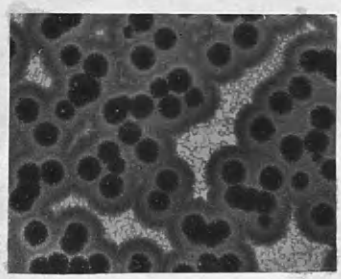
Plaques of phage S1/6.

Agar Concentration	Plaques (diameter)	Illustrations
2 per cent	0.8 - 1.0 mm.	
1.5 per cent	1.5 - 2.0 mm.	
1.0 per cent	3.00 mm. (approx.)	
0.5 per cent	3.5 - 5.0 mm.	

Plating mixture: Agar of stated strength..... 3 ml.
Bacterial suspension(1000M/ml.)0.25ml.
Phage filtrate.....0.02ml.
Plates incubated for 6 hours at 37°C., followed by 15 hours at room temperature.

TABLE 17. - Effect of period of incubation on plaque size and on halo formation.

Plaques of phage S1/6

Period of incubation	Plaques	Illustrations
6 hours at 37 °C., followed by 15 hours at room temperature	Large and clear; 3.5-5mm. diameter; no halo produced.	
24 hours at 37 °C.	Halos formed; Clear centre, 3 mm. diameter. Halos about 2mm. wide.	

Plating Mixture: Agar, 0.5 per cent..... 3 ml.
Bacterial suspension(1000M/ml.)....0.25ml.
Phage filtrate.....0.02ml.

Plates incubated for periods shown.

Conditions for examination of plaques.

As a result of these observations, the plaques were finally studied under the following conditions:-

The plating mixture consisted of,

(a) 3 ml. of 0.5 per cent agar,

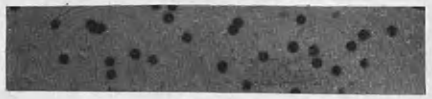
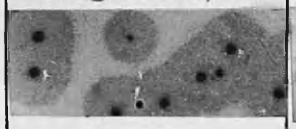
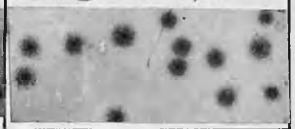
(b) 0.25 ml. of indicator suspension containing approximately 1000 million bacilli per ml.;

(c) 0.02 ml. of a suitable dilution of the phage being studied.

This mixture was poured over the surface of a plate as already described, and the plates were incubated for 6 hours at 37 °C. followed by 15 hours at room temperature.

The plaques produced under these conditions were divided into the five types shown in Table 18. Some of the small plaques showed very narrow halos in some tests. These did not appear consistently, however, and have not been included as characteristic features under the specified conditions.

TABLE 18:- Plaques produced by Ps.aeruginosa phages under the specified conditions.

Plaques	Average diameters	Phages	Illustrations
Small	0.4-0.6 mm.	S3/3, S1/17, S2/14, L1/5, S5/6, S6/6, L1/17, S1/1, S5/3, S10/2, S1/3, S3/2, S5/1, L2/8, L2/17, L1/8, L5/8, L5/17, L12/8, L19/15, L19/6, L19/8, L3/15, L14/6.	Phage L1/5 
Medium	1 - 1.5 mm.	S2/4, S2/15, S2/17, S2/10, S9/15, S2/7, S3/4, S3/15,	Phage S2/4 
Large	3.5-5.0 mm.	S1/6, S2/8p	Phage S1/6 
Small, with wide halo	Centre: 0.5-1.0 mm. Halo: approx. 1-4 mm. wide	L11/14, L11/9	Phage L11/14  Phage L11/9 
Medium, with narrow halo.	Centre: 1 - 1.5 mm. Halo: approx. 1 mm. wide	S3/14, S2/1	Phage S3/14 

Plating Mixture: Agar, 0.5 per cent...3 ml. Phage filtrate.....0.02 ml.
Bacterial suspension, 1000M/ml.....0.25 ml.
All plates incubated for 6 hrs., at 37°C., followed by 15 hrs. at room temperature.

SECTION 4

BACTERIOPHAGE-TYPING OF PS.AERUGINOSA

BACTERIOPHAGE-TYPING OF PS.AERUGINOSA.

Phage-typing tests were carried out on 203 strains of Ps. aeruginosa, testing each strain for sensitivity to the 38 typing preparations by the typing-test technique already described (page 40).

Recording of results.

As stated previously, the term "confluent lysis" is used throughout this work to denote a uniform degree of lysis over the area covered by the phage and therefore includes those cases in which an even layer of resistant growth appears. The amount of resistant growth which arises in some areas of confluent lysis was found to vary greatly. With any phage of the series acting on any one strain, the amount varied from one test to another and even between duplicate spottings on the same plate. On account of this fact, results of typing tests have been recorded as either,

C....confluent lysis free from resistant growth;

R....confluent lysis with resistant growth, the degree of resistant growth being disregarded for the present purpose; or

P....isolated plaques. (Appendix II)

Designation of phage-type.

In order to designate the phage-type of any strain, the typing preparations have been numbered according to the scheme shown in Table 19. In this scheme the typing phages have been arranged in 18 groups, some of which consist of a single phage and others of several. Each group bears a group-letter, while individual phages in the same group are distinguished by an additional number. Phages have been placed in the same group when they were found to produce the same type of plaque and also to have a similar range of activity.

Using this system, the phage-type of any strain can be expressed by the letter(s) and/or numbers corresponding to the typing phages to which the strain is sensitive.

TABLE 19. Type-designation of phage-typing preparations.

Type designation	Phage	Type designation	Phage
A	S3/3	N1	S1/1
B	S1/6	N2	S5/3
C	S1/17	N3	S10/2
D	S2/14	N4	S1/3
E	S2/8p	N5	S3/2
F	L1/5	N6	S5/1
G	S5/6	P1	L2/8
H1	S6/6	P2	L2/17
H2	L1/17	P3	L1/8
K1	S2/4	P4	L5/8
K2	S2/15	P5	L5/17
K3	S2/17	R1	L12/8
K4	S2/10	R2	L19/15
L1	S9/15	R3	L19/6
L2	S2/7	R4	L19/8
L3	S3/4	S	L3/15
L4	S3/15	T	L11/14
M1	S3/14	V	L11/9
M2	S2/1	W	L14/6

PHAGE-TYPES OF PS. AERUGINOSA.

All 203 strains tested were found sensitive to one or more of the available phages. The majority (193) were acted on by the typing preparations and the remainder (10) by certain of the undiluted filtrates.

(1) Strains sensitive to typing phages.

On the basis of phage-type, 193 strains of Ps. aeruginosa have been divided into 14 main groups, some of these being further divided into sub-groups and individual types.

(a) Division into main groups.

The reactions on which the 14 main groups have been differentiated are shown in Table 20.

Strains of Groups I - VII inclusive are characterised by sensitivity to a single phage, those of Groups VIII - XIV by sensitivity to various combinations of phages. Groups VIII, IX, X, and XIII are qualitative groups only, i.e. strains in these groups may show either confluent lysis (with or without resistant growth) or isolated plaques with the phages concerned; additional reactions serve to type the strains quantitatively. In the other groups confluent lysis is obtained, except where the designation of the typing phage appears in brackets ( ); this indicates that the reaction obtained with the phage

TABLE 20.- Phage-types of Ps. aeruginosa: Main groups.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Designation of typing preparations															Phage-type	No. of strains in group
	A	B	C	D	E	F	G	H1	H2	K1	K2	K3	K4	L1	L2		
I	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	78
II	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	B	1
III	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	C	1
IV	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	D	8
V	-	-	-	-(+)	-	-	-	-	-	-	-	-	-	-	-	(E)	3
VI	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	F	6
VII	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	G	1
VIII	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-	BCD/H12/K1234	30
IX	-	-	+	+	-	-	-	-	+	+	+	+	+	-	-	CD/H2/K1234	11
X	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	D/K123	12
XI	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	AD	17
XII	+	-	-	+	-	-	-	-	+	-	-	+	+	-	+	AD/H2/K34/L2	7
XIII	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	ADE/L1	11
XIV	-	-	-	-	+	-	-	-	-	-	-	-	-	(+)	-	E/L(1)	7

It will be noted that the typing preparations K1 and K2 are identical as far as the formation of the main groups is concerned. They differ, however, in their action upon some of the strains in Groups I, XI, and XIII (see Tables 21, 25 and 26).

in question consists of isolated plaques.

The first 15 typing phages only are required for the purpose of placing any strain in its appropriate main group.

(b) Subdivision of main groups.

For convenience, strains will be described as either "basic" or "additive" types. "Basic" types in any group are those strains which show only the reactions common to the group, ie. as shown in Table 20, whereas "additive" types, while showing the group reactions, are also sensitive to other phages which serve to differentiate them into sub-groups and individual types.

Groups II, III, V and VII consist of strains of the basic type only, while groups VIII and IX consist of additive types only. The remaining groups comprise strains of both types. All the phage-types recognised are shown in Tables 21 - 26, in which the division of the groups into basic and additive types, and of the latter into sub-groups and individual types, may be clearly seen.

The phage-types expressed in Tables 20 - 26 do not necessarily represent the full range of sensitivity of any strain to the typing phages. They merely indicate the reactions

(i) by reason of which the strains have been

allotted to their respective groups, and

(ii) by means of which strains within the same group may be differentiated from each other.

The complete range of sensitivity of all strains to the typing preparations is given in Appendix II. This information may prove of value as corroborative evidence for future work on the serology of these strains of Ps. aeruginosa.

TABLE 21. - Phage-type of Ps.aeruginosa: Groups I, II and III.
( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type	PS Strains	No.of strains
I	A	21,26,30,36,37,39,44,46,49,50, 51,52,54,55,56,60,62,69,74,79, 81,123,143,147,153,155,159a, 159b,160,161,162a,162b,163, 164,165,166,167,168,170,171, 172,174,193,230,231,235.	46
I	A K2	87,118	2
I	A K2 M(12)	76	1
I	A L(1)	66a,66b	2
I	A L3	31,32	2
I	A M(1)	18,20,27,28,58,61,128,144,146, 148,150.	11
I	A M(12)	24,169,199,221,227,	5
I	A M12	67a,67b,190,219,220,233.	6
I	A N2	127.	1
I	A N5	224.	1
I	A V	132.	1
II	B	178.	1
III	C	218.	1

TABLE 22. - Phage-types of Ps. aeruginosa: Groups IV, V, VI and VII.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type	PS Strains	No. of strains
IV	D	80, 138, 140	3
IV	D L(2)	194	1
IV	D (E)	139	1
IV	D Pl(2)34	154	1
IV	D S	78a, 78b	2
V	(E)	22, 57, 75	3
VI	F	53, 63, 64, 82, 149	5
VI	F L3	90	1
VII	G	11	1

TABLE 23.- Phage-types of Ps. aeruginosa: Group VIII.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type			PS Strains	No. of strains
VIII	BCD/H12/K1234	E/L1234		130	1
VIII	BCD/H12/K1234	E/L1234	M(12)	232	1
VIII	BCD/H12/K1234	E/L1234	N3	5	1
VIII	BCD/H12/K1234	E/L1234	P34	229	1
VIII	BCD/H12/K1234	E/L1234	R2	15	1
VIII	BCD/H12/K1234	E/L1234	R3/W	6	1
VIII	BCD/H12/K1234	E/L1234	R4/V	117,121	2
VIII	BCD/H12/K1234	E/L(1)234	M12	226	1
VIII	BCD/H12/K1234	E/L(1)2(3)4	N23	34,42,43	3
VIII	BCD/H12/K1234	E/L(12)34	N124	129	1
VIII	BCD/H12/K1234	E/L123		4	1
VIII	BCD/H12/K1234	E/L123	M1	10	1
VIII	BCD/H12/K1234	E/L123	R1	68,88,89	3
VIII	BCD/H12/K1234	(E)/L123	P12345	17	1
VIII	BCD/H12/K1234	E/L(1)23	N1234	136	1
VIII	BCD/H12/K1234	E/L(1)23	V	16	1
VIII	BCD/H12/K1234	L1234		188	1
VIII	BCD/H12/K1234	L1234	N1234/R1	120,125,126	3
VIII	BCD/H12/K1234	L(1)234	N1(2)34/V	182	1
VIII	BCD/H12/K1234	L(1)234	N2	185	1
VIII	BCD/H12/K1234	L123(4)	N1234	95	1
VIII	BCD/H12/K1234	L123		137	1
VIII	BCD/H12/K1234	L(1)23	N1234/G	225	1

TABLE 24.- Phage-types of Ps. aeruginosa. Groups IX and X.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type			PS Strains	No.of strains
IX	CD/H2/K1234	L12	G	33	1
IX	CD/H2/K1234	L12	L_3	197	1
IX	CD/H2/K1234	L12	L(4)	83	1
IX	CD/H2/K1234	L12	S	122	1
IX	CD/H2/K1234	L12	T	14	1
IX	CD/H2/K1234	L(12)		181	1
IX	CD/H2/K1234	L(12)	BE/L3	13	1
IX	CD/H2/K1234	L(12)	R4	189	1
IX	CD/H2/K1234	L(1)23		91	1
IX	CD/H2/K1234	L2		7	1
IX	CD/H2/K1234	L(2)/(E)		186	1
X	D/K123			131	1
X	D/K123	H2/K4	N4	151	1
X	D/K123	H(2)/K(4)	L2	12	1
X	D/K123	H(2)/K(4)	(E)	29	1
X	D/K123	H(2)/K(4)	E/L3	187	1
X	D/K123	(C)/H(2)	L(124)	228	1
X	D/K123	(C)/H(2)	R4	133	1
X	D/K123	(C)/H(2)	S	192	1
X	D/K123	(C)/H(2)	V	9, 86	2
X	D/K123	K(4)	L34	23	1
X	D/K123	K(4)	L4	25	1

TABLE 25.- Phage-types of Ps.aeruginosa. Groups XI and XII.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type	PS Strains	No.of strains
XI	AD	35,38,40,41,47, 59,65,92,175, 183,234.	11
XI	AD ¦ K2	45	1
XI	AD ¦ K2 ¦ L4	222	1
XI	AD ¦ L3	142	1
XI	AD ¦ L3 ¦ N(1)23(4)	135	1
XI	AD ¦ L3 ¦ P145	152	1
XI	AD ¦ N5	236	1
XII	AD/H2/K34/L2 ¦ H1	145,180,196	3
XII	AD/H2/K34/L2 ¦ H1 ¦ L3	184	1
XII	AD/H2/K34/L2 ¦ H1 ¦ L3/M12	237	1
XII	AD/H2/K34/L2 ¦ N4	84, 85	2

TABLE 26.- Phage-types of Ps.aeruginosa. Groups XIII and XIV.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	Phage-type	PS Strains	No.of strains
XIII	ADE/L(1)	93	1
XIII	ADE/L(1) ¦ K2	179	1
XIII	ADE/L(1) ¦ K2 ¦ N2	48	1
XIII	ADE/L(1) ¦ L3 ¦ N2	134	1
XIII	ADE/L(1) ¦ L3 ¦ P12345	8	1
XIII	ADE/L(1) ¦ L4	195	1
XIII	AD(E)/L(1)	77	1
XIII	AD(E)/L(1) ¦ M2	200	1
XIII	AD(E)/L(1) ¦ M2 ¦ S	198	1
XIII	AD(E)/L(1) ¦ M2 ¦ S/V	176	1
XIII	AD(E)/L(1) ¦ M2 ¦ V	177	1
XIV	E/L(1)	94	1
XIV	E/L(1) ¦ L3	191	1
XIV	E/L(1) ¦ N123456	1,2,3	3
XIV	E/L(1) ¦ V	73,141	2

(2) Strains sensitive to undiluted phages only.

