Among the members of the genus *Pseudomonas*, *P. aeruginosa* and *P. pseudomallei* were, until recently, considered the human pathogens. However, since the early 1960s, other *Pseudomonas* species have been found in clinical specimens, and their number and frequency of isolation seem to be growing.

The "simple fluorescent" species, *P. fluorescens* and *P. putida*, belong to those that were thought by earlier authors to be nonpathogenic for man. A few instances of possible human pathogenicity, however, have been reported since 1953 when Pittman described six strains with the characteristics of *P. fluorescens* that had grown in transfusion blood and had caused severe to fatal reactions in the recipients. Rutenburg et al. mentioned three urinary tract infections and one wound infection from which *P. fluorescens* was recovered in pure culture; septicemia developed in the latter case. The authors also reported a mixed urine culture with *P. fluorescens*. Rogers cultured *P. putida* from the blood of an 8-year old boy with a fatal septicemia and otherwise unexplained lymphadenopathy and hepatosplenomegaly. Sutter isolated *P. fluorescens* repeatedly from the blood of a patient with an abdominal abscess following bowel resection. Furthermore, she cultured *P. putida* from a bile drain, from two urines (in pure culture from an asymptomatic patient and in mixed culture from another patient with chronic urinary tract infection), and from a sputum after pneumococcal pneumonia treated with erythromycin and tetracycline. Pathogenicity of the *P. putida* strains could not be established in these cases. Pickett et al. found *P. putida* in urines of ten patients. Two cultures were pure and came from debilitated patients with no clinical signs of urinary tract infection, while three other strains from different sources as well as 43 strains of *P. fluorescens* were not considered etiologically significant by these authors.

In view of such reports, it is not surprising that there is no unanimity as to the pathogenic significance of *P. fluorescens* and *P. putida* for man. This paper is intended as a contribution to the discussion. In the two-year period from March, 1969 to February, 1971, 11 strains of *P. putida* and 4 strains...
of *P. fluorescens* were isolated in the Bacteriology Laboratory of Yale-New Haven Hospital. Their characteristics and possible significance will be outlined.

**ISOLATION AND IDENTIFICATION**

Urine, sputa, and throat swabs were inoculated on aerobic blood agar* and Desoxycholate Agar** plates; pleural fluid, wound, and pus specimens also on anaerobic kanamycin-blood agar plates and into Fluid Thioglycollate Medium;** stool specimens on XLD Agar* and into GN Broth.** For blood cultures, 50 ml of Fluid Thioglycollate Medium and 50 ml of Trypticase Soy Broth**, both with 0.03% of sodium poly-anethol sulfonate,*** were each inoculated with 5 ml of blood. Incubation was for 48 hours at 37°C; only blood cultures were held for seven days and subcultured on anaerobic chocolate agar plates on the fifth day.

Identification followed procedures described elsewhere.8 Table 1 lists features used in the differentiation between the fluorescent species, *P. aeruginosa*, *P. fluorescens*, and *P. putida*. Antimicrobial sensitivity was tested with the Kirby-Bauer method.10 There is some question whether the differences between *P. fluorescens* and *P. putida* justify their distinction as two separate species.12 Both are strictly aerobic, polar multinucleate Gram-negative rods. They grow well on blood agar and on enteric differential agars between 20° and 35°C but will grow poorly or not at all on first isolation at 37°C on these media. The water-soluble green fluorescent pigment, named pyoverdin, fluorescein or fluorescin, is best produced at room temperature on Flo Agar** (or Pseudomonas Agar F****). Some strains exhibit a putrid odor, or beta hemolysis, or both. Positive tests for: oxidase, arginine dihydrolase (but not lysine decarboxylase and deoxyribonuclease), citrate utilization; oxidation of 1% glucose, xylose, arabinose, fructose, galactose, and mannose in OF media.** Occasionally, 1% maltose, sucrose, and mannitol, 10% lactose, and gluconate may be oxidized, and urea may be hydrolyzed.1,2,7,9,10,12-17 Biotypes have been described by Stanier et al.* and by Jessen.14

**CLINICAL DATA**

The strains were isolated from 14 different patients, one from five different sources in the same patient. An additional *P. fluorescens* strain was cultured from the fluid of an ultrasonic nebulizer.

