Prophylaxis and Therapy for *Pseudomonas aeruginosa* Infection with Carbenicillin and with Gentamicin

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The polymixins have for many years been the antibiotics of choice for the treatment of infection with *Pseudomonas aeruginosa*; but although they are active against all strains in vitro their clinical value has been limited. In the treatment of burns, local applications were found to have prophylactic value (Jackson, Lowbury, and Topley, 1951; Cason and Lowbury, 1960), but systemic administration has been less effective in the control of invasive infection (Tumbusch *et al.*, 1961; Kefalides *et al.*, 1964; Jones, Jackson, and Lowbury, 1966).

Two new antibiotics with promising activity against *Ps. aeruginosa* have recently been developed. Gentamicin (Weinstein *et al.*, 1963) is a broad-spectrum antibiotic which is reported to have good activity in vitro and therapeutic value in patients infected with *Ps. aeruginosa* (Bulger, Sidell, and Kirby, 1963; Klein, Eickhoff, and Finland, 1964; Barber and Waterworth, 1966). Carbenicillin (B.R.L. 2064) is a semi-synthetic penicillin with moderate activity in vitro against *Ps. aeruginosa*.

In this paper we report studies which showed that parenteral administration of carbenicillin and of gentamicin protected burned mice against invasive infection with strains of *Ps. aeruginosa*. The use of carbenicillin in the treatment of patients whose burns were infected with *Ps. aeruginosa* was studied in a small controlled trial.

Materials and Methods

Assessments of Prophylaxis and Therapy

Protection of burned mice against virulent strains of *Ps. aeruginosa* was assessed by methods described elsewhere (Jones and Lowbury, 1965; Jones *et al.*, 1966). Mice were anaesthetized with ether and given full-skin-thickness burns of approximately 5% of the body surface. Three hours after burning, the surface of the burned area was inoculated with approximately 700 million viable cells of *Ps. aeruginosa*.

Treatment with antibiotics was started, firstly, immediately after burning and before inoculation with *Ps. aeruginosa*, to assess their prophylactic value, and, secondly, 24 hours, 48 hours, and 72 hours after inoculation with *Ps. aeruginosa*, to assess the therapeutic value of the antibiotics. A group of six burned infected mice was included in each experiment as a control.

All mice were examined and weighed daily for a period of 14 days. Bacterial cultures on blood agar and cetrimide agar (Brown and Lowbury, 1965) were obtained on alternate days from burns of mice receiving the highest dosage of antibiotics. Necropsy examinations, which included cultures of heart blood, were made on all mice that died.

Strains of *Ps. aeruginosa*

Two strains, B4 and P14, which had previously been found virulent for the mouse and which differed in serotype, were used in the experiments. The saline suspensions inoculated on burns were prepared from overnight cultures on nutrient agar. Strains rendered resistant to the antibiotics by habituation in vitro (see below) were also tested.

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Antibiotic Treatment

The antibiotics were administered, in separate experiments, by intraperitoneal and intramuscular routes. Two injections were given each day (at 10 a.m. and 5 p.m.) for five days, and one injection (full dosage) on days 6 and 7. Dosage was adjusted in relation to the surface area of the animal, estimated from its weight (Bushby, 1964; Butler and Richie, 1960); on this basis approximately 660 and 1,000 mg. of carbenicillin per kg. per day and 12 and 18 mg. of gentamicin per kg. per day were selected for treatment of mice. Carbenicillin was also tested at lower dosages (approximately 330 and 160 mg./kg./day). Colistin sulphomethate was tested at 40 and 400 mg./kg./day; in earlier experiments (Jones *et al.*, 1966) approximately 80 mg./kg./day of colistin sulphomethate had been used.

Levels of antibiotics in the serum of burned and of unburned mice were obtained from assays on pooled serum of groups of three mice taken at intervals of half-hour, three hours, and six hours after intraperitoneal administration of the antibiotics. The assay was by a tube dilution method with a sensitive strain of *Staphylococcus aureus* (Oxford) for gentamicin and of *Ps. aeruginosa* (Elsworth) and (in earlier tests) of *Staph. aureus* (Oxford) for carbenicillin; a strain of *Ps. aeruginosa* (B4) was also used for assay of colistin sulphomethate. The inoculum was 0.02 ml. of a 1/1,000 dilution of a broth culture of the test organism.

