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Passage of *Pseudomonas aeruginosa* in Compromised Mice

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There was no appreciable increase in the virulence of *Pseudomonas aeruginosa* PAO-1 after six passes through infections in mice. When strain PAO-1 was passed through mice compromised with iron and methotrexate, the virulence of the passed bacteria increased for normal as well as compromised mice. Bacteria harvested from intraperitoneal passage in compromised mice were more virulent than bacteria harvested from intrathoracic passage. These bacteria expressed 50% lethal dose values distinctive of the respective bacterial isolate when injected intraperitoneally, intrathoracically, or intravenously. Differences between bacteria from intraperitoneal and intrathoracic passage were apparently due to differences in the selective pressures in the two sites of infection, because the intrathoracically passed bacteria assumed the virulence characteristics of the intraperitoneally passed bacteria after intraperitoneal passage in compromised mice.

Pseudomonas aeruginosa is classified as an opportunistic pathogen and, in accordance with this description, causes serious infections in immunosuppressed (5), cancer (6), and burn (12) patients. The characteristics of virulence for opportunists are undefined and may be of doubtful existence since so much of the ability of these bacteria to initiate infection depends upon host defense mechanisms. An epidemiological study of an outbreak in a nursery revealed no relationships between type of *P. aeurginosa* and morbidity (3). In contrast, another survey demonstrated an association between particular O types of *P. aeruginosa* and a high incidence of infection (1). In another report there was a correlation between O types and an increased tendency for bacteremia (15). However, both of these relationships to virulence were periodic, with one O type yielding way to a multitude of other O types.

Although virulence has not been demonstrated for humans, variations in lethality between strains of *P. aeruginosa* have been found in experimental animals. Forseberg and Bullen (7) used a strain of *P. aeruginosa* freshly isolated from a human infection and demonstrated a 50% lethal dose (LD_{50}) for mice of 1.26×10^6 bacteria. This value decreased during 16 serial passages in 4 days to 4.09×10^4. Iron injected into infected mice lowered the LD_{50} for the original strain to 4.74×10^5 and that for the passed strain to less than 10. Bullen et al. (4) used another human isolate to demonstrate an initial LD_{50} of 9.35×10^5 which decreased to 8.8×10^2 after 20 passes through mice. In both studies the investigators found that iron administration had more effect on the lethality of the passed bacteria than the unpassed bacteria.

The relationship of iron to increased lethality suggested that nutritional immunity (14), the ability of the host to withhold an essential nutrient like iron from infecting bacteria, may be an important defense mechanism against *P. aeruginosa*. Liu and Mercer found a correlation between the LD_{50} values of virulent strains and their abilities to grow in serum (10) and were able to reverse the lethality of virulent strains with human transferrin. Nutritional immunity is not the only effective defense mechanism which has been shown to be inhibitable during infections with *P. aeruginosa*. Berk (2) also demonstrated differences in the lethality of strains of *P. aeruginosa* for mice and that methotrexate administration led to greater lethality for only some of the virulent strains.

However, other investigators have not been able to show that animal passage or administration of iron to infected animals increased the virulence of strains of *P. aeruginosa*. Martin et al. (11) did not find increases in lethality during passage through mice and found only slight increases during passage through rats using a strain of *P. aeruginosa* cultured extensively on laboratory medium. Chandler and Fukui (Bacteriol. Proc. **65**:45, 1965) were unable to increase the lethality of a laboratory strain by treating infected mice with iron. The inability of laboratory strains to acquire increased lethality in contrast to isolates from human infection may be due to alterations in control mechanisms or to the loss of genetic material during prolonged maintenance on laboratory medium. The basis

for the expression of virulence by *P. aeruginosa* is unknown, but it may be that laboratory strains may regain virulence only through contact and genetic exchange with virulent *P. aeruginosa*.

P. aeruginosa PAO-1 was originally isolated from a human infection (8) but may be described as a laboratory strain because of decades of existence in laboratory media. This paper describes the aquisition of virulence by this strain during passage through mice which had been treated with iron and methotrexate to establish a host more susceptible to infection by an opportunistic pathogen.

