

CHAPTER III

RESULTS



1. Effect of antibiotics on the growth of bacteria.

The minimal inhibitory concentrations of ampicillin and gentamicin on *Staphylococcus aureus* and *Pseudomonas aeruginosa* were determined and shown in Table 3.

Table 3 MICs of ampicillin and gentamicin by broth dilution method.

Organisms	Antibiotics	
	Ampicillin	Gentamicin
<i>S. aureus</i>	0.06 $\mu\text{g/ml}$	1.0 $\mu\text{g/ml}$
<i>P. aeruginosa</i>	1.8 mg/ml	2.0 $\mu\text{g/ml}$

1.1 *Staphylococcus aureus* and ampicillin

After incubation in the presence of minimal inhibitory concentration of ampicillin in TSB for 4, 16 and 24 hr, the viable cell numbers of *S. aureus* were significantly decreased ($p < 0.01$) while the incubation time increased, as shown in Table 4, page 37, and most of the cells were killed in the first 4 hr of incubation. Moreover, these numbers significantly differed from one-half the MIC treated culture, one-fourth the MIC treated culture, and

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drug-free TSB culture at the same incubation time ($p < 0.01$). In the presence of one-half and one-fourth the MIC of ampicillin in TSB for 4 hr, the viable cell numbers were also markedly decreased and slightly decreased respectively and these numbers significantly differed from drug-free TSB culture ($p < 0.01$). The viable cell number of one-half the MIC-treated culture also significantly differed from one-fourth the MIC-treated culture ($p < 0.01$) after 4 hr of incubation. After subsequent incubations in these subinhibitory concentrations to 16 and 24 hr, the number of cfu per ml markedly increased at each period whereas the viable cell number of drug-free TSB culture only slightly increased from 16 to 24 hr of incubation, as shown in Table 4, page 37 and Fig. 3, page 38. But these numbers were still lower than and significantly differed from those in the drug-free TSB culture at the same incubation time ($p < 0.01$). Moreover, at the same incubation time, the viable cell numbers of one-half the MIC-treated culture also significantly differed from the one-fourth the MIC-treated culture ($p < 0.01$). Fig. 3, page 38 showed the growth curves of *S. aureus*. From 4 to 16 hr of incubation, their growth rate in subinhibitory concentrations of ampicillin were approximately equaled to the control. But from 16 to 24 hr of incubation, the control reached stationary plateau while the one-half the MIC-treated cells still being grow in exponential phase with the faster growing rate than the 4 to 16 hr incubation, and in this period the one-fourth the MIC-treated cells also grew further but in slower rate than that of 4 to 16 hr incubation.

Table 4 Numbers of cfu per ml of *Staphylococcus aureus* after incubation in TSB and TSB containing ampicillin. The number of cfu per ml at starting time for each was 5.50×10^5 .

Incubation time (hr)	Drug-free TSB	TSB containing ampicillin		
		$\frac{1}{4}$ MIC	$\frac{1}{2}$ MIC	1 MIC
4	3.80×10^6	5.20×10^5 ^a	9.60×10^3 ^{a,b}	3.40×10^3 ^{a,b,c}
16	3.30×10^9	3.10×10^8 ^a	6.20×10^6 ^{a,b}	1.23×10^3 ^{a,b,c,d}
24	3.50×10^9	1.90×10^9 ^a	7.40×10^8 ^{a,b}	6.20×10^2 ^{a,b,c,e}

- a Significantly different from the drug free TSB culture at the same incubation time ($p < 0.01$, t-test).
- b Significantly different from the one-fourth the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- c Significantly different from the one-half the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- d Significantly different from one MIC-treated culture after incubation for 4 hr ($p < 0.01$, t-test).
- e Significantly different from one MIC-treated culture after incubation for 16 hr ($p < 0.01$, t-test).