Table 2 lists pertinent patient data. There was no preference for either sex, but nine patients were 50 years or older. Four strains (three from drains and one from a throat) were obviously community acquired; the others were first isolated after 1-25 days of hospitalization. Association with generalized debilitating disease was not consistent. Four of the five patients with positive sputa had a recent history of tracheostomy or intermittent positive pressure breathing (IPPB); the three urine isolations followed

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* Trypticase Soy (BBL) base.
** BBL (BioQuest), Cockeysville, Md.
*** Hoffmann-La Roche Inc., Nutley, N. J.
**** Difco Laboratories, Detroit, Mich.
insertion of Foley catheters. Two patients had been on steroid therapy, and six had previously received penicillin or one of its derivatives.

Table 3 lists the antimicrobial sensitivities. No relationship could be detected between them and the site of isolation, the origin of the strain, the species, or previous therapy.

Only the cultures of two urines (one with a low count), two bloods, and an intravenous catheter were pure. The others were mixed; in six cases, with Gram-negative rods; and in three, with Gram-positive cocci. A causative role of the pseudomonads could, therefore, not be established with certainty in nine cases. Two patients with mixed cultures were seriously ill and died: no. 2 from cardiac failure (at autopsy, no signs of pneumonia were found), and no. 3 from *Proteus mirabilis* septicemia. Although the pseudomonads persisted in their respiratory tracts, they did not seem to contribute to the fatal outcome. Eight patients improved clinically despite lack or inappropriateness of antimicrobial treatment.

Since patient no. 2 did not show a clinically significant urine count, evaluation of a possible etiological role could only be undertaken for *P. putida* strains in four patients. Patients nos. 5, 6, and 11 showed temperature spikes of 101-103°F with febrile periods of 24-48 hours, but no shock or other symptoms of Gram-negative septicemia. Patient no. 11 did have a phlebitis at the intravenous catheter insertion site. A blood culture was not taken, but defervescence followed the removal of the catheter. In patient no. 5, cholecystitis had developed at the time of bacteremia; the patient had been in the recovery stage from extensive cranial surgery for carcinoma of a maxillary sinus. She became afebrile after cholecystectomy and antibiotic treatment with kanamycin and cephalothin. In patient no. 6, the temperature rise occurred postoperatively, and recovery was spontaneous. Both patients no. 5 and no. 6 showed a mild leucocytosis (12.500 and 15.800 WBC per cu.mm) with a shift to the left. All three patients had been on intravenous

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**Table 1. Differential Diagnostic Features Between *P. aeruginosa*, *P. fluorescens*, and *P. putida***

| Characteristics                  | *P. aeruginosa* | *P. fluorescens* | *P. putida* |
|----------------------------------|-----------------|------------------|-------------|
| Polar flagella                   | 1               | more than 1      | more than 1 |
| Nitrate reduction                | +(gas)/(−)      | −/(+)            | −           |
| Growth at 4°C                    | −               | +                | +/−         |
| Growth at 42°C                   | +               | −                | −           |
| Pyocyanin product.               | +/(−)           | −                | −           |
| Gelatin liquefact.               | +/(−)           | +                | −           |
| Egg-yolk reaction                | −               | +                | −           |

+ positive; − negative; / or; ( ) occasional strain
### Table 2. Clinical Data On 14 Patients (Inpatients Unless Otherwise Indicated)