A group of mice was also treated prophylactically with methicillin (5,000 mg./kg./day).

Mixed Infection with Penicillinase-producing Organisms

Mixed infections with *Ps. aeruginosa* and *Staph. aureus* are common in burned patients. To determine whether the presence of penicillinase-producing bacteria interferes with the protective value of carbenicillin against *Ps. aeruginosa*, prophylaxis with the antibiotic was assessed in groups of mice infected by the application to burns of *Ps. aeruginosa* (B4 and P14) and a similar inoculum of a penicillinase-producing strain of *Staph. aureus* (1899).

Antibiotic Sensitivity Tests

Strains of *Ps. aeruginosa* were tested for sensitivity to carbenicillin, gentamicin, and colistin sulphomethate by a tube dilution method; the inoculum was 0.02 ml. of 10^{-3} dilutions of overnight broth cultures in nutrient broth containing doubling dilutions of the antibiotic.

Tests for Inactivation of Carbenicillin by Penicillinase

Penicillinase-producing strains of *Staph. aureus* (B/4324 and B/4327) were inoculated in nutrient broth containing carbenicillin (1,000 µg. per ml.); after incubation for two hours at 37° C. the culture was filtered through a Hemmings filter and tested for residual activity by diffusion from Heatley cylinders on blood agar plates seeded with sensitive strains of *Staph. aureus* (Oxford strain) and *Ps. aeruginosa* (strain B4) (Lowbury, Topley, and Hood, 1952).

Tests for Production of Penicillinase by *Ps. aeruginosa*

Strains of *Ps. aeruginosa*, including those which had acquired resistance to carbenicillin in vitro, were tested for diffusible penicillinase by estimation of residual penicillin after incubation for two hours of fresh cultures in infusion broth containing 100 µg. per ml. of carbenicillin and 100 units per ml. of penicillin G; filtrates were tested on blood-agar plates seeded with *Staph. aureus* (Oxford strain).

Habituation of *Ps. aeruginosa* to the Antibiotics

Strains of *Ps. aeruginosa* (B4 and P14) were tested for the emergence of resistant variants by serial subculture in tubes of broth containing ranges of doubling dilutions of carbenicillin and gentamicin; daily subcultures were made to new test ranges from the tubes containing the highest concentration of antibiotics which did not inhibit growth. Tests for stability of resistance acquired in these strains were made by subculture in the absence of the antibiotics.

Results

Sensitivity of Strains.—Table I shows the distribution of minimal inhibitory concentrations (M.I.C.) of carbenicillin, gentamicin, and colistin sulphomethate for 80 miscellaneous strains of *Ps. aeruginosa*, mostly from burns, and 26 typed strains, including 14 distinct serotypes. With one exception, the M.I.C.s of carbenicillin were in the range 32–128 µg./ml.; the M.I.C. of gentamicin was in the range 1 to 4 µg./ml., and that of colistin sulphomethate was from 1 to 8 µg./ml. Two strains used in the studies on mice (B4 and P14) were inhibited by carbenicillin at M.I.C.s of 64 and 128 µg./ml. and by gentamicin at 0.5 and 4 µg./ml.; both strains were inhibited by colistin sulphomethate at an M.I.C. of 4 µg./ml. The M.I.C.s of methicillin for two strains of *Ps. aeruginosa* were 1,000 and 4,000 µg. per ml.

TABLE I.—Sensitivity of 106 Strains of *Ps. aeruginosa* to Three Antibiotics

Antibiotic	Strains	No. of Strains Inhibited by M.I.C. (µg./ml.)							Total Strains
		128	64	32	16	8	4	2	
Carbenicillin	Miscellaneous	11	47	21	0	0	0	0	80
	Type strains	5	21	0	0	0	0	0	26
Gentamicin	Miscellaneous	0	0	0	0	0	17	48	80
	Type strains	0	0	0	0	0	13	12	26
Colistin sulphomethate	Miscellaneous	0	0	0	0	7	34	33	80
	Type strains	0	6	0	0	1	11	12	26

* Elsworth strain.

Inactivation of Carbenicillin by Penicillinase.—Penicillinase-producing strains of *Staph. aureus* were found to destroy the activity of carbenicillin against both *Staph. aureus* and *Ps. aeruginosa*.

