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Serotyping and the Dienes reaction on *Proteus mirabilis* from hospital infections

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SYNOPSIS The serotype of 320 strains of *Proteus mirabilis* from clinical material was determined. Using 20 O antisera and four H antisera 61% of strains could be fully identified and 90% partially identified. A large number of serotypes were recognized but no difference was found between the serotype of organisms infecting the urinary tract and those from other infections. Biochemically identical organisms found in the same ward generally differed in serology. *Proteus mirabilis* was isolated from the faeces of 84·5% of 84 patients with urinary infection and from none of 20 normal controls. By serology and the Dienes test 61% of the organisms isolated from the urine and faeces of a single patient were identical, indicating that infection arose from the intestine.

Most groups of serologically identical strains could, by the Dienes test, be further divided into a number of subtypes indicating that the strains were different and that cross infection had not been responsible for their spread. With three serological groups, however, the majority of strains belonged to a single Dienes type and it was concluded that these organisms had been spread from a common reservoir or carrier.

Because of the unreliability of the Dienes test when carried out on random organisms it is suggested that reliable results can only be obtained by combining the Dienes test with serotyping.

Because of their importance as causes of hospital infection (Finland, 1960; McCabe and Jackson, 1962) and the desirability, from the epidemiological viewpoint, of being able to identify bacterial strains within a species, Gram-negative bacilli have received increasing attention in recent years. *Esch. coli* (Ujváry 1958; Turck and Petersdorf 1962; Percival, Brumfitt, and de Louvois, 1964), Klebsiella species (Kauffmann, 1949; Brooke, 1951; Edwards and Fife, 1952, 1955), and especially Pseudomonas species (Habs, 1957; Ayliffe, Lowbury, Hamilton, Small, Asheshov, and Parker, 1965; Wahba, 1965; Gillies and Govan, 1966), have all been studied and, by phage or colicine typing, or serology, valuable information on the distribution and epidemiology of these organisms has been obtained.

In spite of the fact that *Proteus mirabilis* infections often present serious clinical and epidemiological problems this species has so far received little attention. *Proteus vulgaris* and *Proteus mirabilis* (Hauser, 1885) were shown by Kauffmann and Perch (1947) to be members of the same antigenic group in spite of being biochemically distinct. Further work on the general bacteriology of these species (Cook, 1948; Elek, 1948; Barber and Waterworth, 1964; Huang, 1966) added to the general information but did not assist in the identification of strains.

Studies by Kippax (1957) on atypical biochemical types, by Belyavin (1951), Krikler (1953), and Story (1954) on the Dienes reaction, and by Pavlatou, Hassikou-Kaklamani, and Zamkioti (1965) and France and Markham (1968) on typing by the use of bacteriophage have attempted to differentiate strains on non-serological grounds.

Following the publication by Perch (1948) of an extended antigenic scheme for *Proteus mirabilis* and *Proteus vulgaris* the only study using this scheme to identify strains isolated from clinical material serologically was by Lányi (1956) in Hungary. In Britain the only serological studies have been by Belyavin, Miles, and Miles (1951) and Krikler (1953) and both used random organisms as their standard strains

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which were not fully investigated antigenically and which are no longer available.

The present study sets out to examine serologically strains of *Proteus mirabilis* from clinical material using the standard organisms of Perch. The clinical strains were isolated from patients who had infections of the urinary tract or infected wounds, and from the faeces of patients with infections of the urinary tract. The objects were to determine the serology of the infecting strains isolated, to establish any serological difference between strains infecting the urinary tract and those infecting wounds, to determine whether patients with urinary tract infections carried organisms of the same serotype in their faeces, and to attempt to recognize any reservoirs of cross infection.

Dienes (1946, 1947) suggested that strains that gave no line of demarcation when swarming together were identical while those that gave a demarcation line were not identical but further studies (Krikler, 1953; Story, 1954) have cast doubt on this assumption. Because the Dienes test is at present the only readily available means of examining strains epidemiologically it was performed on groups of organisms to determine the reliability of the test for recognizing identical strains and to assess its value as a means of dividing strains within a serotype.