Ten strains of Ps. aeruginosa gave no reaction with the typing phages. They were, however, acted upon by one or more of the undiluted phages, and have been placed in the groups shown in the following Table.

TABLE 27.- Strains acted upon by concentrated phages only.

( ) indicates that isolated plaques are obtained instead of confluent lysis.

Group	TPx	Strains
CP:(E)	10^3	119
CP:F	10^2	124, 173, 223.
CP:H12	$10/10^3$	71
CP:H2	10^3	156, 157, 158, 70, 19.

The groups are denoted by the letters CP: followed by the type-designation of the particular phage(s). The strength of the concentrated phage compared to that of the typing preparation is given in the middle column (TPx).

These groupings are purely temporary. In view of the possible lysogenicity of the propagating strains, it was thought that adaptation of the phages to lyse these other strains might result in propagation of the phage carried by the original propagating strain. In order to

ascertain which phage had in fact been propagated during the adaptation process, it would be necessary to subject the resulting phage to neutralisation tests with appropriate antisera. The typing-phages have not yet been characterised serologically, and it was therefore considered preferable to group these strains by means of undiluted phages rather than by phages which might not be of the same serological character as the original.

THE NATURE OF PHAGE-TYPE IN PS. AERUGINOSA.

Examination of Tables 20 - 26 and Appendix II will show certain interesting points regarding phage-type in Ps. aeruginosa.

(1) Heterogeneous nature of phage-type.

(a) Certain types are merely a combination of two or more other types, e.g.

Strains of group I are of phage-type A.

Strains of group IV are of phage-type D.

Strains of group XI are of phage-type AD.

Thus the phage-type of strains in group XI is a combination of those of strains in groups I and IV. Other instances of this may be readily observed.

It is therefore feasible to regard some of the main groups as additive types of another group. Groups XI, XII, and XIII, for example, are really additive types of

group I, viz.:-

Strains of group I are of phage-type A

Strains of group XI are of phage-type A D

Strains of group XII are of phage-type A D/H2/K34/L2

Strains of group XIII are of phage-type A DE/L1

They are, however, more usefully regarded as constituting main groups.

(b) Individual strains of Ps. aeruginosa may belong to different main groups yet have certain features in common, e.g.

	Main group	Sub-group	Type
PS17 is of type	BCD/H12/K1234	(E)/L123	P12345
PS8 is of type	ADE/L(1)	L3	P12345

Both these strains show the reactions designated by the type P12345, yet, because of their other reactions, they must be placed in different groups.

From these observations it is clear that the system adopted for designating phage-type has the great advantage of showing the similarities between strains as well as stressing the differences.

It would appear, also, that a given culture of Ps. aeruginosa may behave towards the typing phages as a mixture of two or more distinct types, and must be characterised by the appropriate combination of phage preparations and not merely by one. Whether or not it would be

possible to separate such a culture into the various fractions responsible for each reaction has not been determined. Investigation of this point would involve the selection of a large number of colonies from the culture under study; the selection and testing of about 50 'colony strains' from PS17 gave no indication that such a separation could be effected. Information on this aspect of phage sensitivity of cultures of Ps. aeruginosa is not necessary for the present purpose.

(c) The precise significance of the differences in phage-type which have been found to exist among strains belonging to the same main group cannot be estimated at present. It is possible that serological analysis of the typing phages will show that some of these consist of the same phage propagated to a different specific titre. This is most likely to be the case with those phages grouped together on the basis of similarity in the plaques produced accompanied by similarity in host-range (Table 19). The reason for grouping such phages together was that they will probably prove to be of the same serological type (Burnet, 1933). If this does, in fact, prove to be the case, then many of the differences in phage-type shown in Tables 20 - 26 will be merely of a quantitative nature.

(2) Stability of phage-type.

Phage-type appears to be as stable a characteristic of Ps. aeruginosa as it has been found with other species.

The results shown in Tables 20 - 26 and in Appendix II have been found constant in at least five successive tests, each test being carried out in duplicate. Some of the strains (including those used as propagating strains) have been tested on more than ten occasions, and the same results obtained.

Cultures isolated from the heart-blood and spleen of mice in pathogenicity tests have always been found to be of the same phage-type as the cultures originally injected.

There is reason, then, to suppose that phage-typing of Ps. aeruginosa will prove as useful in tracing the source and course of infection by this organism as it has proved with staphylococci and other species.

(3) Effect of iridescence on reaction to typing phages.

The phenomenon of iridescence has no effect on the reaction on any strain to the typing preparations. This has been ascertained by performing parallel typing tests on all strains which showed the iridescent phenomenon, the plates in one test being incubated for 6 hours at 37°C., and left at room temperature overnight, and in the other at 37°C. for periods of 12 - 36 hours.

Iridescence appeared in the second test, but not in

the first. In both tests, nevertheless, the same reactions to the typing phages were shown. In order to detect phage reactions on plates showing iridescence it was often necessary to examine them much more carefully than the corresponding plates on which iridescence had not been permitted to appear. The iridescence tended to obscure the presence of isolated plaques, and the longer period of incubation at 37°C. resulted in the production of a greater amount of resistant growth.

The actual appearance of iridescence in any single strain appears to be subject to fluctuation. In some strains, e.g. PS5, PS17, iridescence was produced when the strains were first examined, disappeared from sub-cultures for periods of several months and subsequently reappeared for no apparent reason. Fluctuations of this nature were not accompanied by changes in phage-type.

APPLICATIONS OF PS. AERUGINOSA TYPING.

From the strains examined a few instances can be given which indicate the usefulness of phage-typing in showing how cross-infection with this organism may occur.

Table 28 shows the number of strains of each group which have been isolated at certain hospitals. In most cases a variety of types has been found but, in some cases, strains isolated at or about the same time have been found to be of the same phage-type.

(1) Three strains isolated at the same time from burns of patients in the Glasgow Royal Infirmary were found to be of the same phage-type - viz. strains Ps1, Ps2, and Ps3, all type E/L(1)/N123456 - thereby indicating a common source of infection.

These were the only cases infected with this organism at this time. A single case of infection occurring several weeks later was found to be due to a different type, viz. PS4, type BCD/H12/K1234/EL123.

(2) During an outbreak of Ps. aeruginosa infection in these same wards at a much later date 16 strains were isolated. All these were of phage-type A, 15 of them being the basic type and the remaining strain being the additive type A/M(12). Considered on its own, the fact that two apparently related types were present might suggest that an original strain had undergone a slight variation in its phage-sensitivity during its passage from one host to another. The observations already made on the stability of phage-type render such an occurrence unlikely, and it is probable that two distinct types were present.

(3) The most obvious case of cross-infection concerns three strains isolated at Law Hospital, Carluke. After a long period during which Ps. aeruginosa had been absent from this hospital, strain PS34 was isolated from urine.

Five days later, two strains of the same type (PS42, PS43) were isolated in the same ward from an infected appendix operation wound.

It does not seem likely that any one phage-type is responsible for any particular pathological condition. Table 29 shows the number of each group isolated from the types of infection which have supplied the majority of the strains studied. These figures and the particular case (3) quoted above indicate that any type may be found in any site of infection.

TABLE 28.- Phage-types of Ps. aeruginosa isolated at certain hospitals and laboratories.

Source	Total no. of strains	Number of strains of groups														
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	CP:F
Chase Farm Hospital, Enfield, Middlesex.	4	-	-	-	3	-	-	-	1	-	-	-	-	-	-	-
Royal Infirmary, Glasgow.	21	17	-	-	-	-	-	-	1	-	-	-	-	-	3	-
Hillingdon Hospital, Uxbridge, Middlesex.	12	3	1	-	2	1	-	-	-	-	-	1	-	4	-	-
Kent and Canterbury Hospital.	6	2	-	-	-	-	-	-	1	-	-	2	1	-	-	-
Law Hospital, Carluke.	3	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Melbourne University.	5	-	-	-	-	-	-	-	2	1	-	1	1	-	-	-
Poole Sanatorium, Nunthorpe.	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Park Hospital, London.	6	2	-	-	-	-	-	-	-	1	-	-	3	-	-	-
Southend-on-Sea General Hospital.	4	1	-	-	-	-	-	-	-	2	-	-	1	-	-	-
Victoria Infirmary, Glasgow.	47	27	-	-	1	2	5	-	-	-	2	8	-	1	-	1
Queen Mary's Hospital for Children, Carshalton.	6	3	-	1	-	-	-	-	-	-	-	-	-	2	-	-

TABLE 29.- Phage-types of Ps.aeruginosa isolated from certain pathological specimens.

Source	Total no.of strains	Number of strains of groups															
		I	II	III	IV	V	VI	VIII	IX	X	XI	XII	XIII	XIV	CP:F	CP:H12	CP:H2
Urine	68	35	-	-	3	3	5	2	1	3	11	1	1	1	1	-	1
Burns and wounds	36	21	-	-	-	-	-	4	1	1	3	-	1	4	-	-	1
Faeces	18	2	-	-	1	-	-	4	1	-	1	1	6	-	-	1	1
Ear infections	22	7	1	1	2	-	-	6	1	-	-	2	-	1	1	-	-

PHAGE-TYPE AND OTHER CHARACTERISTICS.

Several attempts have been made by previous workers on Ps. aeruginosa to classify strains of this species into groups, basing their classification on cultural characteristics, notably pigment production. Jordan (1899), for example, suggested that four varieties of the organism might be recognised, viz:-

'A' variety : producing pyocyanin and fluorescin;

'B' variety : producing pyocyanin only;

'C' variety : producing fluorescin only;

'D' variety : nonchromogenic.

Gessard (1919, 1920) also based his classification entirely on pigment production, while Hadley (1924) distinguished six types, characterised by various combinations of pigments and the ability to produce the iridescent phenomenon in successive subcultures.

It is general experience that pigment production is not a constant property of strains, and it has been already observed that the phenomenon of iridescence is also subject to fluctuation. Classifications based on these properties are, therefore, of little value.

Table 30 shows that strains differing in pigment production may belong to the same phage group. Although the number of strains in some of the groups is too small

to justify definite conclusions, it seems fairly certain that there is no connection between phage-type and pigment production or any other cultural or biochemical property. Even strains of the same sub-group or individual type may differ in such properties.

In connection with those strains which show certain "atypical" features, such as the production of indole and hydrogen sulphide, it should be appreciated that the strains which show these features are genuine strains of Ps. aeruginosa of the same phage-type as strains which do not show them.

TABLE 30.- Distribution of various characteristics among the main phage-types of Ps. aeruginosa.

Phage group	T	F	(F)	Pc	(Pc)	Pr	(Pr)	Ir.	P	(P)	GL	(GL)	AGl.	N	(N)	C	Ptn.	H2S	Ind.	H	MBR	NM
I	78	78	-	57	18	1	25	39	76	1	38	39	49	62	11	78	78	2	2	33	75	**1**
II	1	1	-	-	1	-	-	1	1	-	1	-	-	1	-	1	1	-	-	-	1	-
III	1	1	-	-	-	-	-	1	1	-	1	-	-	1	-	1	1	-	-	-	1	-
IV	8	8	-	6	2	-	2	5	8	-	2	6	6	8	-	8	8	-	-	6	8	-
V	3	2	-	1	2	-	2	-	1	1	1	2	2	2	1	3	3	-	-	2	2	1
VI	6	5	-	1	4	-	3	-	3	2	3	3	3	4	2	5	5	1	1	5	5	1
VII	1	-	-	-	1	-	-	-	-	-	1	-	1	1	-	1	1	-	-	1	1	-
VIII	30	30	-	21	8	1	3	14	26	4	15	14	20	24	6	30	29	-	3	15	26	**1**
IX	11	10	1	8	3	-	3	4	11	-	6	5	5	10	1	11	11	-	-	5	11	-
X	12	10	-	8	3	-	1	4	10	-	5	6	8	9	2	12	12	-	-	7	12	-
XI	17	17	-	10	6	-	4	10	13	1	6	10	14	11	5	17	17	-	-	10	16	-
XII	7	7	-	2	5	-	-	1	7	-	4	3	4	5	2	7	7	-	-	3	7	-
XIII	11	10	1	8	3	-	1	4	9	2	6	5	8	7	4	11	11	-	-	5	11	-
XIV	7	7	-	5	1	-	-	5	7	-	4	3	3	7	-	7	7	-	-	3	6	-
CP:H2	5	5	-	2	3	-	-	1	5	-	3	2	2	4	1	5	5	1	1	-	5	-
CP:H12	1	1	-	1	-	-	-	1	1	-	-	1	-	1	-	1	1	-	-	-	1	-
CP:F	3	3	-	-	3	-	-	2	1	2	-	3	3	3	-	3	3	-	-	-	3	-
CP:(E)	1	1	-	-	1	-	-	-	1	-	-	1	-	-	1	1	1	-	-	1	1	-

T : total no. of strains in group.
F : fluorescin } ( ): produced only on
Pc : pyocyanin } special medium.
Pr : pyorubrin in quantity.
Ir : iridescence on solid medium.
P : fatal for mice.
(P): produce local abscess only.
GL : gelatin rapidly liquefied.
(GL): gelatin slowly liquefied.
(Pr): slight pyorubrin production.

AGl : acid from glucose.
N : nitrogen from nitrates.
(N) : nitrite only from nitrates.
C : coagulum in milk.
Ptn. : peptonisation in milk.
Ind : indole from peptone.
H : haemolytic on blood agar.
MBR : methylene blue reduced.
NM : non-motile.

OBSERVATIONS ON AN ALTERNATIVE SYSTEM OF PHAGE-TYPING.

Examination of the results shown in Appendix II will show that all strains sensitive to the typing phages are acted upon by one or more of the phages of type-designation A, B, C, D, E, F, and G. This suggests that a system of grouping of strains might be arranged in which the main groups would be denoted by their range of sensitivity to those seven typing phages. Such a system would have the advantage over the one presented that a strain could be placed in its main group by testing against seven phages instead of fifteen.

For several reasons, however, the present system has been preferred.

In some strains reactions to one or more of the phages A - G were not so well-defined as those to other phages in the series. In group VIIl, for example, the majority of the strains were found sensitive to the phage A. The reaction to this phage obtained in many cases consisted of confluent lysis with a heavy disc of resistant growth. In some cases this was so heavy that the reaction could be detected only by a ring of partial lysis surrounding the secondary growth. In sharp contrast, the reactions indicated by the type designation H12/K1234 were found to be more frequently free, or almost free, from resistant

growth. The phage-type of strains of this group, therefore, gives a truer indication of the pattern of reactions when expressed as 'BCD/H12/K1234' than when expressed by the suggested alternative 'ABCD'.

The same consideration applies in several of the other groups.

There is, also, no reason to suppose that the seven phages A - G will be sufficient to type all strains likely to be isolated in the future. Three of the groups characterised by sensitivity to one of these phages each consist of a single strain. It is possible, therefore, that other strains may be isolated and found sensitive to only one of the other phages in the series.

From the point of view of economy on plates used in typing tests, the application of 15 phages does not involve more plates than would 7, as it is possible to 'spot' twenty or more phages on a 9 cm. plate.

It has seemed reasonable, then, to arrange the main groups on a broader basis than that involving only the phages A - G.

SECTION 5

THE APPARENT RELATIONSHIP OF PS. AERUGINOSA TO OTHER FLUORESCIN-PRODUCING SPECIES.