Prophylaxis against *Ps. aeruginosa*.—The results summarized in Table II show that mice infected with *Ps. aeruginosa* (B4 and P14) by application of the organism to fresh burns were protected against invasion and fatal septicaemia when courses of prophylactic treatment with carbenicillin were given. Intraperitoneal and intramuscular injections were equally effective. Similar treatment with gentamicin prevented invasion and death when the infecting strain was B4, but it had a smaller effect against P14, which was less sensitive to gentamicin. Colistin sulphomethate, on the other hand, showed little or no prophylactic effect at any of the three dosage levels tested, including those reported earlier (Jones *et al.*, 1966), and methicillin was also ineffective. The burns of the infected mice in all series appeared red, with some areas of moisture. Heavy growths of *Ps. aeruginosa* were isolated from swabs taken from the burns of infected mice, whether treated with antibiotics or not. At necropsy the infected mice showed pathological

changes described elsewhere (Jones *et al.*, 1966), including usually the presence of *Ps. aeruginosa* in blood from the heart.

TABLE II.—Prophylaxis by Antibiotics Against *Ps. aeruginosa* Infection in Burned Mice

Treatment Group	Infecting Strain of <i>Ps. aeruginosa</i>	Dosage of Antibiotic (mg./kg./day)	Route of Administration	No. of Mice			Mean Survival Time of mice which died (Days)	<i>Ps. aeruginosa</i> in Blood Culture at Necropsy
				Died	Survived	% Deaths		
Controls: Burned uninfected	—	—	—	3	63	4.5	3.0	—
	Burned infected	—	—	87	33	72.5	5.2	86
Carbenicillin	B4	1,000	IP	0	11	0*	—	—
	B4	660	IP	2	8	20*	12.5	1
	B4	330	IP	5	4	55	6.0	5
	B4	165	IP	6	4	60	5.0	6
	B4	1,000	IM	0	12	0*	—	—
	P14	1,000	IP	1	10	9.0†	(14.5)	1
	P14	660	IP	0	12	0†	—	—
	P14	660	IP	1	12	8.3‡	—	—
Gentamicin	B4	12	IP	1	11	8.0‡	(4.0)	1
	B4	12	IM	0	12	0‡	—	—
	P14	18	IP	4	8	33.3§	8.5	4
	P14	12	IP	7	5	58	3.8	6
Colistin sulphomethate	B4	40	IP	8	4	67	5.0	8
	B4	400	IP	5	1	83	2.4	4
	B4	40	IM	6	6	50	5.7	6
	P14	40	IP	7	5	58	3.8	6
Methicillin	B4	5,000	IP	11	1	92	4.5	11

Significance tests.—The results of experiments marked with the same footnote symbol are grouped together; they were compared with the results in untreated mice infected with the same strain of *Ps. aeruginosa*. * $\chi^2=48.4$, $P<0.001$ (strain B4). † $\chi^2=24.5$, $P<0.001$ (strain P14). ‡ $\chi^2=42.3$, $P<0.001$ (strain B4). § $\chi^2=3.8$, $P=0.05$ (strain P14).

Blood Levels of the Antibiotics.—The concentration of antibiotics in the pooled serum from groups of burned and unburned mice bled at half an hour, three hours, and six hours after administration of a single dose are shown in Table III; inhibitory levels of carbenicillin and of gentamicin were found in the first sample. In mice which had recently received 5% burns the serum level of gentamicin in relation to dosage was high compared with that of carbenicillin. In tests with unburned mice, serum levels half an hour after injection of gentamicin were much lower. No difference between burned and unburned mice was found in serum levels of carbenicillin. The serum from mice treated with colistin sulphomethate did not contain detectable levels of the antibiotic.