MATERIALS AND METHODS

ORGANISMS Three hundred and ninety-one strains of *Proteus mirabilis* isolated from clinical material were investigated. Of these, 218 were from patients with diagnosed urinary tract infection, 102 from patients with other infections, and 71 from the faeces of patients with urinary tract infection. The specimens of faeces were collected two days after the appropriate antibiotic therapy had begun, in order to eliminate contamination.

O ANTISERA From the antigenic scheme of Perch, organisms of cultural type 2, *ie*, *Proteus mirabilis*, were selected from the first 30 O types and the following O antisera were prepared: O_3, O_5, O_6, O_7, Q_9, O_{10}, O_{11}, O_{13}, O_{14}, O_{16}, O_{17}, O_{18}, O_{19}, O_{20}, O_{23}, O_{24}, O_{26}, O_{28}, O_{29}, O_{30}. These sera were manufactured by injecting rabbits with a series of increasing doses of bacterial O antigen, prepared from the standard strains. The specific and non-specific O antibody titres of the sera were determined by a simple doubling dilution-bacterial agglutination technique and the sera diluted or absorbed, as necessary, to render them specific.

H ANTISERA. H Antisera were prepared against organisms possessing H_1, H_2, H_3, and H_4 antigens, these organisms being selected from the standard strains. Formolized six-hour broth cultures were injected into rabbits using a similar method as that for the preparation of O antisera. Dilutions of H antisera were tested on a glass tile against saline suspensions, prepared from swarming cultures on blood agar, of the homologous strain. Cross-reactions could be diluted out leaving a serum that gave rapid macroscopic agglutination with the homologous organism.

EXAMINATION OF STRAINS All organisms were first identified as *Pr. mirabilis* by biochemical reactions, and were then stored at 4°C on solid media until required.

O antigenic suspensions were prepared to each strain by heating saline suspensions of washed overnight broth cultures at 105°C for one hour. These were preserved with formalin and stored at 4°C.

TYPING PROCEDURE The bacterial O antigen of the test organism was diluted in saline to give an optical density of 0·35 to 0·40 at wavelength 480 mμ and a path width of 1 cm. Of this dilution, 0·1 ml was incubated at 50°C for 18 hours with 0·1 ml of each specific O antiserum. After being allowed to stand for one hour at room temperature each tube was examined for agglutination. A saline control was included to recognize autoagglutinating strains.

AUTOAGGLUTINATING STRAINS When a heat-killed O antigen was found to be autoagglutinable, an alcoholic antigen from a desoxycholate citrate agar culture was prepared. Strains found to be autoagglutinable after this procedure were not investigated further.

H SEROTYPE Organisms for H typing were subcultured onto blood agar plates. After overnight incubation saline suspensions were prepared from the swarming portions of each growth. Drops of each bacterial suspension were mixed with drops of specific H antiserum on a glass tile and examined for agglutination after rocking for half a minute.

DIENES TEST Strains of *Proteus mirabilis* to be examined for the Dienes reaction were stab inoculated onto opposite sides of an agar plate and the swarming growth was examined after 18 hours' incubation for a line of demarcation where the two cultures met. The Dienes reaction is negative when strains swarm into each other imperceptibly and positive when a line of demarcation can be seen where they meet.

To determine the reliability of the test for recognizing identical strains a panel of 41 serologically identified organisms was examined. Each organism was tested against the other 40, a total of 800 tests. Pairs of organisms from the urine and faeces of the same patient were also examined to determine the rate of autoinfection. In addition groups of serologically identical strains were examined to assess the value of the Dienes test for further division of organisms within a serotype, and as a possible means of recognizing cases of cross infection. All strains that were Dienes negative were re-examined three or four times for confirmation.