MATERIALS AND METHODS

Bacteria and culture conditions. *P. aeruginosa* PAO-1 (ATCC 15692) was obtained from the American Type Culture Collection and maintained on brain heart infusion agar. Criteria were established for the identification of strain PAO-1 so that it would not be confused with extraneous *P. aeruginosa* which could possibly infect the mice from sources other than the injection. Strain PAO-1 was trained to resistance to streptomycin by repeatedly growing bacteria on 1% tryptic soy agar with increasing concentrations of streptomycin until the strain grew on agar containing 50 μM streptomycin. This characteristic was used in conjuction with the ALA pyocin typing procedure (9) and agglutination with the 17 anti-O typing sera (Difco). Strain PAO-1 gave a variable pyocin type of 62-11-14 or 64-11-14 and maximum agglutination in anti-O 5 serum.

Bacteria were grown for injection at 37°C for 20 h in 1% tryptic soy broth with 0.4 mM $MgCl_2$ added after autoclaving. Bacteria were harvested at 5,900 × *g* at room temperature, washed three times in distilled water, and finally suspended in 10 mM potassium phosphate buffer, pH 7.4, with 0.15 M sodium chloride. Bacteria were diluted in 10 mM potassium phosphate buffer, pH 7.4, with 0.15 M sodium chloride to an absorbance of 0.2 at 600 nm, which corresponded to approximately 5×10^8 bacteria per ml. This dilution was used in constructing the 1:5 dilutions for the LD_{50} assays, and viable numbers of bacteria were determined by plating dilutions on tryptic soy agar.

Passage experiments in normal mice. Virulence was determined by increased lethality of bacteria. Lethality was measured by recording deaths at 10 days after injection and reporting LD_{50} values by the method of Reed and Muench (13). In the initial passage experiments using normal mice, bacteria were injected intraperitoneally (IP), and 24 h later the livers were taken from mice and minced, and the tissue fluid was streaked for isolation of bacteria on blood agar. Bacteria were also injected intrathoracically (IT) in other mice, and 24 h later lung tissue was obtained and treated in a manner identical to liver for the isolation of bacteria. Bacteria from each passage were picked from the plates and designated IP or IT isolates followed by the number of the passage. The cloned isolates were inoculated into tryptic soy broth and onto tryptic soy agar plates containing 50 μM streptomycin. Washed bacteria from growth in tryptic soy broth were used in the agglutination assay, and if the assay was indicative of strain PAO-1, the bacteria were injected for another passage. The isolates were also grown in tryptic soy broth for determination of pyocin type. The numbers of passages on laboratory medium were kept at a minimum, but each isolate was grown on blood agar and in 40 ml of tryptic soy broth before being injected into mice for the next passage.

The results of passage experiments were depicted graphically as progressions of LD_{50} values with each isolate number. Isolate 0 is the unpassed bacterium, and the bacterium harvested from infections with isolate 0 in mice surviving 24 h is isolate 1.

Passage experiments in compromised mice. Mice were injected with 5 μmol of $FeCl_3$ and 1.25 mg of methotrexate per mouse in opposite sides of the peritoneal cavity at the same time as the injection of bacteria. Methotrexate was used to affect immunosuppression, and $FeCl_3$ was used to saturate transferrin and suppress nutritional immunity (14). $FeCl_3$ was used because Fe(III) theoretically cannot be used by bacterial uptake mechanisms without solubilization because it would be largely insoluble at physiological pH in the mice. This form of iron is also less toxic than other forms of iron at the concentrations used in these experiments. Bacteria were passed in and harvested from compromised mice in the same manner as in normal mice except that the lungs and livers were taken from surviving mice at 72 h after injection.

