

Epidemiological aspects of *Proteus* infections with particular reference to phage typing

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SYNOPSIS The properties and characteristics of 296 *Proteus* strains isolated from hospital patients and from sewage have been examined with a view to determining those characters which are of most use in following and detecting cross-infection.

Ninety-six per cent of the organisms isolated were *P. mirabilis* and it was found that by identifying biotypes and by phage typing the species could be divided into a relatively large number of groups which would be useful for epidemiological investigation.

Organisms of the genus *Proteus* may be found in the intestines of normal individuals and are widely disseminated through human communities. They may function as primary as well as secondary pathogens and their incidence in infected burns and wounds has increased in recent years. In urinary tract infections they often replace the primary pathogen following antibiotic therapy.

The aim of this study has been to assess the incidence of *Proteus* infections in a metropolitan hospital and to determine characteristics of strains which might be useful in tracing the source of hospital cross-infection. It has yet to be proved whether hospital cross-infection or auto-infection from the gut is the most important method of infection. In the past, serological typing and the use of the Dienes phenomenon (Dienes, 1946) have been used for epidemiological purposes, but neither method has been very satisfactory. An additional and more reliable method is required and in this study attention has been paid to the possible value of phage typing *Proteus* strains.

MATERIALS AND METHODS

SOURCES OF STRAINS During nine months of 1964, 296 *Proteus* strains were obtained from clinical specimens from hospital patients or from sewage. Of the hospital strains 248 were from in-patients and 23 from out-patients of a 500-bed hospital. The majority of organisms were isolated from urine and faeces and smaller numbers from pus, nasal, or wound swabs and blood cultures. The 48 strains isolated from sewage were the only *Proteus* strains obtained from an examination of 96 samples.

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In a case where more than one strain was obtained from the same patient, only strains which differed from the original in biochemical and cultural characteristics were included for subsequent examination.

IDENTIFICATION OF SPECIES Hydrolysis of urea on Christensen's medium and the conversion of phenylalanine to phenylpyruvic acid (Hill, Fung, and Marcus, 1962), were used as criteria to place non-lactose fermenting Gram-negative rods in the genus *Proteus*. Species identification was made on the basis of indole and H₂S production, liquefaction of gelatine, and the fermentation of glucose, lactose, maltose, mannitol, and sucrose (Table I).

TABLE I
IDENTIFICATION OF *PROTEUS* SPECIES

Substrate or Test	<i>Pr. vulgaris</i>	<i>Pr. mirabilis</i>	<i>Pr. morganii</i>	<i>Pr. rettgeri</i>
Phenylalanine deaminase	+	+	+	+
Urea hydrolysed	+	+	+	+
Lactose fermentation	—	—	—	—
Sucrose fermentation	+	+ or —	—	—
Mannitol fermentation	—	—	—	+
Maltose fermentation	+	—	—	—
Glucose fermentation	+	+	+	+
Indole	+	— or +	+	+
Gelatin	+	+	—	—
H ₂ S	+	+	+	—

BIOTYPES Differentiation of strains of *Pr. mirabilis* into biotypes was based on differences in the fermentation of sucrose and the production of indole.

ANTIBIOTIC SENSITIVITY A standard inoculum of the test strain was spread in a zone at the periphery of a plate of Muller-Hinton medium. Antibiotic discs were placed at the inner edge of the zone and a known sensitive staphylococcal strain was streaked from the centre of the plate to each disc to control its potency. The sensitivity of strains to penicillin, streptomycin, tetracycline, chloramphenicol, sulphafurazole, erythromycin, and nalidixic acid, was determined in this way.

DIENES PHENOMENON Identity of flagellar antigens was tested by the Dienes phenomenon (Dienes, 1946; Story, 1954). Organisms in phase A (Belyavin, 1951) were tested on nutrient agar plates incubated at 37°C overnight.

ISOLATION OF PHAGE These were isolated from lysogenic strains and from sewage. The method of Popovici and Ghioni (1962) was the most satisfactory. Phage was obtained from the supernatant of centrifuged broth cultures which had previously been incubated at 37°C. for 24 hours and left at room temperature for a further 48 hours. Twelve phages were obtained by this method. A further eight phages were isolated from sewage supernatants, but these were the only phages obtained from 58 samples.

PROPAGATION OF PHAGE Strains used for phage propagation were selected by their sensitivity to the action of specific phage.

The soft agar layer (Adams, 1950) and the surface lawn (Blair and Carr, 1953) methods were used to propagate phage. Both gave high titre suspensions and were superior to the cellophane method (Liu, 1958).