THE APPARENT RELATIONSHIP OF PS. AERUGINOSA TO OTHER FLUORESCIN-PRODUCING SPECIES.

Reference has already been made (Section 1) to the fact that, because of morphological and cultural similarity to Ps.aeruginosa (in particular the production of fluorescin) certain species of organisms have been included in many of the early reports relating to this organism (Meader et al., 1925). Of these species the most common is Ps.fluorescens which so closely resembles Ps. aeruginosa that some workers have considered it a variety of Ps. aeruginosa which has become adapted to a saprophytic existence (e.g.Tanner,1918).

Topley and Wilson (1946) suggest the use of the following characters to differentiate between the two organisms:-

	Ps. aeruginosa	Ps. fluorescens
(1)	Optimum temperature 37°C.; grows at 42°C.	Optimum temperature 25°C.; no growth at 42°C.
(2)	Pyocyanin and fluorescin formed.	Fluorescin only formed.
(3)	Liquefaction in gelatin stratiform and saccate.	Liquefaction in gelatin not always present; when present stratiform only.
(4)	Pathogenic to rabbits and and guinea-pigs.	Non-pathogenic to rabbits and guinea-pigs.

At the same time these authors stress the fact that the differences are by no means constant and that differentiation of the two organisms is often impossible.

Opinions on the value of serological distinction between the two organisms are contradictory. Sandiford (1937), for example, expressed the opinion that no cultural or serological distinction between the species was possible, but Munoz et al. (1945), on the other hand, found that Ps. fluorescens and other species which could not be satisfactorily differentiated from Ps. aeruginosa by means of biochemical characteristics could be very easily differentiated by serological means.

An investigation was carried out, therefore, to determine whether or not any phage relationships exist between the two species.

METHODS.

Source of strains.

In this investigation, 39 strains of fluorescin-producing organisms were examined. Of these, 7 were isolated from river water, 5 from canal water, 8 from sewage, 1 from a sample of synthetic cream, 1 was a laboratory culture, and 17 were received from the Blood Products Research Unit of the Lister Institute, where they had been isolated from plasma. One of the strains from river water, and the 17 from plasma were supplied to this laboratory as strains of Ps. aeruginosa.

Isolation of strains from water.

For the isolation of strains from water and sewage, use was made of the medium of Georgia and Poe (1931), referred to in Section 1 as medium 'F'.

The sample of water or sewage was added in 5 ml. volumes to 5 ml. of double-strength broth and incubated for 12-18 hours at 25°C. Tubes containing medium 'F' in approximately 5 ml. volumes were then inoculated with a few drops of these cultures and the tubes incubated for 18-24 hours at 25°C. Those cultures showing fluorescence were plated out to obtain a pure culture of the fluorescin-producing organisms.

Cultural methods.

The strains were examined by the methods described in Section 1 for strains of Ps. aeruginosa, the only difference being that all cultures were incubated at 25°C., which was found to be the optimum temperature for these strains.

Test for sensitivity of strains to Ps. aeruginosa phages.

Each strain being examined was grown in broth for 18 - 24 hours at 25°C., and about 10 drops of the culture spread on agar plates. When the inocula had been absorbed, drops of all the Ps. aeruginosa phages, both concentrated and in typing dilution, were spotted on to the plates.

These were incubated for 12 - 18 hours at 25°C., and then examined for evidence of phage action. As controls, the phages were spotted under the same conditions on plate cultures of their propagating strains.

Tests for lysogenicity of strains for Ps. aeruginosa.

(a) Sterile sintered-glass filtrates were prepared from 18 - 24 hour broth cultures of each strain and these filtrates spotted on to agar plates, each of which had been previously spread with a few drops of a young broth culture of one of the strains of Ps. aeruginosa. The plates were incubated for 6 hours at 37°C., left overnight at room temperature, and examined the following morning.

(b) In order to ensure that negative results were not due to complete removal of phage by adsorption on the filter, the strains were also examined for lysogenicity by a method similar to Fisk's (1942) cross-culture technique. Plates were spread with cultures of Ps.aeruginosa as before and, instead of filtrates, 18 - 24 hour broth cultures of the strains being tested were spotted on to them. The plates were examined after incubation.

Using these methods, the 39 strains of fluorescent species were tested for sensitivity to the 38 phages and also for lysogenicity for each of the 203 strains of Ps. aeruginosa.

RESULTS

(1) None of the fluorescent strains were sensitive to any Ps. aeruginosa phage.

(2) None were lysogenic for any strain of Ps. aeruginosa.

(3) Cultural and biochemical characteristics differed from those of Ps. aeruginosa in the following respects:-

(a) The optimum temperature was 25°C.; although some strains grew at 37°C. none showed growth at 42°C.

(b) No strain produced pyorubrin or pyocyanin even after prolonged subculturing on medium 'P'; all produced fluorescin on agar and in medium 'F'.

(c) The phenomenon of iridescence was not seen with any of these strains.

(d) No strain was pathogenic for mice.

These and other properties of the strains are presented in Appendix III.

Significance of results.

It is generally accepted that phage relationships involve similar antigenic structure. Even one positive indication of phage cross-reaction between Ps. aeruginosa and the fluorescin-producing strains would be sufficient to prove that the reacting strain was related to Ps. aeruginosa. A negative result might be an indication

that no such relationship exists, but it cannot be conclusive, because the organism concerned might be a phage-resistant strain of the same species.

When, however, negative results are consistently associated with some other constant difference, they become more significant.

Fig. 5 shows that one constant difference does exist, namely whether or not growth takes place at 42°C. This is the only property which can differentiate between the two groups. Properties which overlap to even the slightest extent cannot be regarded as satisfactory differential criteria, although they might be valuable as supplementary evidence.

All strains which showed growth at 42°C. were acted on by the available phages. A group of strains which would not grow at 42°C. were insensitive to the same phages. It seems likely, therefore, that the two groups of organisms are distinct species and antigenically unrelated.

These results support the conclusion of Munoz *et. al.* (1945) that *Ps. fluorescens* and *Ps. aeruginosa* can be differentiated serologically. Opposing views can be satisfactorily explained by the possibility that the strains of "*Ps. fluorescens*" examined by some workers were not genuine strains of this species, but merely strains of

Ps. aeruginosa which produced fluorescin but no pyocyanin. Such an error may be easily made since, of the strains of Ps. aeruginosa studied, about 96 per cent produced fluorescin on ordinary agar, while only about 65 per cent produced pyocyanin on the same medium. Unless optimum media are used, therefore, pigment production may be misleading.

The confusion which exists with regard to Ps.aeruginosa and Ps. fluorescens is not so much between typical strains of each species as between Ps. fluorescens and strains of Ps. aeruginosa which fail to produce pyocyanin on ordinary media. This confusion is well illustrated by the fact that 18 strains of fluorescent species were supplied to this laboratory as Ps. aeruginosa.

Mayr-Harting (1948), in a study of the serology of Ps. aeruginosa, excluded from her study strains which would not grow at 37°C. It would appear that incubation at this temperature alone might not differentiate between Ps. aeruginosa and Ps. fluorescens. Of the 39 strains which did not grow at 42°C., 26 showed growth at 37°C., and in 4 cases the growth was moderately good. Mayr-Harting's serological types, however, are true Ps.aeruginosa, since they have been studied here and found sensitive to the phage preparations.

It may be observed that Topley and Wilson (1946) have

furnished a detailed description of Ps. aeruginosa, in which the cultures are described as having been incubated at 25°C., although the description includes the facts that the optimum temperature is "30° - 37°C.", and the limits are "5° - 42°C." This description of Ps.aeruginosa at 25°C., is probably justified by the fact that production of pyocyanin is more rapid at 25°C. than at 37°C. If the strain being examined is a typically-pigmenting one, incubation at 25°C. is satisfactory. If, on the other hand, it is a strain which produces fluorescin only, confusion with Ps. fluorescens is possible.

The results obtained here, therefore, indicate that Ps. aeruginosa and Ps. fluorescens can be differentiated by:

(a) the ability of the former to grow at 42°C.; and

(b) serological methods, these being preferable to differentiation by means of phages, owing to the possibility of encountering phage-resistant strains.

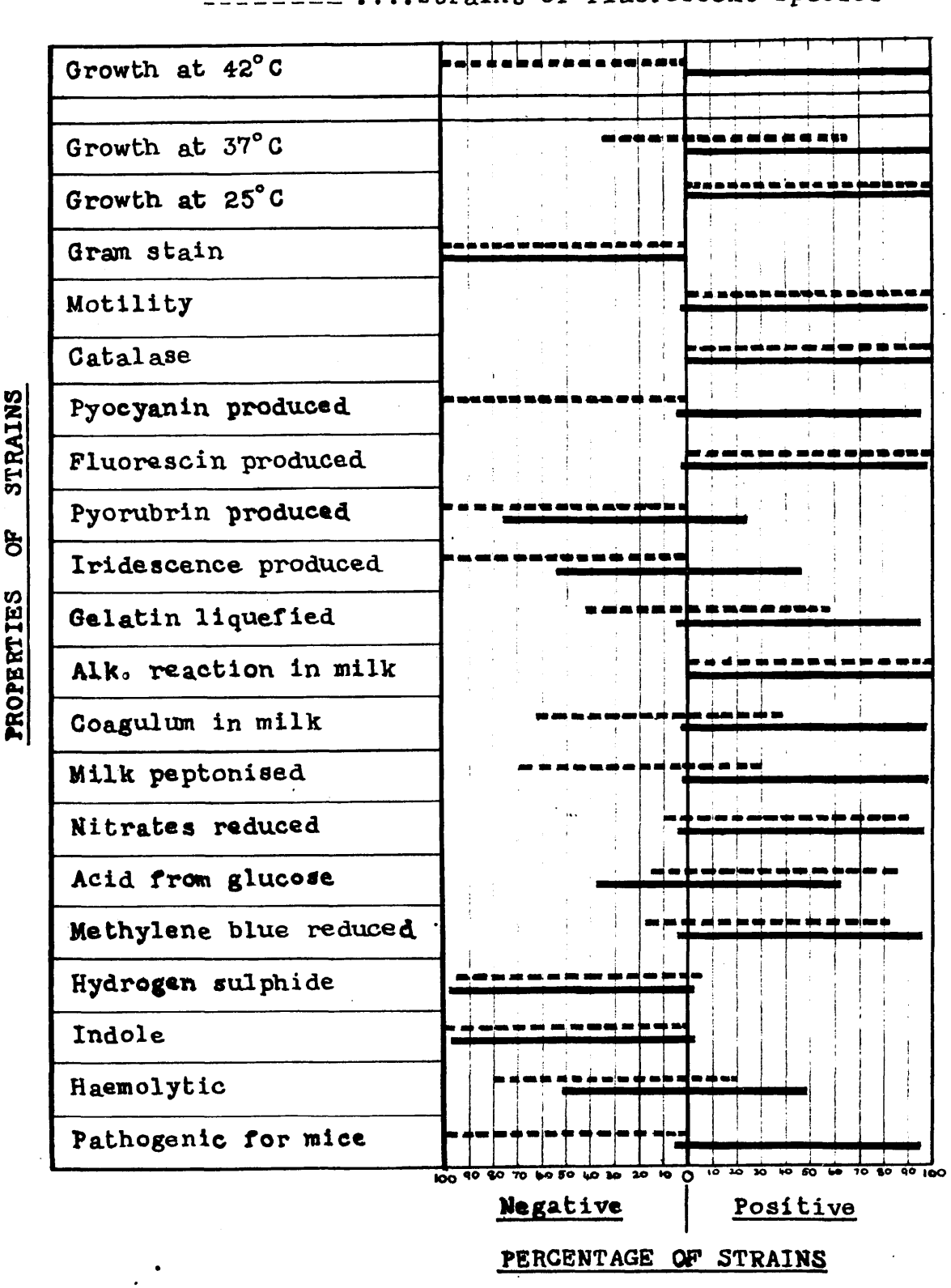


FIG. 5.- Diagrammatic comparison of the properties of strains of Ps. aeruginosa and fluorescent species.

SECTION 6

THE ISOLATION OF BACTERIOPHAGES FOR PS.AERUGINOSA FROM THE LABORATORY ATMOSPHERE.

THE ISOLATION OF BACTERIOPHAGES FOR PS. AERUGINOSA FROM THE LABORATORY ATMOSPHERE.

Throughout this study, all subcultures were examined for possible change in the production of pigment and iridescent areas, since variations of this nature might be accompanied by variation in phage relationships. On one occasion it was observed that one strain, PS8, was contaminated with small plaques. This strain produced no pyocyanin on ordinary nutrient agar and very little fluorescin. Growth was therefore almost completely achromogenic and the contaminating plaques appeared perfectly clear with no trace of metallic iridescence. There was no doubt that they were plaques caused by a typical phage.

A filtrate from this contaminated culture had a strong lytic action for the original strain PS8. The newly acquired lysogenic strain was designated PS8p and was retained for use as an additional indicator strain (Page 48).

It was thought possible that the contaminating phage was derived from the air, a source mentioned by Craigie and Felix (1947). The following experiments were carried out to explore this possibility.

METHODS

Technique for phage isolation.

Strains of Ps. aeruginosa used in these tests were the indicator strains PS1 to PS24.

Tubes containing 10 ml. of nutrient broth were inoculated, each with one drop (0.02 ml.) of a 4-hour broth culture of one of the indicator strains, and incubated at 37°C. for 1 hour. The tubes were then taken from the incubator, the plugs removed, and the contents exposed to the air for periods ranging from three to eighteen days.

On each day of the test, two drops of a 4-hour broth culture of the strain already inoculated were added to the contents of each tube. This was intended as an 'enrichment' process for any phage that may have been introduced from the air. On every third day, 2 - 3 ml. of culture were transferred to a small centrifuge tube and replaced with about 4 ml. of broth, the excess being to make up the small loss due to evaporation. The samples were centrifuged at 3000 r.p.m. for 20 minutes, and the supernatants spotted out on agar plates previously spread with a few drops of a 12-hour broth culture of the corresponding strains. The plates were incubated for 6 hours at 37°C., and left at room temperature overnight. By restricting the spotting test to the one strain, the possibility of a

phage reaction being due to lysogenicity of the indicator strain was eliminated; any phage detected must have been due to air-borne contamination.

The supernatant liquid rather than the whole culture was used in the spotting test because the culture was always contaminated with other organisms. Spotting out the whole culture would have resulted in a grossly contaminated plate and phage reactions might have been obscured.

The cultures were exposed in three laboratories:

No. 1. The laboratory in which the phage work was carried out.

No. 2. The adjoining laboratory, communicating with No. 1.

No. 3. Another laboratory some distance from Nos. 1 and 2.

Control cultures.

Control cultures, similar to those exposed, were kept in plugged tubes on the bench during the tests and inoculated daily at the same time as the exposed cultures. When phage was detected in an exposed culture, the corresponding control was centrifuged and the supernatant tested in the same way as that from the exposed tube.

Propagation of phages isolated.

Where phage contamination was indicated, the supernatant liquid from the cultures showing it was filtered, the filter being washed through with two successive 5 ml.

volumes of broth. The filtrate was inoculated with the sensitive strain and the phage in it propagated, purified, differentiated from, and compared with the phages already obtained from lysogenic strains and sewage, by the methods described in Section 2.

RESULTS

The results of four series of tests are shown in Table 31. Each series consisted of 3 sets of cultures of the 24 indicator strains, a set being exposed in each of the three rooms. Phage was found in 7 of the test cultures, the corresponding controls being negative.