Lethality measurements of the isolates from passage. Passage experiments were conducted in those mice receiving both iron and methotrexate, but LD_{50} values were determined at each passage in mice receiving a variety of treatments. Dilutions of bacteria were injected into four groups of mice: untreated, treated with methotrexate, treated with iron, and treated with iron and methotrexate. Five to seven mice were injected at each dilution with at least one extra mouse included at each dilution in the iron and methotrexate group for the harvest of bacteria in the passage experiment. Control mice received no bacteria, but received injections of iron, methotrexate, and iron and methotrexate. At no time were bacteria other than strain PAO-1 cultured from experimental animals, and control animals were routinely negative for bacteria in the organs. In later experiments, LD_{50} values were determined for the IP and IT isolates in sites of infection other than the site of passage.

Statistical analyses of LD_{50} values were made using the probit analysis of the Statistical Analysis Service. A 95% level of significance was used in analyzing the data.

Bacterial growth in mice. Mice were injected intravenously with numbers of the IP-6 isolate which were below the LD_{50} for normal mice and above the LD_{50} for mice treated with iron (5.0×10^5, see Fig. 4). Viable bacteria in heart blood were measured to determine clearance of bacteria. One group of infected mice remained untreated, a second group received 5 μmol of $FeCl_3$ per mouse IP, and the third group received 1.0 mg of sulfadiazine per mouse subcutaneously at the shoulder in addition to the iron injection. In a separate experiment, injections of bacteria and

sulfadiazine followed the injection of $FeCl_3$ by 24 h. Sulfadiazine was used as a bacteriostatic agent against *P. aeruginosa*. Heart blood was withdrawn at times after injection, and viable bacteria were determined by plating dilutions on tryptic soy agar.

Male Swiss-Webster mice, 6 to 8 weeks of age, were obtained from a colony in the Department of Microbiology, University of Iowa, and were maintained on Tek-Lab mouse chow and tap water.

RESULTS

Passage of strain PAO-1 in normal mice. The LD_{50} of strain PAO-1 was 2.2×10^8 after IP injection and was 4.1×10^7 after IT injection. These values did not change substantially during attempts to obtain bacteria with increased virulence by passage through IP (curve IP, Fig. 1) or through IT (curve IT, Fig. 1) infections in mice.

Passage of strain PAO-1 in compromised mice. Attempts to harvest bacteria from the organs of normal mice at times in excess of 24 h after injection were not routinely successful. To increase the time of exposure of the bacteria to the mammalian environment, strain PAO-1 was passed through infections in mice treated to compromise certain defense mechanisms. Bacteria could be harvested routinely at 72 h from organs of surviving mice which had been treated with iron and methotrexate. Table 1 reveals that iron and methotrexate treatments did not appear to make mice more susceptible to the unpassed bacteria. Passage of strain PAO-1 through mice compromised with iron and methotrexate resulted in isolates from IP passage (curve IP, Fig. 2) and from IT passage (curve IT, Fig. 2) demonstrating increased lethality for compromised mice. The LD_{50} value of IP-8 was so low that further passage was technically difficult because the 1:5 dilutions did not reproducibly yield live mice with bacteria present at 72 h. Comparisons between the lethality of IP-8 in mice receiving different treatments indicate that iron treatment alone (curve a, Fig. 3) was more effective ($P < 0.05$) in lowering the LD_{50} than was methotrexate treatment (curve b, Fig. 3). Lethality of IP-8 in methotrexate-treated mice was not significantly different from that observed in normal mice ($P = 0.094$). Although it appears that iron was the most effective compromising agent for IP-8 (curve a, Fig. 3), methotrexate when combined with iron (IP-8, curve IP, Fig. 2) did have a significant effect ($P < 0.05$) over iron alone.

TABLE 1. *Lethality of passage 0 P. aeruginosa PAO-1*

Mode of injection	LD_{50} values of mice treated with:			
	Untreated	Iron	Methotrexate	Methotrexate + iron
IP	2.2×10^8	2.2×10^8	1.4×10^8	1.9×10^8
IT	4.1×10^7	2.8×10^7	2.5×10^7	6.5×10^7