Purification was achieved by freezing the lysate at -18°C. for 18 hours and thawing, a process which was repeated a second time before final centrifugation. The lysate was stored at -18°C. while dilutions were kept at 4°C.

A set of 20 phages showing stability of lytic spectrum was chosen from a greater number and used for typing.

PHAGE TYPING Phage activity was tested by spotting phage suspension on a surface lawn on a nutrient agar plate. The lawn was prepared by flooding the agar with a three-hour broth culture grown at 37°C. or with a 1 in 10 dilution of the suspension of growth washed from the surface of a nutrient agar slope with 10 ml. of broth. The seeded plates were dried at 37°C. for 30 to 45 minutes. The phage preparations were then applied and the plates incubated for four to six hours at 37°C. followed by overnight storage at 4°C.

Phage suspensions were standardized, using the above method, to a routine test dilution which was that dilution

of phage suspension which just failed to give confluent lysis on the propagating strain. The routine test dilution for the 20 phages in the set lay between 10⁻³ and 10⁻⁷. The lytic spectrum of each phage on all propagating strains was also determined.

In typing the production of 50 or more plaques by a phage suspension at routine test dilution was regarded as positive. Strains which did not react at routine test dilution were re-tested at 1,000 × routine test dilution.

RESULTS

PROTEUS SPECIES The species identified and their source are shown in Table II from which it can be seen that 96% were *Proteus mirabilis*, 4% *Proteus vulgaris*, while a single strain of *Proteus morganii* was isolated. More strains were obtained from urine samples than from any other material.

TABLE II

SOURCE AND NUMBER OF <i>PROTEUS</i> SPECIES ISOLATED				
Source	Total Isolated	<i>Pr. mirabilis</i>	<i>Pr. vulgaris</i>	<i>Pr. morganii</i>
Urine	134	129	4	1
Wound swabs, etc.	23	23	0	0
Nose	14	14	0	0
Faecal	66	59	7	0
Skin and feet	11	11	0	0
Sewage	48	47	1	0
Total	296	283 (96%)	12 (4%)	1

BIOTYPES Division of strains biochemically enabled four biotypes to be recognized. The majority of strains (56.5%) were placed within biotype 1 (Table III).

TABLE III

DIVISION OF STRAINS OF <i>PR. MIRABILIS</i> INTO BIOTYPES				
Test	Biotype 1	Biotype 2	Biotype 3	Biotype 4
Indole	Negative	Negative	Positive	Positive
Sucrose	(AG) ¹	Negative	(AG)	Negative
Total number	160	113	4	6

¹(AG) = delayed acid and gas production

ANTIBIOTIC SENSITIVITIES The results are presented in Table IV. All strains were susceptible to nalidixic acid and most strains were susceptible to streptomycin, chloramphenicol, and sulphafurazole.

TABLE IV

PERCENTAGE SUSCEPTIBILITY OF STRAINS OF *PR. MIRABILIS* TO ANTIBIOTICS

Source	No. of Strains	Penicillin	Streptomycin	Tetracycline	Chloramphenicol	Sulphafurazole	Erythromycin	Nalidixic Acid
Inpatient strains	213	44	94	12	95	79	5	100
Outpatient strains	23	83	100	0	83	98	0	100
Sewage	47	43	100	9	98	58	0	100

DIENES PHENOMENON When a *Proteus* strain was isolated from the rectal swab of a patient who had this organism elsewhere on the body, the paired strains were compared. Of the 63 pairs tested by the Dienes phenomenon, 91% showed no line of demarcation during swarming of pairs on a common plate and it can be assumed that these pairs shared a major flagellar antigen.

Using the same criterion it was found that multiple strains from a single patient, that is, those strains isolated from various sources at different times, and strains isolated from other patients in the same ward, often had similar flagellar antigens but these organisms sometimes had different phage types.

PHAGE TYPING Typing was carried out on 229 strains comprising 170 from hospital patients and 59 strains from other sources. It was found that 174 (76%) strains were lysed by one or more phages of the set at routine test dilution, 13 (6%) typed only at 1,000 × routine test dilution, and 36 (18%) were untypable. Many of the phages lysed *P. vulgaris* as well as *Pr. mirabilis*.