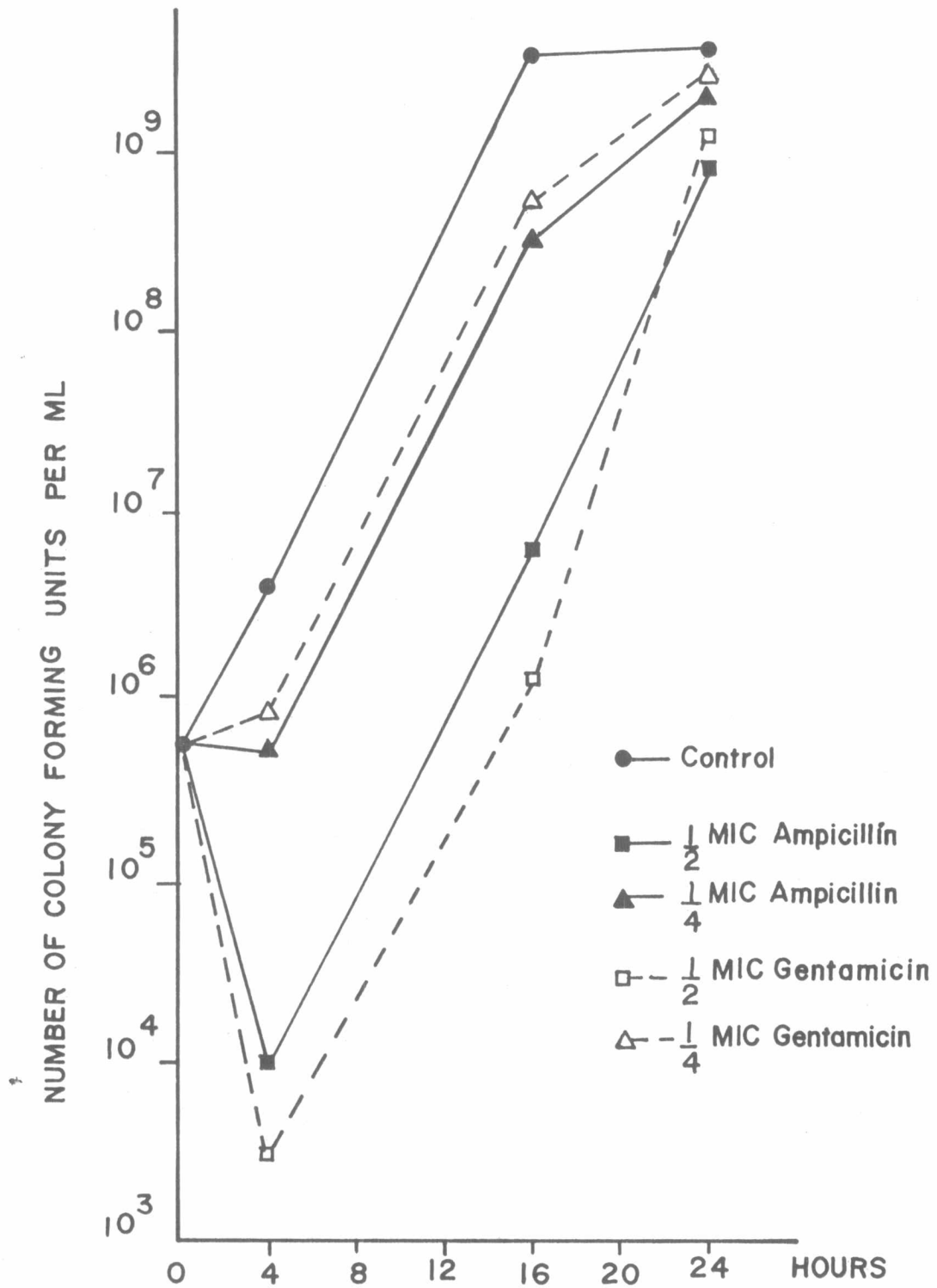


Fig. 3 Growth curves of *Staphylococcus aureus* incubated in the presence of subinhibitory concentrations of ampicillin and gentamicin in TSB for 4, 16 and 24 hours.