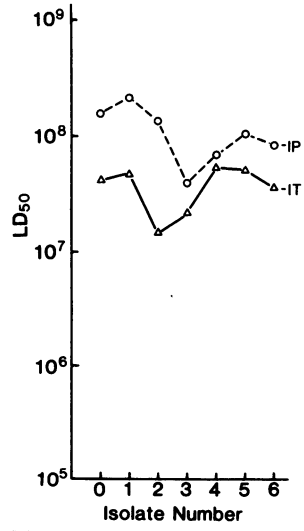

FIG. 1. *LD_{50} values of isolates harvested from passage in uncompromised mice. Bacteria in 1:5 dilutions were injected into six mice per dilution for LD_{50} determinations. Each isolate number indicates bacteria harvested at 24 h from that number of passages from livers of mice injected IP* (○) *and from lungs of mice injected IT* (△).

The LD_{50} of unpassed PAO-1 appeared to be slightly lower in the thoracic cavity than in the peritoneal cavity (Table 1), but the product of IT passage in compromised mice (IT-10, curve IT, Fig. 2) did not attain the lethality of IP-8 in compromised mice ($P < 0.05$). The increased lethality of the IT-10 isolate was more apparent in methotrexate-treated mice (curve a, Fig. 4) than in normal mice (curve c, Fig. 4) ($P < 0.05$). The lethality of the IT-10 isolate was not significantly different ($P = 0.051$) in iron-treated mice (curve b, Fig. 4) from that found in normal mice (curve c, Fig. 4), but the difference between iron-treated and methotrexate-treated mice was significant ($P < 0.05$). Although iron treatment was less effective than methotrexate treatment with IT-10 isolate, iron in combination with methotrexate (IT-10, curve IT, Fig. 2) was significantly more lethal ($P < 0.05$) than methotrexate alone (curve a, Fig. 4).

The possibility that different susceptibilities in the two sites of infection allowed different lethalities for the two passed bacteria was examined by comparing the LD_{50} values of IP-6 and IT-6 isolates injected into the alternative sites (Table 2). Isolate IP-6 injected IP in iron- and methotrexate-treated mice displayed an

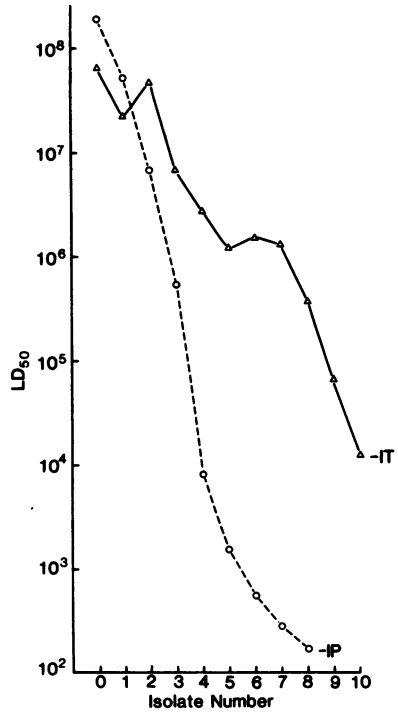

FIG. 2. *LD_{50} values of isolates harvested from passage in iron- and methotrexate-compromised mice. Isolate numbers indicate bacteria harvested at 72 h from that number of IP (○) or IT (△) passages in mice which had received 5 μmol of $FeCl_3$ and 1.25 mg of methotrexate per mouse simultaneously with bacteria.*

LD_{50} of 5.8×10^2, which differed significantly ($P < 0.05$) from iron-treated, methotrexate-treated, or untreated mice. Although there is no difference between the LD_{50} values in iron-treated and methotrexate-treated mice ($P = 0.087$), the LD_{50} in iron-treated mice is significantly different from that found in normal mice ($P < 0.05$). Therefore, the IP-6 isolate is similar in pattern of virulence in iron-treated mice to the IP-8 isolate. When the IP-6 isolate was injected IT, there were no significant differences from the IP injections in similarly treated mice ($P > 0.05$). The most dramatic example is the LD_{50} of 9.3×10^3 in iron- and methotrexate-treated mice after IT injection; this value is similar to the LD_{50} of IP-6 injected IP (5.8×10^2) and significantly different from the LD_{50} of the IT-6 isolate injected in iron- and methotrexate-treated mice (8.1×10^5). Likewise, there were no significant differences ($P > 0.05$) between the LD_{50} values of the IT-6 isolate injected either IT in mice receiving different treatments or between the LD_{50} values in IT and IP infections in similarly treated mice. The IP-6 and IT-6 isolates were also injected intravenously in a limited number of trials and again demonstrated isolate-specific LD_{50} values rather than site-specific values.