TABLE 31.- Results of Tests for air-borne phage.

Test Number	Laboratory Number	Phage detected in days					
		3	6	9	12	15	18
A1	1	PS8	PS10	-	-	-	-
A2	2	PS17	-	-	-	-	-
A3	3	-	-	-	-	-	-
A4	1	PS14	-	-	-	-	-
A5	2	-	PS8	-	-	-	-
A6	3	-	-	-	-	-	-
A7	1	-	PS3	-	-	-	-
A8	2	-	-	-	-	-	-
A9	3	-	-	-	-	-	-
A10	1	PS10	-	-	-	-	-
A11	2	-	-	-	-	-	-
A12	3	-	-	-	-	-	-

(PS. numbers indicate the strains for which phage was detected.)

All the phages isolated in this way proved to be identical with one or another of the phages which were being examined while these tests were going on.

It was observed that the failure of a normal pigmenting strain to produce pigment in an exposed culture was associated with phage contamination occurring within three days of the commencement of the test. The loss of chromogenesis was not permanent; the property could be restored by cultivation of phage-resistant strains from these cultures on medium 'P'.

Significance of results.

The total number of cultures exposed to aerial contamination in these tests was 288; the number of contaminations with phage detected was 7, i.e. approximately 2.5 per cent of the total. In view of the favourable conditions for contamination provided during the tests this proportion cannot be considered high. When normal precautions are observed during subculturing operations, the proportion of accidental contaminations which may be expected will certainly be much lower than this figure. Only one case of accidental phage contamination (that of PS8 reported at the beginning of this section) was observed during the entire course of this work which, with reference to the 24 indicator strains alone, has involved several

thousand subcultures.

The danger of aerial contamination of cultures with phage appears to lie, not in the frequency with which it might be expected to occur, but in the fact that it might pass unobserved. Only if the degree of contamination is slight will plaques be produced. A heavily contaminated culture may consist of organisms all of which have become resistant and lysogenic in respect of the contaminating phage. If such a contaminated culture is used for the propagation of a typing phage, a permanent change in the characteristics of the phage may result. (Craigie and Felix, 1947).

The isolations of phage described here have been achieved in the laboratory in which phage work was being carried out and in the adjoining laboratory. No evidence of phage contamination has been observed in the cultures exposed in laboratory No. 3. It appears, therefore, that as a precautionary measure, strains used for the propagation of valuable typing phages should if possible be subcultured in a room reserved for the purpose and away from the phage laboratory. Maintenance of such strains in at least duplicate cultures will serve as an additional safety measure.

DISCUSSION

DISCUSSION.

The main purpose of this work has been to isolate a range of phages which would enable strains of Ps.aeruginosa to be phage-typed, as a preliminary to trials of therapy with specific phage preparations. This primary object has been achieved and the results, from the point of view of phage-typing, can be regarded as very encouraging. The prospects of success for phage-therapy, on the other hand, are not very promising, on account of the apparent readiness with which phage-resistant growth develops. As stated previously, it may be possible to overcome this difficulty by subjecting the phages to prolonged serial passage or by the use of combinations of phages rather than single preparations. Adopting the latter alternative, greatest possibilities of success will obviously exist in conditions where the infecting strain belongs to Group VIII, since strains of this group show the greatest range of sensitivity to the available phages.

The doubts which surround the possible effectiveness of phage-therapy, however, do not affect the probable value of phage-typing for strains of Ps. aeruginosa. The indications are that phage-typing will prove as valuable with this species as it has done with others.

Comparison of certain findings with those of Warner(1950a).

Warner (1950a), in a report which appeared while this investigation was in progress, showed that about 54 per cent of his strains of Ps. aeruginosa could be distinguished by their sensitivity to a range of phages which he isolated - although typing of the species was not the main purpose of his study. One essential difference between Warner's technique and that adopted here is that, while the 24 indicator strains used here were included in every test during the isolation of the phages, Warner's strains were each omitted from further tests as soon as a phage had been isolated against them. This certainly made sure that phages isolated subsequently would have a different host range, but it also prevented the isolation of different phages for any one strain, and is certainly one reason for the superior host-ranging efficiency of the phages isolated during this work, acting, as they do, on all of the 203 strains studied.

Warner also pointed out that two difficulties oppose the phage-typing of Ps. aeruginosa: firstly, that phage reactions can be obscured by the confluent iridescence that develops on many strains of the organism, and secondly, that since Ps. aeruginosa undergoes variation with apparent ease, strains being used for the maintenance of phage may alter in their phage sensitivity and thus lead to the loss

of useful typing preparations.

The phenomenon of iridescence does not constitute a serious difficulty, since it does not appear when incubation at 37°C. is limited to 6 hours; this period is sufficiently long for lysis of sensitive organisms to take place. It does not appear even after an additional 12 - 15 hours at room temperature, which latter period is necessary to enable the culture surrounding the lysed areas to grow and thus form a suitable background for the detection of the phage reactions. This restricted incubation, too, serves to prevent the over-rapid development of resistant growth in the lysed areas; as a result, the phage reactions are the more clearly defined. This method of incubating cultures has also made it possible to study the plaques produced by the phages; iridescence prevented Warner from doing this in some cases.

As regards the possibility of variation occurring in a propagating strain with consequent loss of the corresponding typing preparation, the stability of phage-type shown by the propagating strains used here seems to indicate that variation is no more likely to occur with Ps. aeruginosa than with any other bacterial species. This is supported by the observation of Gaby (1946) that each of the three basic colony types recognised by him remain stable under ordinary conditions of cultivation,

although in old cultures dissociation may occur. It seems reasonable to suppose that cultures, stabilised by prolonged laboratory cultivation, would prove suitable as propagating strains. Those used here are of this nature. Maintenance of such strains in duplicate cultures and in the dried state would serve to avoid accidental phage contamination, to which some 'spontaneous' variation is undoubtedly due. Moreover, the phages used here are stable for long periods under refrigeration, and fresh filtrates would have to be prepared only occasionally.

Observations made here on the ease with which resistant growth to the phages develops, and on the variable amount produced, agree with those of Warner and confirm the opinion expressed earlier (page 26) that, among the phages of Ps. aeruginosa, strains of low virulence predominate.

Heterogeneous nature of phage-type.

Typing tests have shown that a given culture of Ps. aeruginosa may behave as a mixture of two or more distinct phage-types. Many of those strains sensitive only to one phage will probably prove to be sensitive to other phages which may be isolated in the future. This is suggested by the fact that, with such strains, phage-

resistant growth is nearly always present in some degree or other; this resistant fraction will almost certainly be sensitive to another phage.

This 'mixed' nature of cultures of Ps. aeruginosa and the observation that many minor differences in phage-type exist among strains of the same main group, appear to agree with the finding of Gaby (1946), that a culture of this organism comprises an exceptionally heterogeneous population, differing in colony morphology and in biochemical and serological characteristics.

Improvement of the typing scheme presented.

The phage-typing scheme presented here is capable of improvement in certain directions.

Some of the strains used for propagation of the phages are known to be lysogenic. This is undesirable from the point of view of adaptation of a phage to lyse another strain in a specific dilution, since adaptation may lead to the propagation of the 'contaminant' phage in the filtrate, i.e. of the phage carried by the original propagating strain and which is present in the filtrate in small amount. This risk could be eliminated by the use of propagating strains which are free from phage. Alternatively, serological characterisation of the resulting phage would determine which phage has been propagated.

The provision of definitely phage-free strains will be a matter of considerable difficulty. Topley and Wilson (1946) point out that it would be "unwise to assert that any bacterial strain was certainly not carrying phage". Serological control is therefore the more feasible alternative. This will also be necessary in order to arrive at an accurate grouping of the typing phages, although it is believed that the groupings used here, based on similarities in plaque-type and host range, will prove very similar to those arranged on a serological basis.

Lastly, the range of available phages may be increased, chiefly in order to subdivide the basic types of Group I by more specific reactions. There is no theoretical limit to the number of phages which may be isolated.

Phage activity and the iridescent phenomenon.

This investigation did not set out to answer the question of the possible connection between phage activity and the iridescent areas of some strains of Ps.aeruginosa. The two aspects have, however, been so frequently combined in previous publications on the organism that some remarks on the nature of the iridescent areas, in the light of the results obtained here, are necessary.

This work was commenced with the theory that, whatever their causative agent, the iridescent areas would not

interfere with phage-typing the organism, except from the point of view of obscuring phage reactions on plate cultures. The results have confirmed this assumption. The presence or absence of iridescent areas in a culture had no effect on the sensitivity of the culture to the typing preparations. The suppression of the areas by restricted incubation in order to make the reactions more distinct was therefore justified.

Because of the connection between phage-type and antigenic structure, the fact that iridescence does not affect phage-type is in agreement with the finding of Mayr-Harting (1948) that antisera prepared against a blue iridescent strain and a matt yellow strain (selected on the assumption that they would differ in antigenic structure) gave strong cross-reactions and agglutinated 60 strains of Ps. aeruginosa to approximately the same titre.

Two observations made during this work agree with previous findings on the behaviour of the iridescent areas, namely,

(a) that iridescence is not associated with normal phage action on the organism;

(b) that iridescence and normal lysogenesis are not necessarily connected, since non-iridescent strains are often lysogenic.

Phage and the iridescent areas can be connected only if the latter indicate a special type of lysogenesis in which some cells of the lysogenic strain are sensitive to the associated phage. Normal lysogenesis does not involve lysis of the host (Delbruck, 1946). The fact that a phage cannot always be isolated from an iridescent strain does not necessarily mean that the strain is non-lysogenic, since the demonstration of the presence of a phage presupposes the possession of a suitable sensitive culture on which its action may be demonstrated.

Most of the evidence which has been put forward from time to time against any connection between phage and iridescence is based on differences between the visible properties of the iridescent areas and those of phage plaques. These have been summarised by Warner (1950b), and are briefly as follows:-

(1) Phage plaques are uniform in size while the size of the iridescent areas is variable from day to day on the same medium; phage plaques are usually smaller than the iridescent areas.

(2) Plaques are well-defined and circular in contrast to the iridescent areas which are not always perfectly circular.

(3) Phage plaques all appear within a few hours (less than 16 hours) while iridescent areas may appear at

any time up to 36 hours.

(4) Plaques do not increase in size; iridescent areas do increase.

(5) Plaques are never covered with iridescence, nor associated with crystals in the medium.

None of this evidence indicates any significant difference between the agent causing the phage plaques and that causing the iridescent clearings, since it is all of a quantitative rather than qualitative nature. Some of it, too, is open to question.

Plaques are only uniform in size if plated successively under the same conditions, and are perfectly circular only when discrete. When conditions vary, their size alters, and when a number coalesce the resulting area of clearing may not be circular. Observations on some of the strains used here have indicated that the iridescent areas may be as uniform as phage plaques and may in fact be smaller than some of the latter.

With regard to the time required for the appearance of phage plaques and iridescent areas on cultures, the fact that the latter take longer could mean that they were produced by a phage of very low virulence.

It is not completely accurate to say that plaques do not increase in size. It has been shown here that halos of partially lysed growth increase in width with continued

incubation (page 63), and such a halo must be considered as an essential part of the plaque. It has been the experience here, too, that increase in size of iridescent areas can often be attributed to the coalescence of a number of areas rather than any significant increase in the size of individuals.

The appearance of crystals in agar cultures of Ps. aeruginosa is quite a normal feature of all but very young cultures. Most of the strains studied have shown crystal formation in periods of from 3 days to several weeks. The presence of crystals, therefore, very probably indicates the presence of a large proportion of dead organisms in the culture, without indicating the nature of the agent causing death. The crystals will consist of a substance, perhaps pyocyanin, liberated from the dead organisms. It may be assumed that the reason for crystals not appearing when lysis is caused rapidly by virulent phage is that the crystalline substance is not synthesized before lysis occurs. Pyocyanin, for example, is not produced in the 6 hours at 37°C. within which phage lysis takes place.

Similar considerations apply to the iridescent material itself. Warner (1950b) found that the material resembled a fatty acid, and likened it to pyocyanase (Emmerich and Low, 1899) or the oily chloroform-soluble bacteriolytic substance isolated by Schoental (1941) from old broth

cultures of Ps. aeruginosa. Warner suggested that the iridescent material itself, produced by a variant colony in the midst of normal growth, was responsible for lysis of neighbouring cells. It might well be, however, that the iridescent material is liberated from organisms which have been lysed after developing sufficiently to produce it.

The chief criticism of all these points mentioned, however, is that iridescent areas are compared with normal phage plaques, and the strains producing them with strains sensitive to a normal phage. Such a comparison is hardly justified. Since iridescent areas, if produced by phage, must indicate a special type of lysogenesis, it would be more logical to compare their behaviour towards the strain producing them with that between a normal lysogenic strain and its associated phage. The studies of Lwoff et al. (1950a,b) indicate that lysogenesis and phage lysis are two distinctive phenomena.

The same criticism applies to the additional experimental evidence supplied by Warner (1950b), who found that the production of iridescent areas was inhibited by,

(a) using diluted broth in the preparation of the agar medium;

(b) using a semi-synthetic agar medium;

(c) adding a specified salt solution to the medium;

(d) adding certain concentrations of surface active agents to the medium.

These procedures had no effect on the action of normal phages on sensitive strains. It was not ascertained whether they had any effect of normal lysogenesis.

Warner's assumption that iridescent areas are "probably the result of bacterial variation" is certainly not in accordance with the observation made here that, whether iridescence appeared or not, the phage sensitivity of the strain remained unaltered. One of the common results of bacterial variation is an altered sensitivity to phages.

In the opinion of the author, then, it remains to be proved whether or not the iridescent areas of Ps. aeruginosa and phage are related. It may be that iridescent areas are caused through the action of a weak phage (perhaps incapable of existence apart from its host strain) in a special type of lysogenic culture. Final solution to the problem will probably involve a great deal of study, but the present lack of definite proof on this point is no barrier to successful phage-typing of Ps. aeruginosa.

SUMMARY

SUMMARY

(1) Bacteriophage action on Ps. aeruginosa is characterised by the readiness with which resistant growth develops. In the isolation of phages for this species, refrigeration of the propagating cultures after 6 hours incubation at 30°C. was employed successfully to check the development of resistant growth while allowing lysis of infected bacilli to proceed. Without refrigeration, resistant growth developed rapidly.

(2) Sintered-glass (5/3) filters interfered with the filtration of phage to a less degree than Seitz filters.

(3) Phages for Ps. aeruginosa were isolated from lysogenic strains and sewage, and resolved into 38 phage-typing preparations on the basis of host-range as determined by the specified typing-test technique. In typing tests, broth suspensions were used for spreading plates instead of broth cultures on account of the variable nature of the latter.

(4) The presence of iridescent areas on a surface culture of Ps. aeruginosa may obscure phage reactions on the organism. The phenomenon was prevented from interfering by restricting the period of incubation at 37°C. to 6 hours and leaving the plates at room temperature for a

further 12 - 15 hours. Under these conditions lysis by applied phages readily occurs but iridescence does not develop.

The appearance of iridescence on any culture of Ps. aeruginosa is subject to fluctuation, but has no influence on the phage-sensitivity of the culture.

(5) Among 193 strains of Ps. aeruginosa from a variety of clinical conditions and geographical sources, 14 main phage-types were recognised. An additional 10 strains were divided into 4 groups by the use of more concentrated phages.

(6) Although the 14 main phage-types are well defined, many strains show specific reactions which differentiate them from other strains of the same group.