1.2 *Staphylococcus aureus* and gentamicin

After incubation in the presence of minimal inhibitory concentration of gentamicin in TSB for 4, 16 and 24 hr, the viable cell numbers of *S. aureus* were significantly decreased ($p < 0.01$) while the incubation time increased, as shown in Table 5, page 41, most of the cells were killed in the first 4 hr of incubation. Moreover, these numbers significantly differed from one-half the MIC-treated culture, one-fourth the MIC-treated culture, and drug-free TSB culture at the same incubation time ($p < 0.01$). In the presence of one-half the MIC of gentamicin for 4 hr, the viable cell number was markedly reduced, but after further incubation to 16 and 24 hr, it markedly increased. However, these numbers were significantly lower than the one-fourth the MIC-treated culture and the drug-free TSB culture at the same incubation time ($p < 0.01$). In the presence of one-fourth the MIC of gentamicin for 4, 16 and 24 hr, the viable cell numbers markedly increased at each period whereas the viable cell numbers of the control only slightly increased from 16 to 24 hr of incubation, as shown in Table 5, page 41. But the viable cell numbers of one-fourth the MIC-treated culture were significantly lower than the control values at the same incubation time ($p < 0.01$). Fig. 3, page 38 showed the growth curves of *S. aureus*, their growth rate in subinhibitory concentrations of gentamicin from 4 to 16 hr incubation almost equaled to the control. From 16 to 24 hr of incubation, the control reached stationary plateau whereas the one-half the MIC-treated cells still grew in faster rate than the

4 to 16 hr period of incubation, the one-fourth the MIC-treated cells also grew further but in slower rate than the 4 to 16 hr period of incubation. Moreover, the growth rates from 16 to 24 hr period of incubation of these subinhibitory concentrations of gentamicin-treated cells and ampicillin-treated cells were almost the same.

Table 5 Numbers of cfu per ml of *Staphylococcus aureus* after incubation in TSB and TSB containing gentamicin. The number of cfu per ml at starting time for each was 5.50×10^5 .

Incubation time (hr)	Drug-free TSB	TSB containing gentamicin		
		$\frac{1}{4}$ MIC	$\frac{1}{2}$ MIC	1 MIC
4	3.80×10^6	^a 8.10×10^5	^{a,b} 2.90×10^3	^{a,b,c} 5.95×10^2
16	3.30×10^9	^a 4.93×10^8	^{a,b} 1.13×10^6	^{a,b,c,d} 4.40×10^2
24	3.50×10^9	^a 2.50×10^9	^{a,b} 1.12×10^9	^{a,b,c,e} 1.60×10^2

- a Significantly different from the drug-free TSB culture at the same incubation time ($p < 0.01$, t-test).
- b Significantly different from the one-fourth the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- c Significantly different from the one-half the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- d Significantly different from one MIC-treated culture after incubation for 4 hr ($p < 0.01$, t-test).
- e Significantly different from one MIC-treated culture after incubation for 16 hr ($p < 0.01$, t-test).

1.3 *Pseudomonas aeruginosa* and ampicillin

After incubation in TSB containing minimal inhibitory concentration of ampicillin for 4, 16 and 24 hr, the viable cell numbers of *P. aeruginosa* were significantly decreased ($p < 0.01$) while the incubation time increased, as shown in Table 6, page 44. Moreover, these numbers were significantly lower than one-half the MIC-treated culture, one-fourth the MIC-treated culture and drug-free TSB culture at the same incubation times ($p < 0.01$). In the presence of one-half the MIC and one-fourth the MIC of ampicillin for 4, 16 and 24 hr, the viable cell numbers markedly increased while the incubation time increased whereas the viable cell number of the control from 16 to 24 hr of incubation was reduced. However, the viable cell numbers of these subinhibitory concentrations of ampicillin-treated cultures were significantly lower than the control values at 4 and 16 hr of incubation ($p < 0.01$). But at 24 hr of incubation, the viable cell numbers of one-half and one-fourth the MIC -treated cultures were slightly lower and higher than the control values respectively. The viable cell number of one-half the MIC-treated culture was significantly lower than one-fourth the MIC-treated culture at the same incubation time ($p < 0.01, 0.01$ and 0.05 for 4, 16 and 24 hr of incubation respectively). Fig. 4, page 45 showed the growth curves of *P. aeruginosa*. The control cells grew rapidly during the first 4 hr of incubation, the exponential growth rate decreased when the incubation prolonged to 16 hr and after that the growth curve declined, some cells might die during this period so the number of cfu per