The usefulness was assessed of the various phages in the typing set for epidemiological work. For this purpose the method of analysis employed by Davidson (1961) was used, and results which applied to the 170 hospital strains are shown in Figure 1. This shows the number of cultures lysed by each phage and the proportion of those cultures which were also lysed by each of the other phages. Thus phage 4 lysed 108 of the 170 cultures and 96 (90%) of these 108 cultures were also lysed by phage 7, but only 29 (27%) were lysed by phage 6. The heights of the columns in Fig. 1 are proportional to these percentages.

The lytic patterns fell into eight main groups with a further two patterns which were produced by a mixture of groups I and II and groups I and III phages (Table V).

Thirty strains were tested for stability of phage

TABLE V
PHAGE PATTERN OF TYPING GROUPS

Typing Group	Phage Pattern
I	4/7/11/14/19/20/23/24/25
II	2/6/9/21
III	18/23/24
IV	1/15/16
V	19
VI	19/20/23/25
VII	2/6/15/22
VIII	1/7/11/14/20
I—II	2/4/6/7/9/11/14/19/20/21/23/24/25
I—III	4/7/11/14/18/19/20/23/24/25

type and checked after a period of six weeks at which time no significant alteration in the phage pattern was found.

The distribution of phage types in 170 hospital strains in relation to source and species and biotypes is given in Table VI. It can be seen that although some of the phage groups contained a moderately high proportion of strains, further subdivision of these could be made when the biotype had been determined.

Typing was also carried out on 55 strains isolated from repeat specimens from the same number of patients. Only three of these strains gave a phage typing pattern different from the original strain isolated.

During the course of study it became apparent that in two hospital wards cross-infection with *Proteus* strains had probably taken place. In one ward identical strains of *Pr. mirabilis* (phage group 3, biotype 1) were isolated from four patients, three of whom had been catheterized. This particular phage type had not been encountered in this ward before the initial infection. The Dienes phenomenon could not be used in this circumstance because of the morphological state of the cultures. In a geriatric ward, 15 strains of *Pr. mirabilis* (phage group I-II, biotype 1 and each antigenically related as shown by Dienes phenomenon) were isolated from the urines of as many patients. *Proteus* strains isolated

TABLE VI
DISTRIBUTION OF PHAGE TYPES IN HOSPITAL STRAINS

Group	Source				Pr. mirabilis Biotypes				Pr. vulgaris	Total No.
	Urine	Swabs	Nose	Faecal	1	2	3	4		
I	31	6	1	13	33	13	1	1	3	51(30%)
II	8	2	0	7	10	7	0	0	0	17(10%)
III	7	0	0	2	7	1	1	0	0	9(5%)
IV	1	1	0	0	0	0	1	0	1	2(1%)
V	4	0	0	4	5	3	0	0	0	8(5%)
VI	4	0	0	1	4	0	0	0	1	5(3%)
VII	2	0	0	1	1	2	0	0	0	3(2%)
VIII	2	0	0	0	1	1	0	0	0	2(1%)
I—II	17	3	2	12	16	18	0	0	0	34(20%)
I—III	1	2	0	2	2	2	1	0	0	5(3%)
N.T.	21	3	1	9	18	13	0	0	3	34(20%)