RESULTS

The O antisera produced all had titres of 1/10,000 to 1/20,000 when tested with the standard antigen at an optical density of 0·35 to 0·40. In the majority of cases non-specific reactions were less than 1/640 and

so could be diluted out while still leaving a highly reactive specific serum. However, cross reactions between four sera were higher than 1/640 and the sera had, therefore, to be absorbed.

The H antisera produced all reacted rapidly on the tile at dilutions of 1/500. Their respective O agglutinins were all below 1/300 in titre.

SEROTYPES OF THE 320 STRAINS ISOLATED FROM INFECTIONS Using the 20 O antisera prepared, 197 (61%) of the organisms studied were O typable, while with the four H antisera 287 (90%) of organisms could be recognized. The 197 serotyped organisms could be divided into 49 serotypes by their O and H antigens.

Of the 49 serotypes found, nine, involving 21 strains (11%), had not previously been described. These were O_6H-, $O_{16}H_2$, $O_{17}H_2$, $O_{17}H_3$, $O_{17}H-$, $O_{26}H_1$, $O_{26}H-$, $O_{28}H_1$, and $O_{29}H_1$, H− denoting an H antigen other than H_1, H_2, H_3, or H_4. The remaining 123 strains did not have typable O antigens but could be divided into five groups by their H antigens. Three of these strains were autoagglutinable.

The distribution of O types among organisms isolated from urinary tract and wound infections is shown in Table I. Table I shows that there is no significant difference between the O types infecting wounds and those infecting the urinary tract. In both groups certain serotypes, notably O_3, O_{10}, O_{13}, and O_{26}, occurred more frequently than others. Comparison between the serotype of the organisms and the ward from which they were isolated showed no significant relationship. In only 22% of cases did a particular serotype occur more than once in any one ward.

PROTEUS STRAINS ISOLATED FROM THE FAECES OF PATIENTS WITH PROTEUS URINARY TRACT INFECTIONS *Proteus mirabilis* was isolated from the faeces of 71 (84·5%) of 84 patients with *Proteus mirabilis* urinary tract infection and was not isolated from any of 20 uninfected control patients. The distribution of O types among these organisms is shown in Table I. These organisms show the same distribution of O types as those found in infections. Forty-seven of these 71 strains came from patients with typable organisms in their urine and of these 33 (70%) showed identical organisms in both urine and faeces (Table II). Of the remaining 24 patients nine had organisms of non-groupable O type but identical H

TABLE I

DISTRIBUTION OF GROUPABLE 'O' TYPES FROM THREE SOURCES

O Type	*Urine*	*Wounds*	*Faeces*
O_3	24 (18·0%)	16 (25·0%)	9 (19·0%)
O_5	1 (0·8%)	0	1 (2·2%)
O_6	11 (8·3%)	2 (3·1%)	2 (4·3%)
O_7	1 (0·8%)	0	0
O_9	2 (1·5%)	0	1 (2·2%)
O_{10}	17 (12·8%)	8 (12·5%)	9 (19·0%)
O_{11}	6 (4·4%)	0	1 (2·2%)
O_{13}	12 (9·0%)	6 (9·3%)	4 (8·5%)
O_{16}	4 (3.0%)	0	2 (4·3%)
O_{17}	4 (3·0%)	3 (4·7%)	0
O_{18}	1 (0·8%)	0	1 (2·2%)
O_{20}	3 (2·3%)	2 (3·1%)	0
O_{23}	10 (7·5%)	3 (4·7%)	2 (4·3%)
O_{24}	2 (1·5%)	6 (9·3%)	0
O_{26}	10 (7·5%)	4 (6·3%)	4 (8·5%)
O_{28}	12 (9·0%)	3 (4·7%)	6 (12·6%)
O_{29}	3 (2·3%)	4 (6·3%)	4 (8·5%)
O_{30}	10 (7·5%)	7 (11·0%)	1 (2·2%)
Total groupable	133	64	47
Not groupable	82	38	23
Autoagglutinable	3	0	1
Total	218	102	71