Although the environment did not have substantial effects on LD_{50} values, differences between the two environments may have selected for different isolates during passage. This possibility was investigated by passing the IT-6 isolate through IP infections in iron- and methotrexate-treated mice. The low LD_{50} value of the sixth isolate from this passage in compromised mice (curve a, Fig. 5) was not different ($P > 0.05$) from the LD_{50} of the original IP isolate (IP-6, curve IP, Fig. 2) passed through compromised mice. The lethality of the sixth isolate was greater ($P < 0.05$) in iron-treated mice (curve b, Fig. 5) and iron- and methotrexate-treated mice (curve a, Fig. 5) than in untreated mice (curve d, Fig. 5). Although methotrexate (curve c, Fig. 5) was effective in comparison to untreated mice ($P < 0.05$), there was no significant decrease in LD_{50} when methotrexate was combined with iron (curve a, Fig. 5) over the effect of iron alone.

Clearance of bacteria from iron-treated mice. The purpose of iron injections was to saturate transferrin and inhibit nutritional immunity, but $FeCl_3$ may have multiple effects on

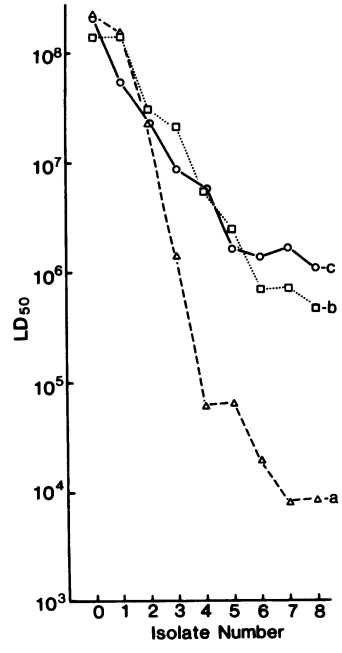

FIG. 3. *LD_{50} values of isolates harvested from IP passage in mice treated with iron and methotrexate. In addition to receiving bacteria which were the same isolates described in curve IP, Fig. 2, mice also received 5 μmol of $FeCl_3$ per mouse (△), 1.25 mg of methotrexate per mouse (□), or no treatment (○).*

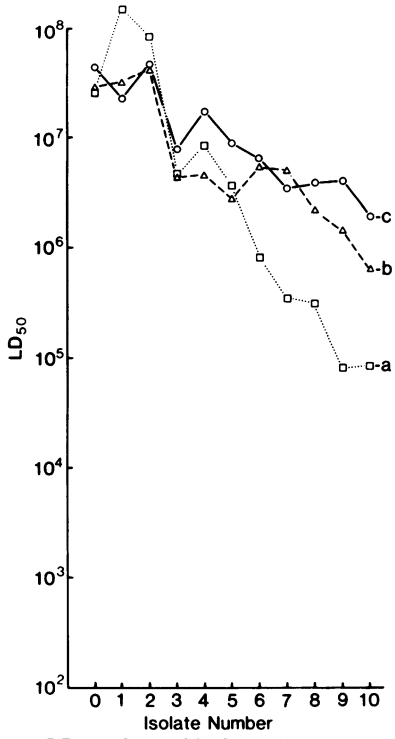

FIG. 4. *LD_{50} values of isolates harvested from IT passage in mice treated with iron and methotrexate. In addition to receiving bacteria which were the same isolates described in curve IT, Fig. 2, mice received 5 μmol of $FeCl_3$ per mouse (△), 1.25 mg of methotrexate per mouse (□), or no treatment (○).*