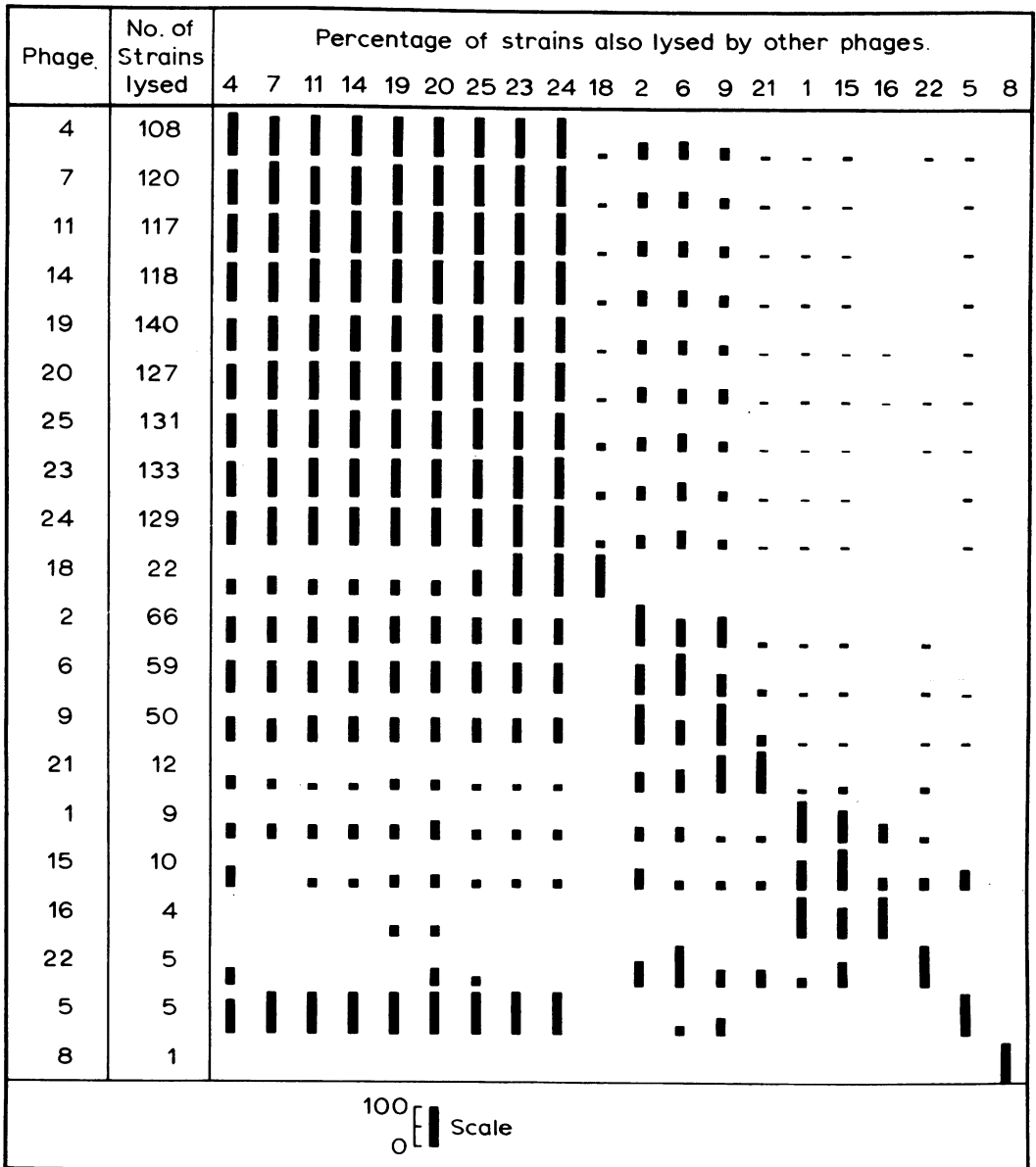


FIG. 1. Analysis of the phage patterns of 170 strains of *Proteus* from hospital sources to determine the frequency with which the phages are associated with one another.

from rectal swabs of six of these patients all differed in phage type and biotype from the urine strains although in one instance there was antigenic similarity.

DISCUSSION

The predominance of the species *Pr. mirabilis* (96%

of 296 strains) amongst *Proteus* strains isolated from various sources is similar to the findings of others. Ten articles published between 1928 and 1964 all indicated the high incidences of this species which usually constituted 70% or more of the collections of strains examined. As in the present study *Pr. vulgaris* had the next highest incidence

while *Pr. morganii* and *Pr. rettgeri* were either rare or absent. The failure to isolate the latter two species from the samples of sewage examined here is additional evidence of the rarity of these organisms in the human population.

On the basis of indole production and sucrose fermentation it was possible to divide strains of *Pr. mirabilis* into four biotypes. Of these, biotype 1 could be regarded as typical of the species and 56% were of this type. Biotype 2 was next in frequency (40% of strains) and was the type most frequently found in a study made by Milner (1963). Biotypes 3 and 4 corresponded to the 'atypical 2' strains described by Kippax (1957) and together made up the remaining 4% of strains.

The majority of strains were sensitive to streptomycin, chloramphenicol, sulphafurazole, and nalidixic acid and resistant to penicillin, tetracycline, and erythromycin.

These observations agree with those made by Story (1954) but differ from the results of Eisenberg, Weiss, and Flippin (1958). The sensitivity of all strains to nalidixic acid is in accordance with the results of Ward-McQuaid, Jichlinski, and Macis (1963) and Lishman and Swinney (1963).

The use and acceptance of the Dienes phenomenon for the typing of strains has been varied. The test makes use of similarities in 'H' antigens, and Storey (1954) concluded that if strains did not intermingle it was strongly suggestive that they were different but that the reverse was not necessarily true. The Dienes phenomenon has been used in epidemiological studies but in general the identification of strains by this method has been unreliable. The results in this study were equivocal and the tests often difficult to read.