TABLE III.—Assays of Antibiotics in Serum of Mice After Intraperitoneal Administration

Antibiotic	Mice	Dosage (mg./kg./dose)	Concentration of Antibiotics in Serum (µg./ml.)		
			½ Hour after Dose	3 Hours after Dose	6 Hours after Dose
Carbenicillin	Burned	500	128	16	<2
	Unburned	330	256	32	<2
	Unburned	500	128	8	>2
Gentamicin	Burned	9	128	8	—
	Unburned	6	64	2	<0.5
	Unburned	6	4	<2	<1
Colistin sulphomethate	Burned	20	<2.6	<2.6	<2.6
	Unburned	20	<1.3	<1.3	<1.3

TABLE IV.—Therapeutic Effects of Carbenicillin and Gentamicin in Burned Mice Infected with *Ps. aeruginosa*

Treatment Group*	Infecting Strain of <i>Ps. aeruginosa</i>	Time when Treatment was Started (Hours after Inoculation with <i>Ps. aeruginosa</i>)	No. of Mice which		% Deaths
			Died	Survived	
Carbenicillin	B4	24	1	5	17
	B4	48	6	6	50
	B4	72	4	2	67
	B4	24	0	6	0
Gentamicin	B4	48	4	8	33
	B4	72	4	2	67

* Dosage.—Carbenicillin: 1,000 mg./kg./day, IP, for 7 days. Gentamicin: 12 mg./kg./day, IP, for 7 days.

Use of Carbenicillin and Gentamicin on Mice with Burns Already Infected.—When courses of treatment with carbenicillin or gentamicin were started 24 hours after inoculation of burns with *Ps. aeruginosa* (strain B4) the survival rate was as high or almost as high as it was in the series given prophylaxis (Table IV). When longer delays occurred before treatment was started a larger proportion of mice died with infection.

Prophylaxis by Carbenicillin against Ps. aeruginosa in the Presence of Penicillinase-producing Staphylococci.—In mice protected with carbenicillin (1,000 mg./kg./day) there were three deaths out of 12 with invasive *Ps. aeruginosa* infection when a penicillinase-producing *Staph. aureus* was also inoculated on the burn (Table V); in those inoculated with *Ps. aeruginosa* alone and similarly protected there were two deaths out of 33. *Ps. aeruginosa*, but not *Staph. aureus*, was found in the blood culture of the mice which died.

TABLE V.—Prophylaxis by Carbenicillin in Burned Mice Infected with *Ps. aeruginosa* and a Penicillinase-producing *Staph. aureus*

Treatment Group	Infecting Strains of		No. of Mice which			<i>Ps. aeruginosa</i> in Blood Culture at Necropsy
	<i>Ps. aeruginosa</i>	<i>Staph. aureus</i>	Died	Survived	% Deaths	
No antibiotic Carbenicillin	B4	1899	3	3	50	3
	P14	1899	5	1	83	4
	B4	1899	1	5	17	1
	P14	1899	2	4	33	2

Habituation of Ps. aeruginosa to Carbenicillin and Gentamicin in vitro.—Strains of *Ps. aeruginosa* showed moderate increases of resistance on repeated subcultures in subinhibitory concentrations of the antibiotics. For strain B4, increases in M.I.C. from 64 to 1,024 µg./ml. of carbenicillin and from 0.5 to 4 µg./ml. of gentamicin were obtained; increases in resistance of strain P14 were from 128 to 2,048 µg./ml. carbenicillin and from 4 to 64 µg./ml. of gentamicin. The strain rendered resistant to carbenicillin grew as small colony variants which produced oxidase and grew on cetrimide agar, but showed poor fluorescence; unlike the sensitive parent strain, they produced a diffusible penicillinase which inactivated benzylpenicillin. There was no reversion to sensitivity on repeated subculture in the absence of carbenicillin. The strains rendered resistant to gentamicin were also of small colony type, but they produced moderate amounts of fluorescein; reversion to sensitivity on subculture did not occur.

Tests with burned mice (Table VI) showed that variants resistant to carbenicillin were avirulent, while those which were resistant to gentamicin appeared to have diminished virulence. Unlike the sensitive parent strains, they were not found on the burns of mice later than on the day of inoculation.