TABLE II

SEROLOGY OF STRAINS FROM URINE AND FAECES OF INFECTED PATIENTS

Urinary Organisms	*No. of Cases*	*Identical Serotype in Faeces*	*Different Serotype in Faeces*	*No Proteus in Faeces*
Typeable strains	54	33 (70%)	14	7
O Non-typeable strains	28	9[1]	13	6
Autoagglutinable strains	2	1[1]	1	0

[1]Possibly identical

TABLE III

DIENES TEST ON 41 SEROLOGICALLY IDENTIFIED STRAINS

No. of Tests Performed	Pairs of Dienes-Negative Organisms	Dienes Test Negative 'Pairs'		
		Serologically Identical	Different O Antigen and Identical H Antigen	Serologically Distinct
800	16	11	4	1

TABLE IV

DIENES TEST ON THE 71 'PAIRS' OF FAECAL AND URINARY STRAINS

Serology	Total	Dienes Test	
		Positive (Line of Demarcation)	Negative (No Line of Demarcation)
Serotyped Pairs			
Pairs identical	32	1	31
Pairs different	19	10	9
O Non-groupable Strains			
With identical H antigen	9	1	8
With different H antigen	3	0	3
O Autoagglutinable Strains			
With different H antigen	2	1	1
One or both strains non-motile	6		
Total	71		

type in both their urine and faeces. In a number of cases an organism of uncommon serotype was isolated from a patient's urine and faeces, thus discounting the possibility that urine and faeces had derived their organisms from separate sources.

A similar incidence of typable organisms was found in the urines of both men and women. In addition identical organisms were found in the faeces with similar frequency in both sexes.

DIENES TEST ON SEROLOGICALLY IDENTIFIED STRAINS When 41 serologically identified strains of *Proteus mirabilis* were examined only 16 pairs of organisms were repeatedly identical by the Dienes test. Eleven of these pairs were serologically identical. The organisms of the remaining five Dienes identical pairs were serologically distinct although in four of them the pairs shared a common H antigen (Table III).

DIENES TEST ON 'PAIRS' OF ORGANISMS FROM URINE AND FAECES The results of the Dienes test performed on the 'pairs' of *Proteus mirabilis* isolated from 71 patients are shown in Table IV. The majority of serologically identical pairs gave no line of demarcation by the Dienes test. Twelve serologically distinct pairs also gave no line of demarcation; however, eight of these pairs had common H antigens. There was also one pair of organisms that were biochemically and serologically identical but showed a line of demarcation between the swarming cultures.

DIENES TEST ON SEROLOGICALLY IDENTICAL STRAINS ISOLATED ON DIFFERENT WARDS The results of the Dienes test on organisms of the same serological group found in urinary tract infections are shown in Table V. With the exception of groups O_3H_1 and $O_{28}H_2$ the number of Dienes types recognized is almost as large as the number of wards from which the organisms were isolated. In some cases sero-

TABLE V

RESULTS OF DIENES TEST ON SEROLOGICALLY IDENTICAL STRAINS FROM URINARY TRACT INFECTIONS IN DIFFERENT WARDS

Serotype	No. of Strains	No. of Different Wards	No. of Dienes Types
O_3H_1	10	5	2
O_3H_2	12	7	7
$O_{10}H_1$	6	2	3
$O_{10}H_2$	6	3	4
$O_{28}H_2$	6	3	4
$O_{28}H_1$	6	5	3
$O_{28}H_2$	7	5	1

logically identical organisms of the same Dienes type were found in a number of wards while in others two or more Dienes types were recognized amongst serologically identical organisms from a single ward. In only half of the cases where two or more serologically identical strains were isolated from the same ward was the Dienes test negative, indicating that the strains were identical. The seven strains of $O_{28}H_2$ isolated came from widely spaced wards and were all, by the Dienes test, identical. Nine organisms belonging to group O_3H_1 were also apparently identical. This indicates that these infections arose from persistent reservoirs or carriers.