| No. | Age, sex | Diagnosis                                      | Source of culture, PF or PP species | Medical treatment prior to culture | Instrumentation | Outcome       |
|-----|----------|-----------------------------------------------|-------------------------------------|-----------------------------------|-----------------|---------------|
| 1   | 65       | M     | Leg hematoma                                  | Drain (1)                          | Pen + Te         | Local surgery | Impr.         |
| 2   | 78       | M     | Acute small bowel infarct. Cong. heart fail. | Sp* Pl. Fl., Stool, Wound, Urine (2) | Am, K, C         | Small bowel resection, Tracheostomy, Centr. ven. cath., Foley cath. | PF persist. in sp. and urine. Death. |
| 3   | 54       | F     | Subarachn. hem., Coma, Pneumon., Sarcoaidosis | Sp* (1) mixed, PP                  | Decadron          | Tracheostomy, Bird respirator, Foley cath. | PP persist. in sp. Death. |
| 4   | 59       | F     | Incompetence of rect. sphinct.                | Urine (2) mixed, PP 3+             | Am               | Sphincter repair, Foley cath. | Urine cult. neg. Foley cath. |
| 5   | 65       | F     | Ca. of maxill. sinus, postop. meningitis, 4 wks. later acute cholecystitis | Blood (1) pure, PP 6000 r X ray. Oxa + K | Li, Cl, Gm, Cf | Maxillectomy, Orbital exenteration, Craniootomy, Tracheostomy. Cholecystectomy. | Impr. |
| 6   | 30       | F     | Ectopic pregn.                                | Blood (1) pure, PP                 | Am               | Salpingo-Oophorectomy, hysterec. | Spont. impr. |
| 7   | 30       | M     | Chron. otitis media (outpt.)                 | Middle (1) mixed, PF              | Pen              | —              | Impr.         |
| 8   | 26       | F     | Tonsillitis (β hem. strep grpA) (outpt.)     | Throat (1) mixed, PP              | Pen              | —              | Impr.         |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 9 | 53 | Pneumonia | Sp* | mixed, | Pen | Cf, Ery | IPPB | Impr. |
|   |   | (Klebsiella) | (2) | PP |   |   |   |
| 10 | 10 | Chron. otitis | Middle | mixed, | — | — | — | Impr. |
| F |   | media (Outpt.) | Ear (1) | PP |   |   |   |
| 11 | 23 | Term pregn. | i.v. cath. | pure, | — | — | Recovery after removal of i.v. catheter |
| F |   | Phlebitis at i.v. site | (1) | PP |   |   |   |
| 12 | 50 | Rectal polyp | Sp** | mixed, | — | — | Removal of polyp | Impr. |
| M |   |   | (2) | PF |   |   |   |
| 13 | 58 | Ca. of rectum | Urine | pure, | — | — | Abd.-perin. resection. Foley cath. | Impr. |
| F |   |   | (1) | 4+PP |   |   |   |
| 14 | 70 | Chr. obstr. and restr. lung dis. | Sp** | mixed, | Predni- | — | IPPB | Impr. |
| M |   |   | (1) | PP | sone |   |   |

Explanations to Table 2

*) plus white cell reaction

**) no white cell reaction

(1) No previous culture had been taken from that site

(2) Previous culture(s) from that site had been negative for PF or PP

2+, between 10,000 and 50,000 bacteria per ml

3+, between 50,000 and 100,000 bacteria per ml

4+, over 100,000 bacteria per ml

Abbreviations:

Am, ampicillin; C, chloramphenicol; cath, catheter; Cl, cephalothin; Cl, colistin; Ery, erythromycin; Gm, gentamicin; impr., improved; K, kanamycin; Li, lincomycin; Oxa, oxacillin; Outpt, outpatient; Pen, penicillin; PF, Pseudomonas fluorescens; PIFl, pleural fluid; PP, Pseudomonas putida; Sp, sputum; Te, tetracycline.
Table 3. Antimicrobial Sensitivities of *Pseudomonas Fluorescens* and *Pseudomonas Putida* Strains

| Drug              | Number of strains tested | Number of strains fully sensitive |
|-------------------|--------------------------|----------------------------------|
| Colistin          | 14                       | 14                               |
| Gentamicin*       | 12                       | 12                               |
| Kanamycin         | 14                       | 11                               |
| Tetracycline      | 14                       | 10                               |
| Chloramphenicol   | 14                       | 4                                |
| Streptomycin**    | 11                       | 2                                |
| Carbenicillin*    | 3                        | 1                                |
| Cephalothin       | 14                       | 0                                |
| Ampicillin        | 14                       | 0                                |
| Nalidixic Acid    | 14                       | 0                                |
| Nitrofurantoin    | 14                       | 0                                |

* became available only after beginning of the study period.
** eliminated during study period in favor of carbenicillin.

Fluids prior to, but also during, the temperature rise. Cultures of the fluid bottles were not taken.

Patient no. 13 had a significant count of *P. putida* in the urine but showed no clinical signs of a urinary tract infection. The urine contained 2-4 WBC per HPF, and the blood count was normal. The culture became negative upon removal of the Foley catheter.

**DISCUSSION**

Two factors worked to our disadvantage. As usual, our cultures were incubated at 37°C, a point above the maximal growth temperature of both species. The relatively large size of the original inocula may have been the reason for growth of the pseudomonads at 37°C; only small inocula consistently fail to grow at 37°C. The recovery rate would almost certainly have been higher at incubation temperatures between 20° and 35°C, but duplicate inoculations are too costly for any laboratory. Secondly, the prevalence of mixed cultures made an evaluation of the causative role of the pseudomonads possible only in four cases.