TABLE VI.—Mortality of Burned Mice Infected with Strains of *Ps. aeruginosa* Rendered Resistant in vitro to Carbenicillin and to Gentamicin

Infecting Strain of <i>Ps. aeruginosa</i>	Sensitivity Pattern	No. of Mice which			<i>Ps. aeruginosa</i> in Blood Culture at Necropsy
		Died	Survived	% Deaths	
B4	Sensitive to carbenicillin and gentamicin	87	33	72.5	86
	Resistant to carbenicillin	0	6	0	—
P14	Sensitive to carbenicillin and gentamicin	41	19	64	41
	Resistant to carbenicillin	0	6	0	—
	Resistant to gentamicin	3	3	50	2

Administration of Carbenicillin to Patients with Burns

To assess the value of carbenicillin in the treatment of burns infected with *Ps. aeruginosa* a controlled therapeutic trial was started. On the first appearance of *Ps. aeruginosa* in cultures from one or more burns, patients were allocated alternately to treatment and control groups. Those in the treatment group were given a seven-day course of carbenicillin (in adults 1 g. six-hourly by intramuscular injection, with probenecid 0.5 g. six-hourly). Local treatment of the burns was with a cream

containing 0.5% silver nitrate and 0.1% chlorhexidine, or with penicillin cream, or with neomycin chlorhexidine tulle gras; in previous experience none of these methods had been effective in removing *Ps. aeruginosa* from burns. The burns were swabbed at changes of dressings (usually every two or three days) and examined for *Ps. aeruginosa* and other bacteria as described elsewhere (Lowbury, 1960; Brown and Lowbury, 1965).

Because of recent success in prophylaxis against *Ps. aeruginosa* in this unit, especially with silver nitrate compresses and cream (Cason *et al.*, 1966), the number of patients available for study in this trial has been very small.

An interim analysis showed that 10 out of 23 burns in nine patients treated with carbenicillin lost *Ps. aeruginosa* during or immediately after the course of treatment. Out of 23 burns in the seven patients of the control series, only one showed the loss of *Ps. aeruginosa* in a corresponding period of time. There was a significant difference in the proportion of burns from which *Ps. aeruginosa* was eliminated in the two groups of the trial ($\chi^2 = 6.3$, $P < 0.02$).

In addition to these patients two others were treated with carbenicillin because the heavy colonization of their burns with *Ps. aeruginosa* was thought to be causing severe clinical infection (not septicaemic). One patient was a man aged 62 with burns of the legs (approximately 12% of the body surface, mostly partial skin loss); he was admitted from another hospital 14 days after injury with cellulitis of both legs, and was severely ill, with confusion, rapid irregular pulse, signs of bronchitis, intestinal distension, and loose green stools; blood examination showed a haemoglobin level of 9.9 g./100 ml., a leucocytosis (W.B.C. 18,000, polymorphonuclear neutrophils 87%, lymphocytes 9%, monocytes 4%), E.S.R. 60 mm./hour (Wintrobe), and a blood urea of 135 mg./100 ml. A heavy growth of *Ps. aeruginosa* was isolated from the burns in pure culture. Blood cultures were sterile, but the severity of the patient's illness was considered to be associated with infection by *Ps. aeruginosa*.

During a course of treatment with carbenicillin (continued for three weeks) the patient's condition improved rapidly, and *Ps. aeruginosa* disappeared from six out of eight of the burns. Assays of carbenicillin in the serum while the patient had renal insufficiency showed 16 µg./ml. at one hour, 32 µg./ml. at three hours, and 64 µg./ml. at five hours after the injection of a dose of the antibiotic. At a later stage, when the kidney function had recovered, serum levels of carbenicillin were lower (16 µg./ml. at three hours and at six hours after administration of the drug).

Another patient with burns heavily colonized with *Ps. aeruginosa* and having normal kidney function was given carbenicillin for seven days with probenecid (2 g./day). Assays of the drug in the patient's serum showed levels of 48 µg./ml. at one hour, 32 µg./ml. at three hours, and 32 µg./ml. at five hours after a dose of carbenicillin. Two of the three burns were cleared of *Ps. aeruginosa* and one showed a reduction in their numbers during the course of treatment.

Sensitivity of *Ps. aeruginosa* to Carbenicillin during the Trial

In two of the eleven patients treated with carbenicillin *Ps. aeruginosa* isolated after the course of treatment was more resistant (M.I.C. 265 µg./ml.) than the strain initially present (M.I.C. 64 µg./ml.). Tests on the "resistant" strains from one of these patients showed no penicillinase production. The phage type of the more resistant variant was the same as that of the strain present before treatment.