The results of the Dienes test on groups of serologically identical strains from wound infections

shows the same distribution as that found with urinary organisms (Table VI). The majority of serological groups contained several Dienes types but the strains of $O_{30}H_2$ found on the three different wards were of identical Dienes type.

TABLE VI

RESULTS OF DIENES TEST OF SEROLOGICALLY IDENTICAL STRAINS FROM WOUND INFECTIONS IN DIFFERENT WARDS

Serotype	*No. of Strains*	*No. of Wards*	*No. of Dienes Types*
O_3H_1	12	5	5
O_3H_2	6	5	3
$O_{10}H_1$	5	3	4
$O_{30}H_2$	5	3	1

DISCUSSION

The desirability of a method for identifying strains of *Proteus mirabilis* has long been recognized. Kauffmann and Perch (1947) published a limited antigenic scheme for *Proteus hauseri* (*Pr. mirabilis* and *Pr. vulgaris*) which was considerably extended by Perch (1948). Belyavin *et al* (1951) and Krikler (1953), using a limited number of strains, few of which had been antigenically classified, were the first workers in Britain to investigate serologically strains of *Proteus mirabilis* from clinical material. Unfortunately, their strains were not identified within Perch's scheme and are no longer available, thus preventing comparison between their work and any subsequent studies.

The only other serological investigation of this group of organisms was by Lányi (1956) in Hungary who examined 1,242 strains of Proteus, 1,041 of which were *Proteus mirabilis*. A large number of Lányi's strains came from cases of infantile enteritis that were not due to any recognized enteric pathogen. Amongst these cases Lányi found a significant increase in the rate of isolation of Proteus belonging to groups O_3 and O_{26}. Although these serotypes are fairly common the possibility that there are infantile enterites producing strains of Proteus similar to those of *Esch. coli* cannot be ruled out. It is perhaps only coincidence that the strains isolated from an outbreak of meningitis in infants at Queen Elizabeth Hospital, Birmingham (unpublished information), also belonged to group O_{26}.

The findings of this study, that even though a large number of antigenic types are responsible for infection, some O groups are isolated from infections more frequently than others, is in agreement with the original work by Perch and the later study by Lányi. At present there is no evidence to show whether these serotypes are more pathogenic or just more frequent than others.

There is some doubt whether Proteus infections arise as a result of cross infection from local reservoirs or by autoinfection from the patient's intestines, particularly with regard to infections of the urinary tract. Kippax (1957) showed that infections in a male urological ward were due to spread from a persistent local reservoir. Krikler (1953), using antisera, and Story (1954) using the Dienes test and biochemical reactions, found, however, that urinary infections were due to autoinfection. The present study also shows a high incidence of autoinfection amongst patients with urinary tract infections. In 31 of 51 (61%) of cases, organisms of identical serotype and Dienes test type could be isolated from both urine and faeces.

The wide distribution of serotypes within a ward or ward block and the low frequency with which serologically identical organisms were found in the same ward shows that cross infection from persistent reservoirs within the wards is infrequent.

The possibility that cross infection occurred from persistent reservoirs within the hospital rather than from individual wards was investigated. Dienes tests carried out on serologically identical organisms isolated from different wards showed that a large number of Dienes types could be recognized and, therefore, that in general cross infection from persistent hospital reservoirs was not responsible for the spread of this organism. There were, however, some cases where hospital reservoirs could have been responsible. The wide distribution of Dienes identical strains of serotypes $O_{28}H_2$ and O_3H_1 amongst urinary infections and $O_{30}H_2$ amongst wound infections was probably due to spread from common reservoirs.

It would, therefore, seem most probable that both auto- and cross infection play a part in the spread of *Proteus* and that possibly under certain circumstances one mode of spread predominates.