Nevertheless, our observations led to some important conclusions:

1. Apyocyanogenic strains of *P. aeruginosa* may be diagnosed as *P. fluorescens*; conversely, any fluorescein-producing pseudomonad may be diagnosed as *P. aeruginosa*. A differential diagnosis, should, therefore be attempted (see Table 1).

2. At present, *P. fluorescens* and *P. putida* are sensitive not only to colistin and gentamicin but frequently also to kanamycin and tetracycline.
VON GRAEVENITZ, WEINSTEIN

P. fluorescens and P. putida
cycline.\textsuperscript{1,19} P. aeruginosa, on the other hand, is rarely affected by kanamycin or tetracycline. Of all strains isolated during the study period, 3\% were sensitive to kanamycin, and 1.5\% were sensitive to tetracycline. Similar figures have been reported elsewhere.\textsuperscript{19}

3. Like most pseudomonads, P. fluorescens and P. putida are found in water, soil, and on plants.\textsuperscript{2,10,14,20} Man could become colonized or infected from these sources including intravenous fluids (possible sources for our septicemia cases?). In hospitals, well-known factors that favor the emergence of Gram-negative rods could provide for persistence and transmission of these bacteria, as is at least suggested by our cases no. 2, 3, 4, 12, and 13. Human carriers of the two species in the oropharynx have been observed.\textsuperscript{21} Case no. 2 suggests that the intestinal tract may become a source, as it is in the case of P. aeruginosa.\textsuperscript{5}

Nevertheless the pathogenic potential for man of the two species is, compared to that of P. aeruginosa, obviously limited. Reports of isolation from human sources\textsuperscript{1,4,9,16-17} are much more frequent than reports of infections in which these species could be incriminated etiologically. Such infections when they occur generally run a mild course, as indicated earlier and demonstrated by our cases. Pathogenicity of P. fluorescens for cold-blooded animals, however, has been well documented.\textsuperscript{22}

Liu\textsuperscript{23} has shown that the inability of P. fluorescens to produce generalized infection in warm-blooded animals is due to its failure to grow at their internal body temperature. Inoculation of the burned mouse tail resulted in extensive local necrosis, but positive heart blood or organ cultures could not be obtained one to three days following inoculation. Similarly, injection of the bacterium into rabbits and mice by various routes led to no significant pathological changes. The organism was apparently rapidly eliminated from the circulation. Inoculation of mice with P. aeruginosa, a species well adapted to growth at 37\textdegree C, also led to necrosis, but bacteremia and a sometimes fatal lung invasion were observed as well.

In most of our patients, the pseudomonads apparently were either eliminated in an analogous way or were unable to compete for pathogenicity with other bacteria present. Patients no. 2 and no. 3 demonstrate that adaptation to body temperature, similar to that observed \textit{in vitro} in pseudomonads with lower growth maxima,\textsuperscript{29} may occur \textit{in vivo} as well. Since few follow-up cultures were taken, it cannot be determined whether persistence through such adaptation is associated with compromised host defense, as cases no. 2 and no. 3 would suggest.

Reactions to transfused blood contaminated with fluorescent pseudomonads\textsuperscript{5} are easily explained by growth of the organisms at 4\textdegree C and endotoxin release; they are, as the time interval between transfusion and re-
action proves, not due to pseudomonas growth in the recipient’s blood or tissues.

We did not see infections in our patients with compromised defenses in which either \textit{P. fluorescens} or \textit{P. putida} played an etiologically defined role. On the basis of our observations and those of other authors,\textsuperscript{1,7} the pathogenic potential of these pseudomonads in “normal” man must be regarded as low, presumably due to their poor chance for survival at body temperature.

SUMMARY

Eleven strains of \textit{P. putida} and four strains of \textit{P. fluorescens} were isolated in a two-year period from various clinical specimens. Characteristics of the organisms and of the patients from whom they were isolated are described. Two patients with pure cultures of \textit{P. putida} from blood and one with a pure culture from an intravenous catheter had short-lived fever episodes. One patient with a pure urine culture of the same organism after Foley catheter insertion was asymptomatic. It is suggested that these bacteria with growth maxima below 37°C are unable to sustain prolonged infection in man, although they may adapt to body temperature. Evidence of a strong pathogenic potential in patients with compromised defenses could not be presented as most of the patients were not debilitated.

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