Some strains behave towards the typing preparations as mixtures of two or more distinct phage-types, and must be characterised by the appropriate combination of phages instead of by single preparations.

(7) No apparent connection exists between phage-type and any cultural or biochemical property.

(8) Of the 203 strains examined, 12 exhibited atypical features such as non-motility and the production of indole and hydrogen sulphide. Phage-typing tests confirmed that

these strains were Ps. aeruginosa.

(9) No phage-relationships could be detected between two groups of organisms, one consisting of 203 strains of Ps. aeruginosa and the other of 39 strains of other fluorescin-producing species. A constant cultural difference between the two groups is the ability of Ps. aeruginosa to grow at 42°C.

(10) By means of a suitable technique, phages were isolated from the laboratory atmosphere. They proved to be identical with some of those being propagated at the time of the tests. Under normal working conditions the possibility of phage contamination occurring from this source appears to be slight.

(11) It is considered that the evidence yet presented, here or elsewhere, is insufficient to establish whether or not phage activity is responsible for the phenomenon of iridescence.

REFERENCES

REFERENCES.

Adcock, J.D. and Plumb, R.T. (1947) Jour.Amer.Med.Assoc., 133,579.

Albert, A., Francis, A.E., Garrod, L.P., and Linnell, W.H. (1938) Brit.J.Exp.Path.,19,41.

Alston, J. M. (1944) Brit.Med.J.,i,654.

Applebaum, M. and Patterson, M.B., (1936) J.Infect.Dis., 58,195.

Asheshov, I. (1926) C.R.Soc.Biol.,95,1029.

Asheshov, I.N., Asheshov, I., Lahiri, M.N., and Chatterji,S.K. (1933) Indian J.med.Res.,20,1159.

Asheshov, I.N., Wilson, J., and Topley, W.W.C. (1937) Lancet,i,319.

Barron, J.N. and Mansfield, O.T. (1944) Brit.med.J.,i,521.

Behrens, O.K. (1949) Immuno-Chemistry Research Dept., Lilly Research Laboratories, Indianapolis 6, Ind.,U.S.A. Personal communication.

Bergey: (1948) Manual of Determinative Bacteriology, 6th edition. (Bailliere,Tindall and Cox),page 89.

Bernoulli, R. (1943) Arch.Ges.Virusforsch.,2,533.

Berry, H. (1944) Lancet,ii,175.

Bodenham, D.C. (1943) Lancet,ii,725.

Botterell, E.H. and Magner, D. (1945) Lancet,i,112.

Browning, C.H. (1943) Brit.med.J.,i,341.

Burnet, F.M. (1930) "A System of Bacteriology in Relation to Medicine", London (Medical Research Council) Vol.7,p.494. (1933) J.Path.Bact.,36,307.

Cairns, H. (1944) Brit. J. Surg., 32,199.

Cherry, W.B. and Watson, D.W. (1949) J.Bact.,58,601.

Colebrook, L., Clark, A.M., Gibson, T., and Todd, J.P. (1942-43) "Studies of Burns and Scalds" (M.R.C.) Special Report Series, No.249, Part II, page 22.

Coleman, M.F., Reid, J.J., and Farrell, M.A. (1943) J.Bact.,45,38.

Cooper, F.B., Gross, P., and Lewis, M. (1939) Proc.Soc. exp.Biol.Med.,40,34.

Craigie, J., and Felix, A. (1947) Lancet,i,823.

Craigie, J., and Yen, C.H. (1938) Canada publ.Hlth.J., 29,448,484.

Delbruck, M. (1946) Biol.Rev.,21,30.

Dickinson, L. (1948) J.Gen.Microbiol.,2,154.

Dubos, R.J., Strauss, J.H. and Pierce, C. (1943) J.exp.Med., 78,161.

Emmerich, R. and Low, O. (1899) Z.Hyg.Infektkr.,31,1.

Evans, F.T. (1945) Lancet,i,115.

Fastier, L.B. (1945) J.Bact.,49,633; ibid.,50,301.

Felix, A. and Callow, B.R. (1943) Brit.med.J.,ii,127.

Fisk, R.T. (1942) J.Infect.Dis.,71,153,161.

Fisk, R.T. and Mordvin, O.E. (1944) Amer.J.Hyg.,40,232.

Florey, M.E., Ross, R.W.N.L., and Turton, E.C. (1947) Lancet,i,855.

Gaby, W.L. (1946) J.Bact.,51,217.

Garretson, W.T. and Cosgrove, K.W. (1927) J.Amer.Med.Assoc., 88,700.

Georgia, F.R. and Poe, C.F. (1931) J.Bact.,22,349.

Gessard, C. (1822) C.R.Acad.Sci.,94,563.
(1890) Ann.Inst.Pasteur,4,88.
(1891) ibid., 5,65.
(1892) ibid., 6,801.

Gessard, C. (1917) C.R.Acad.Sci.,165,1071.
(1919) Ann.Inst.Pasteur,33,241.
(1920) ibid., 34,88.
(1925) C.R.Acad.Sci.,174,1301.

Gough, J., Berry, H. and Still, B.M. (1944) Lancet,ii,176.

Hadley, P. (1924) J.Infect.Dis.,34,260.

Harper, G.J. (1943) Lancet,ii,569.

Hauduroy, P. and Peyre, E. (1923) C.R.Soc.Biol.,88,688.

d'Herelle, F. (1926) The Bacteriophage and its Behaviour, London, (Bailliere, Tindall, and Cox).

Hunter, C.A. and Ensign, P.R. (1947) Amer.Jour.Publ.Hlth., 37,1166.

Jones, D., Metzger, H. J., Schatz, A., and Waksman, S.E., (1944) Science, 100,103.

Jordan, E.O. (1899) J.exp.Med.,4,627.

Knop, C.Q. (1946) Proc.Staff Meet. Mayo Clin.,21,273.

Kohn, F., Hall, M.H. and Gross, C.D. (1943) Lancet,i,140.

Krueger, A.P. and Scribner, E.J. (1941) J.Amer.Med.Assoc., 116,2160.

Larkum, N.W. (1926) J.Bact.,12,203.

Lewin, W. (1948) Brit.Jour.Surg.,35,266.

Lilley, A.B. and Bearup, A.J. (1928) Med.Jour.Austral.,i,362.

Lwoff, A., and Gutmann, A. (1950a) Ann.Inst.Pasteur,78,711.

Lwoff, A., Siminovitch, L., Kjeldgaard, N., Rapkine, S., Ritz,E. and Gutmann, A. (1950b) Ann.Inst. Pasteur,79,815.

Lisch, H. (1924) Zbl. Bakt.,Orig.,93,421.

Mamelle, A. (1918) C.R.Soc.Biol.,81,1137.

Mayr-Harting, A. (1948) J.Gen.Microbiol.,2,31.

Meader, P.D., Robinson, G.H. and Leonard, V. (1925) Amer.J.Hyg.,5,682.

Melton, G. and Beck, A. (1939) Lancet,i,867.

Moragues, V. and Anderson, W.A.D. (1943) Ann.Internal.Med., 19,146.

Morley, G.H. and Bentley, J.P. (1943) Lancet,i,138.

Munoz, J., Scherago, M. and Weaver, R.H. (1945) J.Bact., 49,524.

Okuda, S. (1923) Arch.Hyg.,Berl.,92,109.

Paine, T.F., Murray, R., Seeler, A.O. and Finland, M. (1947) Ann.Internal Med.,27,494.

Pandalai, N.G. (1941) J.Path.Bact.,53,150.

Pesch, K.L. and Sonnenschien, C. (1925) Klin.Wschr.,4,1585.

Pulvertaft, R.J.V. (1943) Lancet,ii,341.

Rabinowitz, G. (1934) J.Bact.,28,237.

Robinson, H.J., Smith, D.G. and Graessle, O.E. (1944) Proc.Soc.exp.Biol.Med.,57,226.

Robson, J.M. and Scott, G.I. (1941) Nature,148,167.
(1942) Brit.med.J.,i,5.

Ruzicka, S. (1898) Zbl.Bakt.,24,11.

Ryan, T.C., Jones, D.T., White, W.L., Orr,T.E., Walters, W. and Hayes, T.G. (1946) Ann.Surgery,124,1047.

Sakula, J. (1943) Lancet,ii,758.

Sandiford, B.R. (1937) J.Path.Bact.,44,567.

Schaffer, A.J. and Oppenheimer, E.H. (1948) Southern Med.Jour., 41,460.

Schoental, R. (1941) Brit.J.exp.Path.,22,137.

Schultz, E.W., Thomassen, P.R. and Marton, L. (1948) Proc.Soc.exp.Biol.Med.,68,451.

Schwarz, L.H. and Lazarus, J. (1947) J.Bact.,53,506.

Sherwood, N.P., Johnson, T.L. and Radotincky, I. (1926) Univ.Kansas Sci.Bull.,15,91.

Tanner, F.W. (1918) J.Bact.,3,63.

Topley, W.W.C., and Wilson, G.S. (1946) "Principles of Bacteriology and Immunity", 3rd edition, (Edward Arnold and Co.), pp.346,510.

Vuylsteke, C.A. (1947) Brit.med.J.,i,179.

Warner, P.T.J.C.P. (1950a) Brit.J.exp.Path.,31,112.
(1950b) ibid., 31,242.

Williams, R.E.O., Clayton-Cooper, B., Faulkner, H.C. and Thomas, H.E. (1944) Lancet,i,787.

Wilson, G.S. and Atkinson, J.D. (1945) Lancet,i,647.

Zaytzeff - Jern, H. and Meleny, F.L. (1936) J.Lab.Clin.Med., 22,284.

APPENDIX I

THE PATHOLOGICAL AND GEOGRAPHICAL SOURCES, AND THE PROPERTIES OF THE STRAINS OF PS.AERUGINOSA.

KEY TO TABLE 32

(1) DETAILS OF SOURCE

"S" : denotes the hospital or laboratory from which the culture or swab was received.

"Ref." : denotes the reference (name or number) attached to the culture by the hospital or laboratory supplying it.

"Isolated": refers to the geographical source of the culture; this differs, in some cases, from the situation of the hospital or laboratory.

Key to source

A ..... Astley-Ainslie Hospital, Edinburgh.

B ..... Bristol University (Dr. A. Mayr-Harting).

C1..... Chase Farm Hospital, Enfield, Middlesex.

C2..... Charing Cross Hospital Medical School, London.

C3..... Ciba Laboratories Ltd., Basle, Switzerland.

D ..... R.A.M. College, Millbank.

E ..... Edgware General Hospital, Middlesex.

F ..... Farnborough Hospital, Kent.

G ..... Glasgow Royal Infirmary.

H ..... Hillingdon Hospital, Uxbridge, Middlesex.

I ..... Institute of Laryngology and Otology, London.

J ..... Glasgow Veterinary College.

Key to source (contd.)

K ..... Kent and Canterbury Hospital, Canterbury.

L ..... Law Hospital, Carluke.

M ..... Melbourne University.

N ..... National Collection of Type Cultures.

O ..... Poole Sanatorium, Nunthorpe.

P ..... Park Hospital, London.

Q ..... Queen Mary's Hospital for the East End.

R ..... Royal Infirmary, Manchester.

S ..... Southend-on-Sea General Hospital.

T ..... Wellcome Foundation.

U ..... University of Louvain, Belgium.(Prof.R.G. Bruynoghe)

V ..... Victoria Infirmary, Glasgow.

W ..... Westminster School of Medicine.

X ..... Royal College of Physicians Laboratory,Edinburgh.

Y ..... Queen Mary's Hospital for Children, Carshalton, Surrey.

KEY TO TABLE 32

(2) PROPERTIES OF STRAINS

Numbers in any part of the table refer to period of incubation in days at 37 °C.

Pigment production:

F .. fluorescin + .. on agar

Pc.. pyocyanin (+).. on optimum medium only

\+ .. predominant pigment

Pr.. pyorubrin ± .. slight within 4 weeks

\- .. negative within 4 weeks

Ir. - Iridescent areas:

(24 hrs. at 37 °C.)+ .. less than 5 areas on slope culture

++.. between 5 and 15 areas

LA.. large areas of confluent iridescence

CI.. confluent iridescence almost covering culture

P - Pathogenicity for mice:

(intramuscular inj.) ++.. fatal within 24 hours

\+ .. fatal between 1 and 7 days

L .. cause local abscess only

\- .. non-pathogenic

GL - Gelatin liquefaction:

\+ .. complete within 24 hours

± .. commencing between 1 and 7 days

(±).. commencing between 7 and 28 days

\- .. negative up to 28 days

NR - Nitrate reduction:

N .. to nitrogen

\+ .. to nitrite only

MBR - Methylene-blue reduction:

\+ .. complete reduction

± .. partial reduction

\- .. no reduction

G - Glucose: A .. acid produced

R .. indicator reduced

BCP Milk: Alk. reaction alkaline

C .. coagulum formed

P .. complete peptonisation

Pa.. partial peptonisation

H - Haemolysis: + .. strong

± .. weak

I - Indole in peptone water

HS - Hydrogen sulphide in lead acetate agar

M - Motility

Table 32: Source and properties of strains of Ps.aeruginosa.

All strains (a) Gram negative; (b) Catalase positive; (c) Opt. Temp. 37°C.; (d) Grow at 42°C.