The results obtained from the 800 tests on a panel of 41 serotyped strains once again raises serious doubts about the absolute reliability of the Dienes test when carried out on random organisms. These results, which confirm those of previous workers (Krikler, 1953; Story, 1954), show that negative Dienes reactions among random strains are rare (16/800 or 2%). In view of the five false negative results obtained it would seem that when comparing Dienes reactions on random strains it is only possible to say that a positive Dienes test shows the organisms to be different. The results of Dienes tests on the pairs of organisms from urine and faeces also showed a high incidence of false negative results (13 of 24 or 54%). In both groups there was a high incidence of shared H antigens amongst organisms giving a false negative Dienes reaction. In spite of these results when the Dienes test is carried out on strains

that are antigenically identical it is of great value in further dividing a particular serotype and it is clear that only by combining serology and the Dienes test can reliable results be obtained.

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REFERENCES

Ayliffe, G. A. J., Lowbury, E. J. L., Hamilton, J. G., Small, J. M., Asheshov, E. A., and Parker, M. T. (1965). *Lancet*, **2**, 365.
Barber, M., and Waterworth, P. M. (1964). *J. clin. Path.*, **17**, 69.
Belyavin, G. (1951). *J. gen. Microbiol.*, **5**, 197.
——, Miles, E. M., and Miles, A. A. (1951). *Ibid.*, **5**, 178.
Brooke, M. S. (1951). *Acta path. microbiol. scand.*, **28**, 313 and 328.
Cook, G. T. (1948). *J. Path. Bact.*, **60**, 171.
Dienes, L. (1946). *Proc. Soc. exp. Biol. (N.Y.)*, **63**, 265.
—— (1947). *Ibid.*, **66**, 97.
Edwards, P. R., and Fife, M. A. (1952). *J. infect. Dis.*, **91**, 92.
——, —— (1955). *J. Bact.*, **70**, 382.
Elek, S. D. (1948). *J. Path. Bact.*, **60**, 183.
Finland, M. (1960). *New Engl. J. Med.*, **263**, 207.
France, D. R., and Markham, N. P. (1968). *J. clin. Path.*, **21**, 97.
Gillies, R. R., and Govan, J. R. W. (1966). *J. Path. Bact.*, **91**, 339.
Habs, I. (1957). *Z. Hyg. Infekt.-Kr.*, **144**, 218.
Hauser, G. (1885). *Ueber Fäulnissbacterien*. Vogel, Leipzig.
Huang, C. T. (1966). *J. clin. Path.*, **19**, 438.
Kauffmann, F. (1949). *Acta path. microbiol. scand.*, **26**, 381.
—— (1966). *The Bacteriology of Enterobacteriaceae*, 3rd ed. Munksgaard, Copenhagen.
——, and Perch, B. (1947). *Acta path. microbiol. scand.*, **24**, 135.
Kippax, P. W. (1957). *J. clin. Path.*, **10**, 211.
Krikler, M. S. (1953). Ph.D. Thesis, London University.
McCabe, W. R., and Jackson, G. C. (1962). *Arch. intern. Med.*, **110**, 847 and 856.
Lányi, B. (1956). *Acta microbiol. Acad. Sci. hung.*, **3**, 417.
Pavlatou, M., Hassikou-Kaklamani, E., and Zankioti, M. (1965). *Ann. Inst. Pasteur*, **108**, 402
Perch, B. (1948). *Acta path. microbiol. scand.*, **25**, 703.
Percival, A., Brumfitt, W., and de Louvois, J. (1964). *Lancet*, **2**, 1027.
Story, P. (1954). *J. Path. Bact.*, **68**, 55.
Turck, M., and Petersdorf, R. G. (1962). *J. clin. Invest.*, **41**, 1760.
Wahba, A. H. (1965). *Brit. med. J.*, **1**, 86.
Ujváry, G. (1958). *Zbl. Bakt., I. Abt. Orig.*, **170**, 394.