ml at 24 hr incubation was markedly reduced. In the presence of one-half and one-fourth the MIC of ampicillin in TSB, the treated cells showed different growth curves from the control. The former showed the growth rate that increased after further incubation from 4 to 16 hr and the cells still grew faster than before when the incubation time prolonged from 16 to 24 hr. The later showed the growth rate that slightly increased after further incubation from 4 to 16 hr but after that the cells still grew but in decreasing rate. Finally, at 24 hr of incubation, the viable cell numbers of these subinhibitory concentrations of ampicillin-treated cultures were not significantly different from the control value.

Table 6 Numbers of cfu per ml of *Pseudomonas aeruginosa* after incubation in TSB and TSB containing ampicillin. The number of cfu per ml at starting time for each was 5.42×10^5 .

Incubation time (hr)	Drug-free TSB	TSB containing ampicillin		
		$\frac{1}{4}$ MIC	$\frac{1}{2}$ MIC	1 MIC
4	3.64×10^7	3.16×10^6 ^a	1.07×10^6 ^{a,b}	4.07×10^4 ^{a,b,c}
16	2.65×10^9	1.08×10^9 ^a	2.45×10^7 ^{a,b}	1.00×10^4 ^{a,b,c,d}
24	2.18×10^9	2.55×10^9	1.90×10^9 ^b	5.65×10^2 ^{a,b,c,e}

- a Significantly different from the drug-free TSB culture at the same incubation time ($p < 0.01$, t-test).
- b Significantly different from the one-fourth the MIC-treated culture at the same incubation time ($p < 0.05$, t-test).
- c Significantly different from the one-half the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- d Significantly different from one MIC-treated culture after incubation for 4 hr ($p < 0.01$, t-test).
- e Significantly different from one MIC-treated culture after incubation for 16 hr ($p < 0.01$, t-test).