host defense mechanisms. To test the effects of $FeCl_3$ on clearance, bacteria were measured in heart blood after intravenous injection. The LD_{50} value of the IP-6 isolate injected intravenously was very close to that found with IP injection. Bacteria were injected at a concentration which would be lethal if injected with $FeCl_3$, but nonlethal if injected into untreated mice. The initial clearance rates in normal mice (curve a, Fig. 6) and iron-treated mice (curve c, Fig. 6) were similar. Sulfadiazine was injected to establish a bacteriostatic concentration and allow time for the mice to recover from initial effects of the injected iron. The clearance of bacteria in sulfadiazine- and iron-treated mice was equivalent to that found in normal mice and was followed by bacteriostasis for 25 h, at which time growth resumed (curve b, Fig. 6). The rate of growth at 25 h was similar to that found in mice treated with iron (curve c, Fig. 6), whereas untreated mice maintained low levels of bacteria until the termination of the experiment (curve a, Fig. 6). In a separate experiment, mice were injected with iron, but bacteria and sulfadiazine were injected 24 h later. The same demonstration of immediate clearance followed by growth at 25 h suggested that bacterial growth resulted from a decrease in sulfadiazine concentration and not from optimal effects of the iron injection at 25 h. However, it appears that $FeCl_3$ remains at levels effective for the promotion of bacterial growth for at least 25 h after IP injection.

DISCUSSION

Animal passage is a commonly used laboratory technique for increasing the virulence of pathogenic bacteria. Strain PAO-1 appears to be similar to laboratory strains of *P. aeruginosa* described by other investigators (11; Chandler and Fukui, Bacteriol. Proc. **65**:45, 1965) in being incapable of responding with increased lethality to passage through mice (Fig. 1). However, passage of strain PAO-1 through infections in compromised mice yielded isolates, IP-8 and IT-10, with increased virulence for normal mice. It is important to note that the original strain PAO-1 did not appear to be representative of an opportunistic pathogen. Compromising the defense mechanisms of mice with iron and methotrexate did not make them more susceptible to

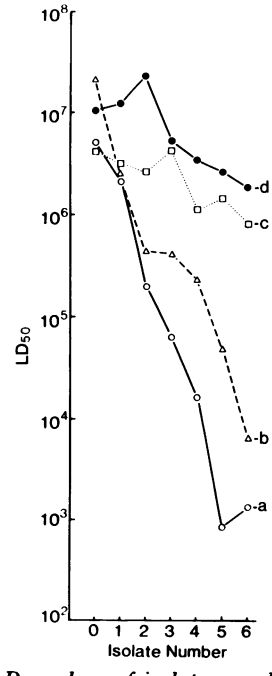

FIG. 5. *LD_{50} values of isolates resulting from IP passage in iron- and methotrexate-compromised mice of the IT-6 isolate (described by curve IT, Fig. 2, and by Fig. 4). In addition to receiving bacteria and no other treatment (●), mice were also treated with 5 μmol of $FeCl_3$ and 1.25 mg of methotrexate per mouse (○), 5 μmol of $FeCl_3$ per mouse (△), and 1.25 mg of methotrexate per mouse (□).*

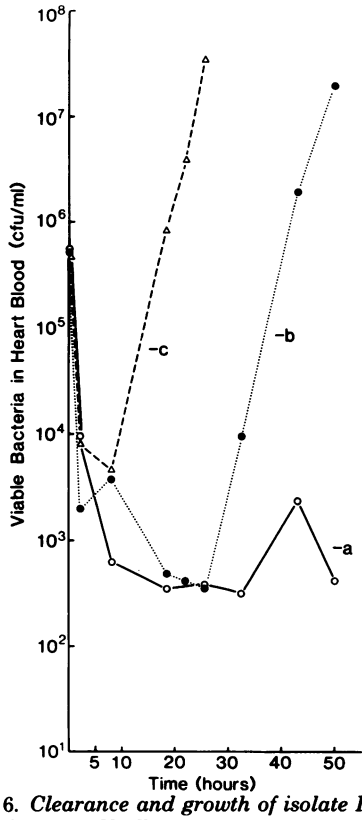

FIG. 6. *Clearance and growth of isolate IP-6 (described by curve IP, Fig. 2, and Fig. 3) measured by viable bacteria in heart blood. Mice were injected with approximately 5 × 10⁵ IP-6 bacteria and received no additional treatment* (○), *5 μmol of $FeCl_3$ per mouse* (△), *or 5 μmol of $FeCl_3$ and 1.0 mg of sulfadiazine per mouse* (●).