Discussion

Infection of burns with *Ps. aeruginosa* is commonly acquired in hospital. Usually the organism remains localized, colonizing

slough and exudate, with no ill effect except some interference with skin grafts and consequent delays in healing (Jackson *et al.*, 1951). Occasionally it causes invasive infection with cellulitis, invasion of the walls of small blood vessels, and in some cases septicaemia (Fraenkel, 1906; Jackson *et al.*, 1951; Rabin *et al.*, 1961; Teplitz *et al.*, 1964a, 1964b; Sevvit, 1964).

This complication is often fatal, and antibiotic treatment is likely to fail even when it removes the organism from the lesions, because of irreparable damage which has occurred by the time invasion is recognized (Jones *et al.*, 1966). For this reason it is important that high-risk cases should receive effective prophylaxis. Silver nitrate compresses (Moyer *et al.*, 1965; Cason, Jackson, Lowbury, and Ricketts, 1966) and isolators (Cason *et al.*, 1966) are useful barriers against contamination, but they are not invariably successful. A second line of defence for such cases is systemic prophylaxis by antisera or by antibiotics.

The two antibiotics studied in this paper, like the specific antisera recently described by Jones *et al.* (1966), protected mice against invasion by *Ps. aeruginosa*. All strains of *Ps. aeruginosa* which were tested were at least moderately sensitive to carbenicillin and highly sensitive to gentamicin; variants which became resistant to carbenicillin on habituation *in vitro* had lost virulence for mice, and an apparent reduction in virulence was found also in gentamicin-resistant variants obtained in this way.

Gentamicin is more active than carbenicillin *in vitro* against *Ps. aeruginosa*, and smaller doses are recommended for treatment. Carbenicillin on the other hand, though only moderately active *in vitro*, was more successful than might have been expected in its prophylactic action against *Ps. aeruginosa* in mice.

In burned patients treated with carbenicillin and probenecid the highest blood levels were rarely higher than the minimal inhibitory concentration for the infective strains; nevertheless, a significantly larger proportion of burns were cleared of *Ps. aeruginosa* in patients treated with carbenicillin than in the untreated controls. This clearance was associated with persistence of or replacement by other organisms in the burns.

Study on a larger series of patients is needed for an assessment of this unexpectedly favourable result; meanwhile it is possible that the local application of silver nitrate and chlorhexidine, though ineffective by itself, may have contributed to the effect of systemic treatment with carbenicillin against *Ps. aeruginosa*.

None of the patients studied in the trial had septicaemia; a considerably higher dosage would undoubtedly be required for successful treatment in such cases.

Gentamicin was less effective than carbenicillin in prophylaxis against the less sensitive of the two strains tested, though the dosage was sufficient to give inhibitory serum levels in the mice; gentamicin (unlike carbenicillin) was found in much higher concentration in the serum of mice which had recently been burned than in unburned mice. The combined use of carbenicillin and gentamicin, like that of gentamicin and ampicillin (Bulger and Kirby, 1963), might be expected to have a synergistic action and deserves study. Both agents may be useful in the protection of patients treated with cytotoxic drugs and other high-risk cases.

The failure of colistin sulphomethate to protect mice was associated with an absence of detectable colistin in the serum of mice receiving treatment with the antibiotic. Kitamoto, Fukaya, and Honma (1962) have also reported an absence of colistin in the serum of treated mice, though the antibiotic was detected in the kidneys at necropsy. In burned patients, however, appreciable blood levels and evidence of some therapeutic value from very large dosage of colistin sulphomethate have been reported (Jones *et al.*, 1966).

Summary

A series of 106 strains of *Pseudomonas aeruginosa* were sensitive in tube dilution tests to gentamicin (M.I.C. 1 to 4 µg./ml.) and moderately sensitive to a new semisynthetic penicillin, carbenicillin (M.I.C. 32 to 128 µg./ml.); one strain was found to be more sensitive to carbenicillin. Carbenicillin was inactivated by the penicillinase of *Staph. aureus*.

Groups of mice were infected by inoculation of small burns with two strains of *Ps. aeruginosa*; about 70% of the mice infected with these strains died with septicaemia. Courses of treatment with carbenicillin gave serum levels in burned or unburned mice up to 128 µg./ml., and the mice were completely protected against both strains. Gentamicin gave peak blood levels in recently burned mice of 64–128 µg./ml. but lower levels (4–8 µg./ml.) in unburned mice; gentamicin protected the animals against the more sensitive strain of *Ps. aeruginosa* only (M.I.C. 0.5–1 µg./ml.). Similar results were obtained with treatment by carbenicillin or by gentamicin, starting at the time of infection or 24 hours later. In most experiments colistin sulphomethate gave no evidence of protection.