PS. No.	Details of Source				Pigment			Ir.	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
1	Burn	G	-	Glasgow	+	+	-	+	++	+	-	N	Alk.C. P6	-	-	+	+	+	XIV
2	Burn	G	-	Glasgow	+	+	-	+	+	+	-	N	Alk.C. P6	-	-	+	+	+	XIV
3	Burn	G	-	Glasgow	+	+	-	++	+	+	-	N	Alk.C. P4	-	-	+	±	+	XIV
4	Burn	G	-	Glasgow	+	+	±	-	++	+	-	N	Alk.C. P6	-	-	+	+	+	VIII
5	-	T	CN 1362	-	+	+	±	LA	+	±	-	N	Alk.C. P6	-	-	+	+	+	VIII
6	-	N	254	-	+	+	-	CI	L	±	-	N	Alk.C. P6	-	-	+	±	+	VIII
7	-	N	1540	-	+	+	±	-	+	+	Al	N	Alk.C. P6	-	-	+	+	+	IX
8	-	N	1784	-	(+)	(+)	-	-	L	+	Al	N	Alk.C. P6	-	-	+	±	+	XIII
9	-	N	1999	-	+	+	-	-	++	+	Al	N	Alk.C. P7	-	-	+	±	+	X
10	-	N	2000	-	+	+	-	-	+	+	Al	N	Alk.C. P6	-	-	+	+	+	VIII
11	-	N	2002	-	-	(+)	-	-	-	+	A2	N	Alk.C. P6	-	-	+	±	+	VII

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir.	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
12	-	N	2003	-	+	+	-	-	++	±	-	N	Alk.C. P7	-	-	+	±	+	X
13	-	N	2005	-	+	+	-	-	++	+	-	N	Alk.C. P6	-	-	+	±	+	IX
14	-	N	2034	-	(+)	(+)	-	-	++	+	-	N	Alk.C. P6	-	-	+	±	+	IX
15	-	N	2157	-	+	+	-	CI	++	+	R	N	Alk.C. P6	-	-	-	+	+	VIII
16	-	N	6750	-	+	+	-	CI	L	+	-	N	Alk.C. P6	-	-	-	±	+	VIII
17	-	N	7244	-	+	+	-	++	++	±	-	N	Alk.C. P6	-	-	-	±	+	VIII
18	Urine	R	S4M	Manchester	+	+	±	-	++	±	Al	N	Alk.C. Pa7	-	-	-	+	+	I
19	Urine	R	4M	Manchester	+	+	-	-	++	±	Al	+	Alk.C. P5	+	+	-	+	+	[CP:H3]
20	Urine	R	O.P.	Manchester	+	(+)	±	-	++	±	Al	N	Alk.C. Pa7	-	-	-	+	+	I
21	Swab from hernia wound	R	S4M	Manchester	+	(+)	-	-	+	±	-	N	Alk.C. Pa7	-	-	-	+	+	I
22	Urine	V	5894/50	Glasgow	+	(+)	±	-	-	±	A4	N	Alk.C. P4	-	-	±	-	-	V
23	Urine	R	O.P.	Manchester	+	(+)	-	-	+	+	-	N	Alk.C. P5	-	-	+	±	+	X

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir.	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
24	Burn	G	-	Glasgow	+	+	-	LA	++	±	-	N	Alk.C. Pa7	-	-	±	+	+	I
25	Urine	V	3376/50	Glasgow	+	+	±	LA	-	±	-	+	Alk.C. Pa7	-	-	-	±	+	X
26	Urine	V	3379/50	Glasgow	+	+	±	CI	+	±	A2	N	Alk.C. Pa7	-	-	-	+	+	I
27	Urine	V	3192/50	Glasgow	+	+	±	-	+	+	A1	-	Alk.C. P7	-	-	±	±	+	I
28	Urine	V	4261/50	Glasgow	+	+	±	-	+	+	A4	-	Alk.C. P7	-	-	±	+	+	I
29	Sputum	V	4731/50	Glasgow	-	+	-	LA	+	±	A3	N	Alk.C. Pa7	-	-	-	+	+	X
30	Unknown	-	(1)	Unknown	+	+	±	-	-	±	A1	+	Alk.C. P7	-	-	±	+	+	I
31	Unknown	-	(2)	Unknown	+	+	±	CI	++	±	A2	N	Alk.C. Pa7	-	-	-	+	+	I
32	Unknown	-	(3)	Unknown	+	+	±	CI	+	±	-	N	Alk.C. Pa7	-	-	-	+	+	I
33	Unknown	-	(4)	Unknown	+	+	±	+	+	±	-	N	Alk.C. Pa7	-	-	-	+	+	IX
34	Urine	L	-	Carluke	+	(+)	-	-	+	±	A1	+	Alk.C. P3	-	+	+	+	+	VIII
35	Urine	V	4998/50	Glasgow	+	+	-	CI	L	±	A2	+	Alk.C. Pa7	-	-	±	+	+	XI

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir.	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
36	Urine	V	6035/50	Glasgow	+	+	-	-	++	+	A3	N	Alk.C. P3	-	-	±	+	+	I
37	Urine	V	5357/50	Glasgow	+	+	±	+	+	±	-	+	Alk.C. Pa7	-	-	-	+	+	I
38	Urine	V	5382/50	Glasgow	+	+	-	-	+	+	A3	+	Alk.C. P3	-	-	±	+	+	XI
39	Urine	V	5305/50	Glasgow	+	+	-	Cl	+	±	A2	N	Alk.C. Pa7	-	-	-	±	+	I
40	Urine	V	5788/50	Glasgow	+	+	±	LA	-	±	A1	+	Alk.C. Pa7	-	-	+	±	+	XI
41	Urine	V	6211/50	Glasgow	+	+	±	LA	-	±	A3	+	Alk.C. Pa7	-	-	-	-	+	XI
42	Appendix operation wound	L	-	Carluke	+	+	-	-	+	+	A1	+	Alk.C. P3	-	+	+	+	+	VIII
43	as PS42	L	-	Carluke	+	+	-	-	+	+	A1	+	Alk.C. P3	-	+	+	+	+	VIII
44	Urine	V	6292/50	Glasgow	+	+	-	-	+	+	A3	+	Alk.C. P4	-	-	+	±	+	I
45	Urine	V	6554/50	Glasgow	+	+	±	LA	-	±	A1	N	Alk.C. Pa7	-	-	-	±	+	XI
46	Urine	V	6576/50	Glasgow	+	+	-	-	++	±	A2	+	Alk.C. P3	-	-	+	+	+	I
47	Urine	V	6638/50	Glasgow	+	+	±	-	+	+	A1	+	Alk.C. P7	-	-	+	+	+	XI

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir.	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
48	Ulcer	V	6941/50	Glasgow	+	+	±	+	L	+	A4	+	Alk.C. P4	-	-	+	±	+	XIII
49	Urine	V	7067/50	Glasgow	+	+	±	-	+	+	A2	+	Alk.C. P3	-	-	+	+	+	I
50	Urine	V	7530/50	Glasgow	+	+	-	CI	+	±	A2	N	Alk.C. Pa7	-	-	-	±	+	I
51	Urine	V	7720/50	Glasgow	+	+	-	CI	+	+	A2	N	Alk.C. Pa7	-	-	-	±	+	I
52	Urine	V	7787/50	Glasgow	+	+	-	CI	+	+	A1	N	Alk.C. Pa7	-	-	-	-	+	I
53	Urine	V	8145/50	Glasgow	+	(+)	±	-	L	+	-	N	Alk.C. P4	-	-	+	±	+	VI
54	Urine	V	8146/50	Glasgow	+	+	±	CI	+	+	-	N	Alk.C. P5	-	-	-	+	+	I
55	Urine	V	8148/50	Glasgow	+	+	±	CI	++	±	-	N	Alk.C. P5	-	-	-	+	+	I
56	Urine	V	8352/50	Glasgow	+	(+)	-	-	++	+	-	+	Alk.C. P5	-	-	+	±	+	I
57	Urine	V	8379/50	Glasgow	+	(+)	±	-	L	+	-	+	Alk.C. P5	-	-	-	±	+	V
58	Urine	V	9943/50	Glasgow	+	(+)	-	-	++	+	-	N	Alk.C. P3	-	-	+	+	+	I
59	Urine	V	8893/50	Glasgow	+	+	-	CI	++	(±)	A1	N	Alk.C. P7	-	-	-	+	+	XI

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
60	Urine	V	10335/50	Glasgow	+	+	-	CI	++	(±)	-	N	Alk.C. Pa7	-	-	-	+	+	I
61	Urine	V	10332/50	Glasgow	+	(+)	-	-	++	+	-	N	Alk.C. P3	-	-	+	+	+	I
62	Urine	V	10333/50	Glasgow	+	+	-	CI	++	±	-	N	Alk.C. P7	-	-	±	+	+	I
63	Urine	V	10528/50	Glasgow	+	(+)	±	-	++	±	-	N	Alk.C. P3	-	-	+	+	+	VI
64	Urine	V	10601/50	Glasgow	+	(+)	±	-	+	+	-	+	Alk.C. P4	-	-	+	+	+	VI
65	Urine	V	10646/50	Glasgow	+	+	-	LA	++	±	Al	N	Alk.C. P4	-	-	-	+	+	XI
66a	Exteriorised empyema	O	-	Nunthorpe	+	(+)	±	-	++	±	Al	N	Alk.C. P7	-	-	-	+	+	I
66b	As above	O	-	Nunthorpe	+	(+)	±	-	++	±	Al	N	Alk.C. P7	-	-	-	+	+	I
67a	Ear Swab	V	10699/50 A	Glasgow	+	(+)	±	-	+	+	Al	+	Alk.C. P7	-	-	+	+	+	I
67b	Ear Swab	V	10699/50 B	Glasgow	+	(+)	±	-	+	+	Al	+	Alk.C. P7	-	-	+	+	+	I
68	Wound discharge	W	Ps 1	London	+	+	-	-	++	+	Al	N	Alk.C. P2	-	-	+	+	+	VIII
69	Preoperative swab	W	Ps 2	London	+	(+)	±	-	++	±	Al	N	Alk.C. Pa7	-	-	-	+	+	I

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
70	Faeces	W	Ps 3	London	+	+	-	+	+	±	A1	N	Alk.C. P7	-	-	-	+	+	[CP:H2]
71	Faeces of patient on aureomycin therapy	W	Ps 4	London	+	+	-	LA	+	±	-	N	Alk.C. Pa7	-	-	-	+	+	[CP:H12]
73	Urine	X	-	Edinburgh	+	+	-	++	++	(±)	A2	N	Alk.C. P5	-	-	-	+	+	XIV
74	Unknown	H	Ps 1	Uxbridge	+	+	-	-	++	+	A2	+	Alk.C. P5	-	-	+	+	+	I
75	Urine	H	Ps 2	Uxbridge	-	+	-	-	+	±	A1	N	Alk.C. Pa7	-	-	±	±	+	V
76	Chronic otitis media	H	Ps 3	Uxbridge	+	+	-	-	++	(±)	A1	N	Alk.C. Pa7	-	-	-	+	+	I
77	Urine	H	Ps 4	Uxbridge	+	(+)	-	-	++	±	-	+	Alk.C. P7	-	-	+	±	+	XIII
78a	Neonatal otitis media	H	Ps 5A	Uxbridge	+	+	±	++	++	(±)	A4	N	Alk.C. P6	-	-	+	±	+	IV
78b	As above	H	Ps 5B	Uxbridge	+	+	±	++	++	(±)	A4	N	Alk.C. P6	-	-	+	±	+	IV
79	Urine	V	10947/ 50	Glasgow	+	+	-	CI	+	(±)	A2	N	Alk.C. P6	-	-	-	+	+	I
80	Urine	V	10993/ 50	Glasgow	+	+	-	CI	+	(±)	A2	N	Alk.C. P6	-	-	±	+	+	IV
81	Urine	V	10994/ 50	Glasgow	+	+	-	CI	+	±	A2	N	Alk.C. Pa7	-	-	±	±	+	I

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
82	Urine	V	11056/50	Glasgow	+	(+)	-	-	++	±	A3	N	Alk.C. P7	-	-	+	+	+	VI
83*	-	D	NCTC 1999 A	-	+	(+)	-	-	++	±	A2	N	Alk.C. P7	-	-	-	+	+	IX
84	Unknown	D	B	University Coll.Hosp.	+	(+)	-	-	+	±	A2	N	Alk.C. P7	-	-	-	+	+	XII
85	Faeces	D	C	Military Hospital, Horley.	+	(+)	-	-	+	(±)	A2	N	Alk.C. P7	-	-	-	+	+	XII
86*	Septic Finger	D	D	Military Hospital, Millbank.	+	(+)	-	-	++	(±)	A2	N	Alk.C. P7	-	-	+	+	+	X
87	Faeces	D	E	Military Hospital, Millbank.	+	(+)	-	-	+	±	A1	N	Alk.C. P5	-	-	-	+	+	I
88	Faeces	D	F	Military Hospital, Millbank.	+	(+)	±	-	+	±	A2	N	Alk.C. P7	-	-	-	+	+	VIII
89	"Camp Water"	D	G	Military Hospital, Malta.	+	(+)	-	-	+	±	A2	+	Alk.C. P6	-	-	-	+	+	VIII
90	Unknown	S	19190	Southend-on-Sea.	-	-	-	-	-	(±)	A1	+	Alk.	+	+	+	+	-	VI
91	Unknown	S	19431	Southend-on-Sea.	+	+	-	-	+	±	A1	N	Alk.C. P5	-	-	+	+	+	IX
92	Faeces	F	-	Farnborough, Kent.	+	+	-	++	+	±	A1	N	Alk.C. P7	-	-	-	+	+	XI
93	Faeces	F	-	Farnborough, Kent.	+	+	-	-	+	(±)	A2	+	Alk.C. P5	-	-	±	+	+	XIII

* In view of phage-type, these cultures are thought to have been wrongly labelled before being sent to this laboratory (cf. strain PS 9).

Table 32 (contd.): Source and properties of strains of Ps.aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
94	Burn	E	-	Edgware	+	(+)	-	-	+	±	A2	N	Alk.C. P5	-	-	-	+	+	XIV
95	Otitis media	E	-	Edgware	+	+	-	-	L	+	A2	N	Alk.C. P6	-	-	±	+	+	VIII
117	Aural swab	I	3233/ 50	London	+	+	-	CI	+	-	A1	N	Alk.C. P7	-	-	-	+	+	VIII
118	Aural swab	I	3641/ 50	London	+	+	-	-	+	±	A2	N	Alk.C. P7	-	-	+	+	+	I
119	Gum swab	I	3688/ 50	London	+	(+)	-	-	+	±	-	+	Alk.C. P5	-	-	+	+	+	[CP(E)]
120	Aural swab	I	3851/ 50	London	+	(+)	-	-	+	+	A1	N	Alk.C. P7	-	-	+	±	+	VIII
121	Aural swab	I	3924/ 50	London	+	-	-	CI	+	(±)	A1	N	Alk.C. P7	-	-	-	+	+	VIII
122	Aural swab	I	3927/ 50	London	+	+	-	CI	+	(±)	A3	N	Alk.C. P7	-	-	-	±	+	IX
123	Aural swab	I	3974/ 50	London	+	+	-	CI	+	+	A2	N	Alk.C. P7	-	-	+	+	+	I
124	Aural swab	I	4002/ 50	London	+	(+)	-	-	L	(±)	A1	N	Alk.C. Pa7	-	-	-	+	+	[CP:F]
125	Aural swab(left)	I	4049/ 50	London	+	+	-	CI	++	(±)	-	+	Alk.C.	-	-	+	-	+	VIII
126	Aural swab(right)	I	4050/ 50	London	+	(+)	-	-	++	+	A2	+	Alk.C. P7	-	-	+	-	+	VIII

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
127	-	B	4656 n.m.	-	+	+	-	LA	L	±	-	N	Alk.C. Pa7	-	-	-	-	-	I
128	-	B	Gerard, Paris	-	+	+	+	LA	+	(±)	A2	N	Alk.C. Pa7	-	-	-	+	+	I
129	-	B	DuBois, Paris	-	+	+	-	-	++	±	-	N	Alk.C. Pa7	-	-	-	+	+	VIII
130	-	B	y Type B n.m.	-	+	+	-	+	+	±	A5	N	Alk.C. P7	-	-	±	-	-	VIII
131	-	B	Molyneux Type(A+B)	-	+	+	-	LA	+	(±)	A2	-	Alk.C. P7	-	-	-	+	+	X
132	-	B	P8 Type A(+)	-	+	+	-	-	+	(±)	-	N	Alk.C. P6	-	-	-	+	+	I
133	-	B	1602 Type A	-	+	+	-	LA	+	(±)	A4	N	Alk.C. Pa7	-	-	-	+	+	X
134	-	B	Lewis Type C	-	+	+	-	CI	+	(±)	A2	N	Alk.C. P5	-	-	-	+	+	XIII
135	-	B	Emery Type B m.	-	+	+	-	+	+	(±)	A1	N	Alk.C. P7	-	-	+	+	+	XI
136	-	B	470 Type(B+C)	-	+	(+)	-	CI	+	(±)	A1	N	Alk.C. Pa7	-	-	-	+	+	VIII
137	Leg Ulcer	Cl	B.5042	Enfield	+	+	+	-	+	+	A1	N	Alk.C. Pa7	-	-	+	+	+	VIII
138	Nasal pus	Cl	B.5091	Enfield	+	+	-	-	+	+	A2	N	Alk.C. P7	-	-	+	+	+	IV