strain PAO-1 (Table 1), but, after passage in compromised mice, the IP-8 and IT-10 isolates were more typical of opportunistic pathogens in being more lethal for compromised mice (Fig. 2, 3, and 4). It is also important that the lethality of the IP-8 isolate appeared to be similar to freshly isolated strains from human infections (4, 7) which possessed LD_{50} values for mice in the range of 10^6 and demonstrated lower LD_{50} values for iron-treated mice. It is not clear why initial LD_{50} values of human isolates were low in comparison with laboratory strains (11; Chandler and Fukui, Bacteriol. Proc. **65**:45, 1965). A previous report indicated that virulence is specific for the mammalian species of passage; a bacterium from passage in rabbits did not show increased virulence for mice (4). However, low initial LD_{50} values (10^6 to 10^7 with intraperitoneal injection) in mice have also been observed in this laboratory with *P. aeruginosa* from human burns.

Intraperitoneal injections and passage were used in these studies because this is the site which has been used most often to demonstrate the effects of iron on bacterial virulence (4, 7). The original intent of IT passage was to obtain an isolate adapted to growth in the lung. Mouse passage subjected the bacteria to selective environments, and it appears that distinct selective pressures in the peritoneal cavity or liver and in the thoracic cavity or lung yielded different isolates. Although continued passage of the IT-10 isolate may result in comparable lethality with the IP-8 isolate, there are distinct differences between the IP-8 and the IT-10 isolates in mice receiving iron treatments. Iron treatment was the most effective compromising agent for the IP-8 isolate but not for the IT-10 isolate. Methotrexate was most effective for the IT-10 isolate. Although one treatment was more effective than the other for the lethality of each isolate, the combination of the two compounds was more effective for both isolates.

The indication that the isolates are different came from the finding of isolate-specific virulence characteristics which were affected negligibly by the site of infection. The IP-6 isolate retained low LD_{50} values in iron-treated mice when injected IT (Table 2). If the low LD_{50} value of the IP-6 isolate had been due to the site of infection, the IT-6 isolate would also have had a lower LD_{50} when injected IP. An LD_{50} in the range of 10^6 for the IT-6 isolate for both sites suggested that the peritoneal cavity is not simply more susceptible to infection. This phenomenon of adaptation to particular sites of infection may be characteristic of certain noninvasive and opportunistic pathogens.

The effects of the selective pressures which resulted in the IP-8 isolate were demonstrated by the acquisition of virulence similar to IP-6 by the IT-6 isolate when it was passed through six infections IP in compromised mice (Fig. 5). Al-

TABLE 2. *Lethality of passage 6 P. aeruginosa PAO-1*

Strain of passage 6	Treatment of mice	LD_{50} values	
		IP[a]	IT[a]
IP	Untreated	1.4×10^6	1.6×10^6
	Iron	1.9×10^4	6.9×10^4
	Methotrexate	7.0×10^5	2.4×10^6
	Methotrexate and iron	5.8×10^2	9.3×10^3
IT	Untreated	8.6×10^6	6.2×10^6
	Iron	8.9×10^6	5.3×10^6
	Methotrexate	1.1×10^7	1.5×10^6
	Methotrexate and iron	4.2×10^6	8.1×10^5

[a] Route of injection.

though iron was not significantly involved in the virulence of IT-6, the sixth product of IP passage was most lethal in mice injected with iron. The acquisition of lethality in iron-treated mice was not due to the proximity of IP-injected iron. Intrathoracic passage of strain PAO-1 in compromised mice with 200 nmol of $FeCl_3$ injected IT yielded an isolate after six passes with an LD_{50} value in iron-treated mice of 1.6×10^6 with no significant difference from the IT-6 isolate (curve IT, Fig. 2).