A small controlled trial in progress on patients with burns has shown that *Ps. aeruginosa* was removed from a significantly larger proportion (10/24) of burns in patients treated with carbenicillin and probenecid than in untreated controls (1/23). The highest blood levels of carbenicillin (48–64 µg./ml.) were in the same range as its minimal inhibitory concentration against the infecting strains.

A strain of *Ps. aeruginosa* which acquired resistance to carbenicillin *in vitro* was converted to a small colony form and showed a loss of virulence for burned mice. In a similar experiment with gentamicin the resistant variants were also small colony forms, but retained some virulence for mice.

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REFERENCES

- Barber, M., and Waterworth, P. M. (1966). *Brit. med. J.*, 1, 203.
- Brown, V. I., and Lowbury, E. J. L. (1965). *J. clin. Path.*, 18, 752.
- Bulger, R. J., and Kirby, W. M. M. (1963). *Amer. J. med. Sci.*, 246, 717.
- , Sidell, S., and Kirby, W. M. M. (1963). *Ann intern. Med.*, 59, 593.
- Bushby, S. R. M. (1964). In R. J. Schnitzer and F. Hawking's *Experimental Chemotherapy*, 1, 50. New York.
- Butler, A. M., and Richie, R. H. (1960). *New Engl. J. Med.*, 262, 903.
- Cason, J. S., Jackson, D. M., Lowbury, E. J. L., and Ricketts, C. R. R. (1966). *Brit. med. J.*, 2, 1288.
- , and Lowbury, E. J. L. (1960). *Lancet*, 2, 501.
- Feller, I. (1965). *Second International Congress for Research in Burns*, Edinburgh, p. 56.
- Fraenkel, E. (1906). *Virchows Arch. path. Anat.*, 183, 405.
- Jackson, D. M., Lowbury, E. J. L., and Topley, E. (1951). *Lancet*, 2, 137.
- Jones, R. J., and Lowbury, E. J. L. (1965). *Lancet*, 2, 623.
- , Jackson, D. M., and Lowbury, E. J. L. (1966). *Brit. J. plast. Surg.*, 19, 43.
- Kefalides, N. A., Arana, J. A., Bazan, A., Velarde, N., and Rosenthal, S. M. (1964). *Ann. Surg.*, 159, 496.
- Kitamoto, O., Fukaya, K., and Honma, H. (1962). *J. Antibiotics (Tokyo)*, Series B, 15, 7. (Cited in R. J. Schnitzer and F. Hawking's *Experimental Chemotherapy*, 1964, 3, 217. New York.)
- Klein, J. O., Eickhoff, T. C., and Finland, M. (1964). *Amer. J. med. Sci.*, 248, 528.
- Lowbury, E. J. L. (1960). *Brit. med. J.*, 1, 994.
- , Topley, E., and Hood, A. M. (1952). *Lancet*, 1, 1036.
- Moyer, C. A., Brentano, L., Gravens, D. L., Margraf, H. W., and Monafio, W. W. (1965). *Arch. Surg.*, 90, 812.
- Rabin, E. R., Graber, C. D., Vogel, E. H., Finkelstein, R. A., and Tumbusch, W. A. (1961). *New Engl. J. Med.*, 265, 1225.
- Sevvit, S. (1964). *Acta chir. plast.*, 6, 173.
- Teplitz, C., Davis, D., Mason, A. D., and Moncrief, J. A. (1964a). *J. surg. Research*, 4, 200.
- , Walker, H. L., Raulston, G. L., Mason, A. D., and Moncrief, J. A. (1964b). *Ibid.*, 4, 217.
- Tumbusch, W. J., Vogel, E. H., Butkiewicz, J. V., Graber, C. D., Larson, D. L., and Mitchell, E. T. (1961). *J. Trauma*, 1, 22.
- Weinstein, M. J., Luedemann, G. M., Oden, E. M., and Wagman, G. H. (1963). *Antimicrobial Agents and Chemotherapy*, p. 1.