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	Gl	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
139	Urine	Cl	C5132	Enfield	+	(+)	-	-	++	±	A2	N	Alk.C. P5	-	-	+	±	+	IV
140	Urine	Cl	C5198	Enfield	+	+	-	+	+	±	A6	N	Alk.C. P7	-	-	+	+	+	IV
141	Ear	X	(1)	Edinburgh	+	+	-	++	+	(±)	-	N	Alk.C. Pa7	-	-	-	+	+	XIV
142	Urine	X	(2)	Edinburgh	+	-	-	-	++	±	A2	N	Alk.C. P5	-	-	+	+	+	XI
143	Urine	V	11405/50	Glasgow	+	+	-	-	++	+	A2	-	Alk.C. P5	-	-	+	+	+	I
144	Urine	V	11457/50	Glasgow	+	+	-	-	++	+	-	-	Alk.C. P7	-	-	±	±	+	I
145	Chronic otitis media	P	20831/50	London	+	+	-	-	+	+	-	+	Alk.C. Pa7	-	-	-	+	+	XII
146	Urine	P	20902/50	London	+	+	±	-	++	+	-	N	Alk.C. P7	-	-	+	+	+	I
147	Urine	P	20902/50	London	+	+	-	-	+	+	A3	N	Alk.C. P7	-	-	+	±	+	I
148	Urine	V	11705/50	Glasgow	+	+	-	-	+	±	-	N	Alk.C. Pa7	-	-	-	+	+	I
149	Urine	V	11706/50	Glasgow	+	+	-	-	L	+	A2	N	Alk.C. P7	-	-	±	+	+	VI
150	Urine	V	11781/50	Glasgow	+	(+)	-	-	++	+	-	+	Alk.C. P7	-	-	+	+	+	I

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
151	Cerebrospinal fluid	C2	L	London	+	(+)	-	-	+	+	A2	+	Alk.C. P7	-	-	+	+	+	X
152	Surface of melanoma of leg	C2	W	London	+	(+)	-	-	+	+	A1	N	Alk.C. P7	-	-	+	±	+	XI
153	Urine	Q	-	London	+	(+)	±	-	++	+	-	N	Alk.C. P7	-	-	±	±	+	I
154	Varicose Ulcer	Q	-	London	+	+	-	CI	++	±	-	N	Alk.C. Pa7	-	-	-	±	+	IV
155	Urine	Q	-	London	+	(+)	-	-	++	+	A4	N	Alk.C. P7	-	-	-	±	+	I
156	Wound (nephrectomy)	Q	-	London	+	(+)	-	++	++	+	A1	N	Alk.C. P7	-	-	-	+	+	[CP:H2]
157	Varicose ulcer	Q	-	London	+	(+)	-	-	++	+	-	N	Alk.C. P7	-	-	+	±	+	[CP:H2]
158	Varicose ulcer	Q	-	London	+	(+)	-	-	+	+	-	N	Alk.C. P7	-	-	-	+	+	[CP:H2]
159a	Burn	G	-	Glasgow	+	+	±	++	++	+	A1	N	Alk.C. P7	-	-	-	+	+	I
195b	Burn	G	-	Glasgow	+	+	±	++	++	+	A1	N	Alk.C. Pa7	-	-	-	+	+	I
160	Burn	G	-	Glasgow	+	+	-	++	++	(±)	A1	N	Alk.C. Pa7	-	-	-	+	+	I
161	Burn	G	-	Glasgow	+	+	-	++	++	±	A1	N	Alk.C. P7	-	-	±	+	+	I

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
162a	Burn	G	-	Glasgow	+	+	±	++	++	+	A1	N	Alk.C. P7	-	-	-	+	+	I
162b	Burn	G	-	Glasgow	+	+	±	++	++	+	A1	N	Alk.C. Pa7	-	-	-	+	+	I
163	Burn	G	-	Glasgow	+	+	-	++	++	(±)	-	N	Alk.C. Pa7	-	-	-	+	+	I
164	Burn	G	-	Glasgow	+	+	-	++	++	±	-	N	Alk.C. P7	-	-	±	+	+	I
165	Burn	G	-	Glasgow	+	+	-	++	++	+	-	N	Alk.C. P7	-	+	-	+	+	I
166	Burn	G	-	Glasgow	+	+	±	++	++	+	A1	N	Alk.C. P7	-	-	+	+	+	I
167	Burn	G	-	Glasgow	+	+	-	++	++	±	A2	N	Alk.C. Pa7	-	-	±	+	+	I
168	Burn	G	-	Glasgow	+	+	-	++	++	+	A1	N	Alk.C. Pa7.	-	-	±	+	+	I
169	Burn	G	-	Glasgow	+	+	-	++	++	(±)	A3	N	Alk.C. P7	-	-	-	+	+	I
170	Burn	G	-	Glasgow	+	+	±	++	++	±	A6	N	Alk.C. P2	-	-	-	±	+	I
171	Burn	G	-	Glasgow	+	+	-	++	++	+	-	N	Alk.C. P4	-	-	-	+	+	I
172	Burn	G	-	Glasgow	+	+	-	LA	++	+	-	N	Alk.C. P4	-	-	-	+	+	I

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	Gl	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
173	Urine	V	11985/50	Glasgow	+	(+)	-	++	L	±	A1	N	Alk.C. Pa7	-	-	-	±	+	[CP:F]
174	Urine	H	-	Uxbridge	+	(+)	-	-	+	+	-	N	Alk.C. P4	-	-	+	-	+	I
175	Urine	H	-	Uxbridge	+	(+)	-	LA	++	+	-	N	Alk.C. P4	-	-	+	+	+	XI
176	Faeces	H	-	Uxbridge	+	+	-	-	+	±	A3	+	Alk.C. P7	-	-	-	-	+	XIII
177	Faeces	H	-	Uxbridge	+	+	-	-	+	+	-	N	Alk.C. P7	-	-	-	±	+	XIII
178	Otitis media	H	-	Uxbridge	+	(+)	-	++	++	+	-	N	Alk.C. Pa7	-	-	-	±	+	II
179	Burn	H	-	Uxbridge	+	(+)	-	+	++	+	-	N	Alk.C. P4	-	-	+	±	+	XIII
180	Urine	P	-	London	+	(+)	-	-	++	+	-	N	Alk.C. P4	-	-	+	±	+	XII
181	Urine	X	-	Edinburgh	+	+	-	+	+	±	A5	N	Alk.C. Pa7	-	-	-	±	+	IX
182	Unknown	M	I	Lab.culture No.unknown	+	(+)	-	-	++	(±)	A2	N	Alk.C. P4	-	-	-	±	+	VIII
183	Unknown	M	II	Melbourne	+	(+)	-	-	++	-	A2	N	Alk.C. P4	-	-	-	±	+	XI
184	Otitis media	M	III	Melbourne	+	+	-	LA	++	±	-	N	Alk.C. P7	-	-	-	+	+	XII

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
185	Enteritis	M	IV	Melbourne	+	+	-	-	++	+	-	N	Alk.C. P6	-	-	+	-	+	VIII
186	Surgical case	M	V	Melbourne	+	+	-	LA	++	+	-	N	Alk.C. Pa7	-	-	-	+	+	I
187	Bovine lung	J	14540	Glasgow	+	+	-	-	++	+	-	N	Alk.C. P4	-	-	+	+	+	X
188	Intestine of sea-lion	J	14526	Glasgow	+	+	-	-	++	+	-	N	Alk.C. P4	-	-	-	+	+	VIII
189	Unknown	S	-	Southend-on Sea	+	(+)	-	-	++	+	-	+	Alk.C. P4	-	-	±	+	+	IX
190	Unknown	S	-	Southend-on Sea	+	-	-	-	++	-	-	N	Alk.C. P7	-	-	-	+	+	I
191	Unknown	C3	B.pyo. path.	Basle, Switzer-land	+	-	-	-	+	+	A2	N	Alk.C. Pa7	-	-	-	-	+	XIV
192	Unknown	C3	B.pyo. apath.	Basle, Switzer-land	-	-	-	-	-	-	A2	N	Alk.C. Pa7	-	-	-	+	+	X
193	Empyema	W	7/11	London	+	+	-	+	++	(+)	-	N	Alk.C. P4	-	-	-	+	+	I
194	Faeces gastro-enteritis	W	Archer	London	+	(+)	-	-	++	+	-	N	Alk.C P7	-	-	-	±	+	IV
195	Faeces gastro-enteritis	W	Brock-man	London	+	+	-	-	++	+	A2	N	Alk.C P7	-	-	-	±	+	XIII
196	Vaginal swab	P	21459/ 50	London	+	(+)	-	-	++	+	A2	+	Alk.C P7	-	-	+	±	+	XII

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source: Specimen	S	Ref.	Isolated	Pigment: F	Pc	Pr	Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
197	Faeces	P	22405/50	London	+	+	±	-	++	+	-	N	Alk.C. P4	-	-	+	±	+	IX
198	Faeces gastro-enteritis	Y	12739a	Carshalton, Surrey	+	+	-	-	++	+	A2	N	Alk.C. P4	-	-	-	±	+	XIII
199	Faeces gastro-enteritis	Y	12739b	Carshalton, Surrey	+	+	-	LA	++	±	-	N	Alk.C. P5	-	-	-	±	+	I
200	Faeces gastro-enteritis	Y	-	Carshalton, Surrey	+	+	-	+	++	(±)	A1	N	Alk.C. Pa7	-	-	-	+	+	XIII
218	Ear (right)	Y	-	Carshalton, Surrey	+	-	-	CI	++	+	-	N	Alk.C. Pa7	-	-	-	±	+	III
219	Ear (left)	Y	436(3)	Carshalton, Surrey	+	-	-	LA	++	±	-	N	Alk.C. Pa7	-	-	-	+	+	I
220	Ear (right)	Y	437(3)	Carshalton, Surrey	+	-	-	-	++	±	-	N	Alk.C. Pa7	-	-	-	+	+	I
221	Osteomyelitis	A	(1)	Edinburgh	+	(+)	-	-	++	±	A2	N	Alk.C. Pa7	-	-	-	±	+	I
222	Urine	A	(2)	Edinburgh	+	(+)	-	CI	++	±	-	N	Alk.C. P7	-	-	-	±	+	XI
223	Testicle abscess	A	(3)	Edinburgh	+	(+)	-	++	++	±	A2	N	Alk.C. P7	-	-	-	+	+	[CP:F]
224	Chronic leg lesion	A	-	Edinburgh	+	+	-	CI	++	±	A1	N	Alk.C. P4	+	-	-	+	+	I
225	Faeces	A	-	Edinburgh	+	+	-	CI	+	±	A2	N	Alk.C. P4	-	-	-	+	+	VIII

Table 32 (contd.): Source and properties of strains of Ps. aeruginosa.

PS. No.	Details of Source				Pigment			Ir	P	GL	G	NR	BCP Milk	H_2S	I	H	MBR	M	Phage group
	Specimen	S	Ref.	Isolated	F	Pc	Pr												
226	Pulmonary abscess	U	Sierens	Louvain	+	+	-	++	+	+	A2	N	Alk.C. P4	-	-	-	+	+	VIII
227	Pyelocystitis	U	De Ridder	Louvain	+	+	-	-	++	+	A2	-	Alk.C. P4	-	-	±	+	+	I
228	Urine	U	Van der Capellen	Louvain	+	+	-	-	++	+	A3	N	Alk.C. P4	-	-	±	±	+	X
229	Faeces	U	Dello	Louvain	+	(+)	-	+	++	+	A1	N	Alk.C. P4	-	-	-	+	+	VIII
230	Empyema	U	Dupont	Louvain	+	+	-	-	+	+	A1	N	Alk.C. P2	+	+	-	+	+	I
231	Pyelocystitis	U	Van Glabbeck	Louvain	+	+	-	LA	+	±	A1	N	Alk.C. P4	-	-	-	+	+	I
232	Chronic Cystitis	K	1801/51	Canterbury	+	+	-	LA	L	±	A2	N	Alk.C. P4	-	-	-	+	+	VIII
233	Skin graft wound	K	16871/50 Whigham I	Canterbury	+	(+)	-	-	+	+	A2	N	Alk.C. P2	-	-	+	+	+	I
234	Cystostomy wound	K	3801/51 Northbourne	Canterbury	+	(+)	-	-	++	+	-	N	Alk.C. P2	-	-	+	±	+	XI
235	Urine	K	17647/50 Mount	Canterbury	+	(+)	-	-	++	+	A2	N	Alk.C. P4	-	-	+	+	+	I
236	Operation Wound	K	19174/50 Whigham I	Canterbury	+	(+)	-	-	++	+	A2	-	Alk.C. P2	-	-	+	+	+	XI
237	Ulcer of foot	K	17595/50 O.P.	Canterbury	+	(+)	-	-	++	+	A2	N	Alk.C. P4	-	-	+	+	+	XII

APPENDIX II

COMPLETE RANGE OF SENSITIVITY OF STRAINS OF PS. AERUGINOSA TO THE TYPING PREPARATIONS.

Table 33: Complete range of sensitivity of strains to typing preparations.

C: Confluent lysis free from resistant growth. P: Isolated plaques.
R: Confluent lysis with resistant growth. -: No reaction.
___ denotes those reactions used to express phage-type.

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
1	C---C--	- -	- - - -	P - - -	C R	C C C C C C	- - - - -	- - - -	----
2	C---C--	- -	- - - -	P - - -	C R	C C C C C C	- - - - -	- - - -	----
3	C---C--	- -	- - - -	P - - -	C R	C C C C C C	- - - - -	- - - -	----
4	RPCCC--	C C	C C C C	C C C -	- -	- - - - - -	- - - - -	- - - -	----
5	RCCCCC-	C C	C C C C	C C C C	R R	R - C R - -	R - - - -	- - - -	R-R-
6	RCCCC-R	C C	C C C C	C C C C	C R	- R - - - -	- P P - -	- - R -	--RR
7	--PRP--	- P	R R R R	- C - -	- -	R R - R - -	- - - - -	- - - -	----
8	RR-RCP-	- -	- - - -	P P C -	R R	- - - - - -	C C C C R	R - - R	----
9	--PR---	P P	P R P -	P P - -	- -	- - - - - -	- - - - -	- - - -	--C-
10	RPCCC--	C C	C C C C	C C C -	R -	- - - - - -	- - - - -	- - - -	----
11	------C	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
12	R--C---	- P	R R R P	- C - -	R R	- - - - - -	- - - - -	- - - -	----
13	RCPCC--	- P	R R P P	P P C R	- -	- - - - - -	- - - - -	--- - -	----
14	C-RC---	R R	R C C P	R R - -	R R	- - - - - -	- - - - -	- - - -	-C--
15	RCCCC--	C C	C C C C	C C C C	- -	- P - - - -	- P P - -	- R R R	R---
16	RPRCCP-	R R	C C C C	P C C -	R -	- R - - - -	P - R R P	P - - R	P-R-
17	RCCCPP-	C C	C C C C	C C C -	R -	- P - - P -	C C C C C	- - - R	--R-
18	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
19	-------	- R	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

☐ reaction with concentrated phage only.