Although there were no apparent effects of IT- or IP-injected iron on the lethality of IT isolates or unpassed bacteria, there may still be effects of iron on phagocytic cells. Studies on bacterial clearance of the IP-6 isolate suggested that iron injection did not affect clearance (Fig. 6). Sulfadiazine was used to inhibit bacterial growth in mice, but viable numbers of bacteria remained at similar levels in the presence and absence of iron until 25 h. Growth at 25 h in the iron-treated mice occurred because of the falling concentration of sulfadiazine, and it appears that there was sufficient iron at 25 h after injection to enhance bacterial growth.

Enhancement of the virulence of passed bacteria by iron treatment of animals has been reported for isolates from human infections (4, 7). This report extends the knowledge of increased virulence due to animal passage to a laboratory strain of *P. aeruginosa*. It is pertinent that the site of infection is an important factor in the selection of virulent isolates of this bacterium. The molecular bases of virulence of the IP-8 and IT-10 isolates are not known. Findings of particular O serotypes in multiple patients over long periods (1, 15) and findings by this laboratory of particular pyocin types in burn patients over periods of months suggest that the proper conditions for bacterial passage through compromised humans exist in hospitals. Experiments are underway to determine the bases of virulence expressed by the passed isolates of PAO-1 and to compare these bacteria with strains from human infections.

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LITERATURE CITED

1. **Baltimore, R. S., A. S. Dobek, F. R. Stark, and M. S. Artenstein.** 1974. Clinical and epidemiological correlates of *Pseudomonas* typing. J. Infect. Dis. **130**:553–559.
2. **Berk R. S.** 1977. Experimental mouse infections caused by *Pseudomonas aeruginosa*, p. 169–172. *In* D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.
3. **Bobo, R. A., E. J. Newton, L. F. Jones, L. H. Farmer, and J. J. Farmer.** 1973. Nursery outbreak of *Pseudomonas aeruginosa*: epidemiological conclusions from five different typing methods. Appl. Microbiol. **25**:414–420.
4. **Bullen, J. J., C. G. Ward, and S. N. Wallis.** 1974. Virulence and the role of iron in *Pseudomonas aeruginosa* infection. Infect. Immun. **10**:443–450.
5. **Feingold, D. S.** 1970. Hospital acquired infections. N. Engl. J. Med. **283**:1384–1391.
6. **Fishman, L. S., and D. Armstrong.** 1972. *Pseudomonas aeruginosa* bacteremia in patients with neoplastic disease. Cancer **30**:764–773.
7. **Forseberg, C. M., and J. J. Bullen.** 1972. The effect of passage and iron on the virulence of *Pseudomonas aeruginosa*. J. Clin. Pathol. **25**:65–68.
8. **Holloway, B. W.** 1955. Genetic recombination in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **13**:572–581.
9. **Jones, L. F., J. P. Zakanycz, E. T. Thomas, and J. J. Farmer.** 1974. Pyocin typing of *Pseudomonas aeruginosa*: a simplified method. Appl. Microbiol. **27**:400–406.
10. **Liu, P. V., and C. B. Mercer.** 1963. Growth, toxigenicity and virulence of *Pseudomonas aeruginosa*. J. Hyg. Camb. **61**:485–491.
11. **Martin, C. M., J. H. Handl, and M. Finland.** 1963. Enhancement of acute bacterial infections in rats and mice by iron and their inhibition by human transferrin. J. Infect. Dis. **112**:158–163.
12. **Pruitt, B. A.** 1974. Infections caused by *Pseudomonas* species in patients with burns and in other surgical patients. J. Infect. Dis. **130**:S8–S13.
13. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
14. **Weinberg, E. D.** 1974. Iron and the susceptibility to infectious disease. Science **184**:952–956.
15. **Young, V. M., and M. R. Moody.** 1974. Serotyping *Pseudomonas aeruginosa*. J. Infect. Dis. **130**:S47–S52.