Because of the prevalence of *Proteus* infections in hospitals it is desirable to have a satisfactory method to identify strains which may be responsible for cross-infection. In most situations strains of *Pr. mirabilis* predominate and species identification will be of limited epidemiological value. The determination of biotypes of *Pr. mirabilis* is of limited value also since the majority of strains will be in one or other of two of the four types. The antibiotic sensitivity patterns of strains will depend on the extent to which the use of the various drugs has either selected resistant mutants or promoted the transfer of the resistance factors from other members of the Enterobacteriaceae. Antibiotic resistance patterns cannot therefore be used to identify a specific strain. The Dienes phenomenon at best identifies only major flagellar antigens and has no reference to somatic antigens. Perch (1948) was able to divide 536 strains into 98 serotypes by the identification of somatic and flagellar antigens. Sero-

logical identification would be very satisfactory but is not practical for use in a routine laboratory because of the wide range of antisera required.

It seemed that phage typing might help in solving the problem of strain identification. We have found that 81% of the 229 strains (including 221 strains of *Pr. mirabilis* and eight strains of *Pr. vulgaris*) were lysed by one or more of the 20 phages used. The phage patterns observed were stable in all of 30 strains tested. These results were comparable with those reported by Pavlatou, Hassikou-Kaklamani, and Zantioti (1965), who used 15 phages to divide 213 strains into 14 lysotypes. A retrospective analysis of our results showed that six phages were closely related and could be removed from the set without disadvantage thus leaving 14 phages (*viz.*, 1, 2, 5, 7, 8, 9, 15, 16, 18, 19, 20, 21, 22, 23) to divide strains into eight typing groups. During the time when the lytic property of these phages on various strains was being investigated it was observed that cross-infection had occurred in two hospital wards due to *Pr. mirabilis*.

All strains from both wards were of the same biotype but the phage group was distinctive for strains from each ward. Antigenic similarity of strains from one ward could not be determined but strains isolated from the other were shown to have related antigens as determined by the Dienes phenomenon. Thus *Proteus* strains involved in two epidemic episodes were broadly grouped by biotype and specifically identified by phage grouping, while some additional evidence of similarity was provided by the Dienes phenomenon.

It is a reasonable assumption that infections in two wards were due to cross-infections caused by specific *Proteus* strains. If auto-infection from the patient's gut had been the predominant cause it would be expected that a variety of strains would have been involved. Sporadic cases of infection have been encountered where the faecal strain has been identical with the lesion strain but since faecal swabs were not taken before the lesion was manifest the significance of these observations is equivocal. In order to determine the incidence of auto-infection it would be necessary to identify faecal *Proteus* strains at the time of the patient's admission to hospital and before any lesion had been established.

It is concluded that phage typing of strains of *Proteus* can be useful for epidemiological purposes when it is supplemented by species and biotype determination.

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REFERENCES

- Adams, M. H. (1950). *Meth. med. Res.*, **2**, 1.
 Belyavin, G. (1951). *J. gen. Microbiol.*, **5**, 197.
 Blair, J. E., and Carr, M. (1953). *J. infect. Dis.*, **93**, 1.
 Davidson, I. (1961). *Res. vet. Sci.*, **2**, 396.
 Dienes, L. (1946). *Proc. Soc. exp. Biol. N. Y.*, **63**, 265.
 Eisenberg, G. M., Weiss, W., and Flippin, H. F. (1958). *Amer. J. clin. Path.*, **30**, 20.
 Hill, G. A., Fung, J., and Marcus, S. (1962). *J. Bact.*, **84**, 191.
 Kippax, P. W. (1957). *J. clin. Path.*, **103**, 211.
 Lishman, I. V., and Swinney, J. (1963). *Brit. J. Urol.*, **35**, 116.
 Liu, P. V. (1958). *Amer. J. clin. Path.*, **29**, 176.
 Milner, P. F. (1963). *J. clin. Path.*, **16**, 39.
 Pavlatou, M., Hassikou-Kaklamani, E., and Zantioti, M. (1965). *Ann. Inst. Pasteur*, **108**, 402.
 Perch, B. (1948). *Acta path. microbiol. scand.*, **25**, 703.
 Popovici, M., and Ghioni, E. (1962). *Arch. roum. Path. exp.*, **21**, 307.
 Story, P. (1954). *J. Path. Bact.*, **68**, 55.
 Ward-McQuaid, J. F. N. C., Jichlinski, D., and Macis, R. (1963). *Brit. med. J.*, **2**, 1311.

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