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
20	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
21	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
22	----P--	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
23	CP-R---	- -	R R R P	- P R R	R R	- - - - - -	- - - - -	- - - -	--R-
24	R------	- -	- - - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----
25	R--R---	P -	P P P P	- - - R	- -	- - - - - -	- - - - -	- - - -	----
26	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
27	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
28	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
29	---CP--	P P	P R P P	- P - -	P -	- - - - - -	- - - - -	- - - -	----
30	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
31	RP-----	- -	- - - -	- - R -	- -	- - - - - -	P - - - -	- - - -	----
32	RP-----	- -	- - - -	- - R -	- -	- - - - - -	P - - - -	- - - -	----
33	R-RC--C	P R	R R R R	R R - R	- -	- R R - - -	- - - - -	- - - R	----
34	RPRCC--	R C	C C C C	P C P C	- -	- R R - - -	- - - - -	- - - -	----
35	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
36	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
37	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
38	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
39	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
40	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
41	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
42	RPRCC--	R C	C C C C	P C P C	- -	- R R - - -	- - - - -	- - - -	----
43	RPRCC--	R C	C C C C	P C P C	- -	- R R - - -	- - - - -	- - - -	----
44	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
45	R--R---	- -	- R - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
46	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
47	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
48	R--RC--	- -	- R - -	P - - -	- R	- R - - - -	- - - - -	- - - -	----
49	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
50	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
51	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
52	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
53	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
54	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
55	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
56	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
57	----P--	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
58	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
59	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
60	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
61	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
62	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
63	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
64	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
65	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
66a	R-P----	P P	- - - -	P - - -	- -	- R - - - -	- - - - -	- - - -	----
66b	R-P----	P P	- - - -	P - - -	- -	- R - - - -	- - - - -	- - - -	----
67a	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
67b	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
68	RRRCR--	R C	R C R R	R C C -	- -	- - - - - -	P - R R P	R R - R	----
69	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
70	-------	- C	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
71	-------	C C	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
73	----C--	- -	- - - -	P - - -	- -	- - - - - -	- - - - -	- - P P	--R-
74	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
75	----P--	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
76	R------	- -	- R - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----
77	R--RP--	- -	- - - -	P - - -	- -	- - - - - -	- - - - -	- - - -	----
78a	---RR--	- -	- - - -	P - - -	- -	P P - P P P	P - - - -	- - - -	R---
78b	---RR--	- -	- - - -	P - - -	- -	P P - P P P	P - - - -	- - - -	R---
79	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
80	---R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

☐ reaction with concentrated phage only.

Table 33: Complete range of sensitivity of strains to typing preparations (contd.)

PS No.	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
	Type-designation of phage-typing preparations.								
81	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
82	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
83	R-CC---	R C	C C C C	C C - P	- -	- - - - - -	- - - - -	- - - -	----
84	R--C---	- R	- - C R	- C - -	- -	- - - R - -	- - - - -	- - - -	----
85	R--C---	- R	- - C R	- C - -	- -	- - - R - -	- - - - -	- - - -	----
86	--PR---	P P	P R P -	P P - -	- -	- - - - - -	- - - - -	- - - -	--C-
87	R------	- -	- R - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
88	RRRCR--	R C	R C R R	R C C -	- -	- - - - - -	P - R R P	R R - R	----
89	RRRCR--	R C	R C R R	R C C -	- -	- - - - - -	P - R R P	R R - R	----
90	-----R-	- -	- - - -	- - R -	- -	R - R - - -	- - - - -	- - - -	----
91	R-RR---	R R	R R R R	P R R -	- -	- - - - - -	- - - - -	- - - -	P---
92	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
93	RP-CC--	P P	- - - -	P - - -	- -	- - - - - -	- - - - -	- - - -	----
94	----C--	- -	- - - -	P - - -	- -	- - - - - -	- - - - -	- - - -	----
95	RCCC---	C C	C C C C	C C C P	- -	R R R R - -	- - P - -	- - - -	----
117	RCCCR--	C C	C C C C	C C C C	- R	- - - - - -	R - - - -	- P - R	P-R-
118	R------	- -	- R - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
119	----P--	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
120	RCCC---	C C	C C C C	C C C C	- -	R C C R - -	- P P - -	R R R R	----
121	RCCCR--	C C	C C C C	C C C C	- R	- - - - - -	R - - - -	- P - R	P-R-
122	--RCR-R	P R	C C C P	R R - -	- -	R - R R - -	R - R R -	P - - R	C-R-

[ ] reaction with concentrated phage only.

Table 3.3: Complete range of sensitivity of strains to typing preparations (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
123	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
124	-----R-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
125	RCCC---	C C	C C C C	C C C C	- -	R C C R - -	- P P - -	R R R R	----
126	RCCC---	C C	C C C C	C C C C	- -	R C C R - -	- P P - -	R R R R	----
127	R------	- -	- - - -	- - - -	- -	- R - - - -	- - - - -	- - - -	----
128	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
129	RCPCC--	P P	C C R P	P P C R	- -	C R - C - -	P P P P P	- P - P	--R-
130	-CCCC--	C C	C C C C	C C C C	- -	- - - - - -	- - - - -	- - - -	----
131	---R---	- -	R R R -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
132	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	--R-
133	R-PR--R	R P	R P R -	R R - -	- -	- - - - - -	P - - R R	R P - C	----
134	RC-CC--	- -	- - - -	P - C -	- -	P R - - - -	- - - - -	- - - -	----
135	RP-C---	- -	- - - -	- - R -	- -	P C R P R -	P - - - -	- - - -	P---
136	RCPCCP-	R R	R C R R	P R C -	- -	R C C R - -	P P P P P	- - - P	----
137	RRCC-P-	P R	C C C C	C C R -	- -	- - - - - -	- - - - -	- - - -	----
138	---R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
139	---RP--	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
140	---R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
141	----C--	- -	- - - -	P - - -	- -	- - - - - -	- - - - -	- - P R	--R-
142	CP-R---	- -	- - - -	- - R -	P P	- - - - - -	- - - - -	- - - -	----
143	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

☐ reaction with concentrated phage only.

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
	Type-designation of phage-typing preparations.								
144	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
145	RP-C---	C C	- - C C	- C - -	- -	- - - - - -	- - - - -	- - - -	----
146	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
147	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
148	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
149	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
150	R------	- -	- - - -	- - - -	P -	- - - - - -	- - - - -	- - - -	----
151	C--R---	R R	R R R R	- R C R	R R	R - - R - -	- - - - -	- - - -	--R-
152	CP-C-P-	- -	- - P P	- - R -	- -	- - - - - -	R - - R R	- - - -	----
153	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
154	-P-R-P-	- -	- - - -	- - R -	- -	- - - - - -	R P R R -	R P - R	----
155	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
156	-------	- R	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
157	-------	- R	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
158	-------	- R	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
159a	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
159b	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
160	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
161	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
162a	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
162b	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

☐ reaction with concentrated phage only.

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
163	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
164	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
165	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
166	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
167	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
168	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
169	R------	- -	- - - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----
170	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
171	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
172	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
173	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
174	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
175	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
176	C--RP--	- -	- - - -	P - - -	- R	- P - P - P	P - - - -	- - - -	R-R-
177	R--RP--	- -	- - - -	P - - -	- R	- - - - - -	- - - - -	- - - -	--R-
178	-R-----	- -	- - - -	- - - -	- -	- R R - - -	- - - - -	- - - -	----
179	R--RC--	- -	- R - -	P - - -	- -	- - - - - -	- - - - -	- - - -	----
180	RP-C---	C C	- - C C	- C - -	- -	- - - - - -	- - - - -	- - - -	----
181	R-PC---	P C	C C R R	P P - -	- -	- - - - - -	- - - - -	- - - -	----
182	RCPC-P-	R C	C C C C	P C C R	- -	R P P R P P	- - - - -	- P C C	--R-
183	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----

□ reaction with concentrated phage only.

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
184	RP-C---	C C	- - C C	- C C -	- -	- - - - - -	P - - - P	- - R R	----
185	RRPC-P-	R C	C C C C	P C C R	- -	- R - - P P	- - - - -	- P R R	--RP
186	R-PCP--	- P	R R R P	- P - -	- -	- - - - - -	- - - - -	- - - -	----
187	RP-CC-P	P P	R C P P	P P C R	- -	- - - - - -	- - - - -	- - - -	----
188	RRRC---	R R	C C P R	R R R R	- -	- - - - - -	- - - - -	- - - -	----
189	R-PRRP-	P P	R R P P	P P R -	- -	P - - R - -	- - - - -	- - - R	--R-
190	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
191	RP--C--	- -	- - - -	P - R R	- -	- - - - - -	- - - - -	- - - -	----
192	R-PP--R	P P	P P P -	P P - -	R R	- - - - - -	- - - - -	- - - -	R-R-
193	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
194	--PR---	- P	- P P -	- P - -	- -	- - - - - -	- - - - -	- - - -	----
195	R--RR--	- -	- - - -	P - - R	- -	- - - - - -	- - - - -	- - - -	----
196	RP-C---	C C	- - C C	- C - -	- -	- - - - - -	- - - - -	- - - -	----
197	R-CC---	C C	C C C C	C C C -	- -	- P - - - -	- - - - -	- - - -	----
198	R--RP--	- -	- - - -	P - - -	- R	P P P - - -	P - - - -	- - - -	R---
199	C------	- -	- - - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----
200	R--RP--	- -	- - - -	P - - -	- C	- - - - - -	- - - - -	- - - -	----
218	-PC-P--	- -	- - - -	- - P -	- -	- - - - - -	- - - - -	- - - -	----
219	C------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
220	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
221	R------	- -	- - - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----

Table 33: Complete range of sensitivity of strains to typing preparations. (contd.)

PS No.	Type-designation of phage-typing preparations.								
	ABCDEFG	H1H2	K1K2K3K4	L1L2L3L4	M1M2	N1N2N3N4N5N6	P1P2P3P4P5	R1R2R3R4	STVW
222	RP-C---	- -	P R - -	- - P R	- -	- - - - - -	- - - - -	- - - -	----
223	-----C-	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
224	RP-----	- -	- - - -	- - - -	- -	- - - P R -	- - - - -	P - - P	----
225	RRPC--C	P P	P C C P	P C C -	R R	C C C C - -	- - - - -	- - - R	----
226	RCPCC--	C C	C C C C	P C C C	R R	- - - - - -	P P P R P	- - - P	----
227	R------	- -	- - - -	- - - -	P P	- - - - - -	- - - - -	- - - -	----
228	--PP---	P P	P P R -	P P - P	- -	- - - - - -	- - - - -	- - - -	----
229	CCCCCP-	C C	C C C C	C C C C	R R	- - - - - -	P P C C P	P P - P	----
230	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
231	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
232	RCCCC--	C C	C C C C	C C C C	P P	- - - - - -	- - - - -	- - - -	--R-
233	R------	- -	- - - -	- - - -	R R	- - - - - -	- - - - -	- - - -	----
234	R--R---	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
235	R------	- -	- - - -	- - - -	- -	- - - - - -	- - - - -	- - - -	----
236	R--R---	- -	- - - -	- - - -	- -	P P P - R -	- - - - -	- - - -	----
237	RR-C---	R C	- - C C	- R R -	R R	- - - - - -	- - - - -	P P P P	----

□ reaction with concentrated phage only.

APPENDIX III

SOURCE AND PROPERTIES OF THE STRAINS OF FLUORESCENT SPECIES

Table 34: Source and properties of strains of fluorescent species.

Properties common to the group

(1) Gram -ve; (2) Motile.
(3) Optimum temperature: 25°C.; do not grow at 42°C.
(4) Produce fluorescin; cannot be adapted to produce pyocyanin.
(5) Do not show iridescent areas.
(6) Non-pathogenic for mice.
(7) Catalase positive.
(8) Indole not produced.

Variable Properties

PS No.	Source	Temp.			GL	G.	BCP Milk	H	MBR	NR	H2S
		25	37	42							
72	Moorland stream	T	±	0	±	A1	Alk.	+	±	+	-
96	N.C.T.C.3756	T	±	0	±	A2	Alk.	-	±	-	-
97	Sewage	T	±	0	-	A1	Alk.	±	-	+	-
98	Sewage	T	±	0	-	A1	Alk.	-	+	+	-
99	Sewage	T	±	0	-	A1	Alk.	-	-	+	-
100	Sewage	T	±	0	-	A1	Alk.	-	-	+	-
101	Sewage	T	±	0	-	A1	Alk.	-	±	+	-
102	Sewage	T	±	0	±	A1	Alk.	-	±	+	-
103	Sewage	T	0	0	-	A1	Alk.	-	±	+	-
104	Sewage	T	±	0	-	A1	Alk.	-	±	+	-
105	River water	T	0	0	±	A1	Alk.	-	±	+	-
106	River water	T	0	0	±	A1	Alk.	-	±	+	-
107	River water	T	+	0	±	A1	Alk.	±	±	+	-
108	River water	T	0	0	±	A1	Alk.	-	±	+	-

Table 34: Source and properties of strains of fluorescent species (contd.).

PS No.	Source	Temp. 25	37	42	GL	G.	BCP Milk	H	MBR	NR	H2S
109	River water	T	O	O	±	Al	Alk.	-	±	+	-
110	River water	T	O	O	±	A3	Alk.	-	+	+	-
111	Canal water	T	O	O	-	Al	Alk.	-	±	+	-
112	Canal water	T	±	O	-	Al	Alk.	-	+	+	-
113	Canal water	T	±	O	-	Al	Alk.	±	±	+	-
114	Canal water	T	+	O	-	Al	Alk.C6	±	±	+	-
115	Canal water	T	+	O	-	Al	Alk.C6	±	±	+	-
116	Synthetic cream	T	±	O	+	Al	Alk.C7	±	-	+	-
201	Plasma	T	±	O	±	-	Alk.	-	+	+	-
202	Plasma	T	±	O	+	Al	Alk.C.Pa7	-	+	+	-
203	Plasma	T	±	O	+	Al	Alk.C.Pa7	±	+	+	-
204	Plasma	T	±	O	±	-	Alk.C.Pa7	-	+	+	-
205	Plasma	T	O	O	±	A3	Alk.C.Pa7	-	+	N	-
206	Plasma	T	±	O	±	A2	Alk.C. P7	-	+	+	-
207	Plasma	T	O	O	±	Al	Alk.C.Pa7	-	+	N	-
208	Plasma	T	O	O	-	A3	Alk.C.Pa7	-	±	N	-
209	Plasma	T	±	O	±	-	Alk.C.Pa7	-	+	+	-
210	Plasma	T	O	O	±	A3	Alk.	-	+	+	-
211	Plasma	T	±	O	-	A2	Alk.	-	+	+	+
212	Plasma	T	O	O	-	A2	Alk.	-	±	N	-
213	Plasma	T	±	O	±	-	Alk.C.P7	-	-	-	+

Table 34: Source and properties of strains of fluorescent species (contd.).

PS No.	Source	Temp. 25	37	42	GL	G.	BCP Milk	H	MBR	NR	H2S
214	Plasma	T	+	0	-	Al	Alk.	-	+	+	-
215	Plasma	T	0	0	±	A3	Alk.C.Pa7	-	+	+	-
216	Plasma	T	±	0	(±)	A2	Alk.C.Pa7	-	-	-	-
217	Plasma	T	±	0	±	-	Alk.C.P7	-	+	+	-

Temperature Relations:

T.....optimum growth.
+.....moderate growth.
±.....scanty growth.
0.....no growth.

GL. - Gelatin Liquefaction:

+...complete within 24 hours.
±...commencing between 1 and 7 days.
(±)..commencing between 7 and 28 days.
-...negative up to 28 days.

G - Glucose:
A...acid produced.

H - Haemolysis:
+...strong.
±...weak.

BCP Milk:
Alk.reaction alkaline.
C...coagulum formed.
Pa..partial peptonisation.
P...complete peptonisation.

MBR - Methylene-blue reduction:
+.....complete reduction.
±.....partial reduction.
-.....no reduction.

NR - Nitrate Reduction:
+...nitrite only detected.
N...nitrogen produced.

Numbers in table refer to days of incubation at 25°C.

APPENDIX IV

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