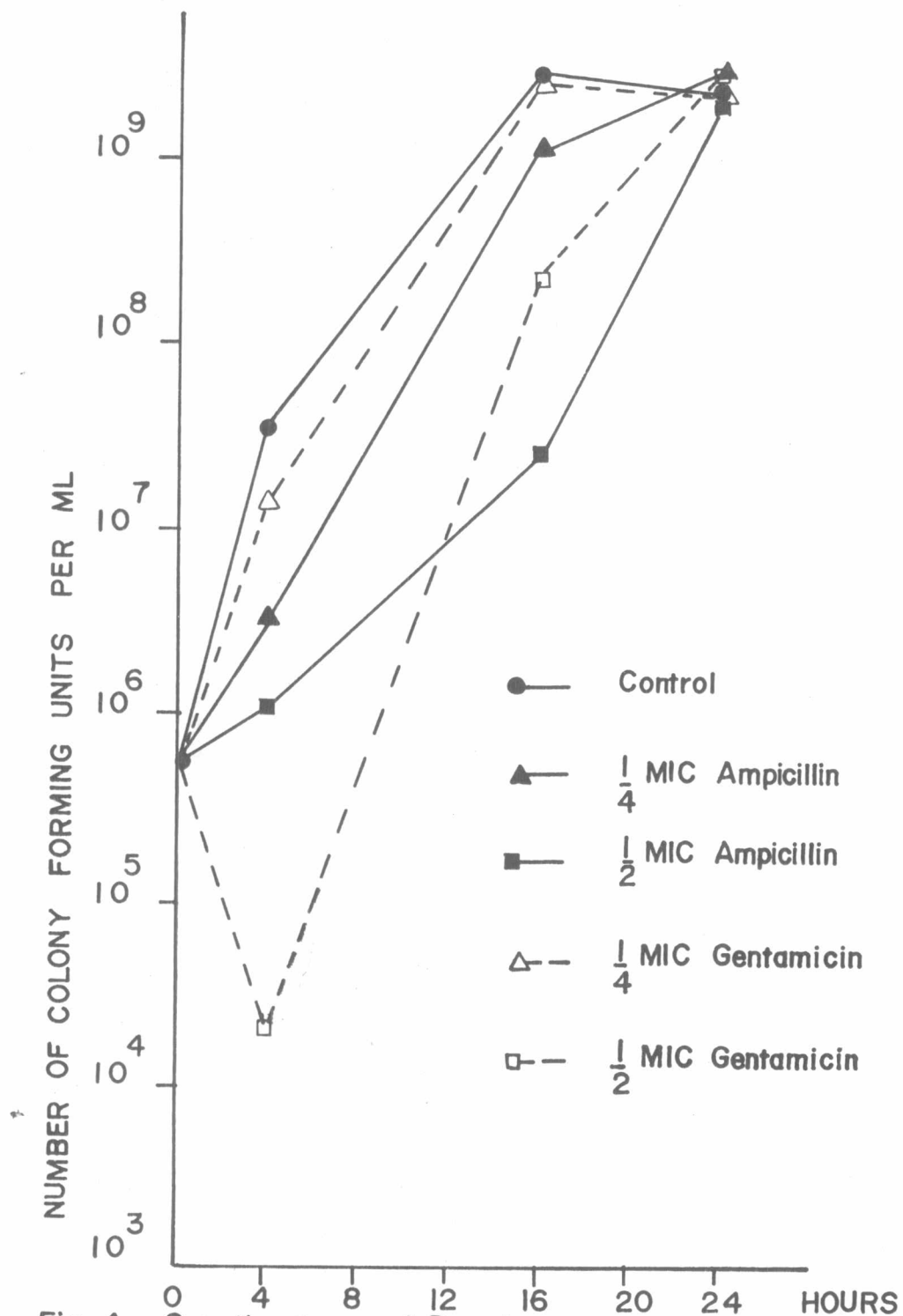


Fig. 4 Growth curves of *Pseudomonas aeruginosa* incubated in the presence of subinhibitory concentrations of ampicillin and gentamicin in TSB for 4, 16 and 24 hours.

1.4 *Pseudomonas aeruginosa* and gentamicin

After incubation in TSB containing minimal inhibitory concentration of gentamicin for 4, 16 and 24 hr, the numbers of viable cells of *P. aeruginosa* were significantly decreased as the incubation time increased ($p < 0.02$), as shown in Table 7, page 48. Moreover, these numbers also significantly differed from one-half the MIC-treated culture, one-fourth the MIC-treated culture and drug-free TSB culture at the same incubation time ($p < 0.01$). In the presence of one-half the MIC of gentamicin in TSB for 4 hr, the viable cell number was markedly reduced and significantly lower than the control value and the one-fourth the MIC-treated culture ($p < 0.01$). After the incubation time was prolonged to 16 and 24 hr, the viable cell number of one-half the MIC-treated culture markedly increased and finally, at 24 hr incubation, this viable cell number was slightly higher than the control and the one-fourth the MIC treated values. However, at 16 hr incubation, the viable cell number of one-half the MIC-treated culture was significantly lower than the one-fourth the MIC-treated culture and the control ($p < 0.01$). In the presence of one-fourth the MIC of gentamicin in TSB, the viable cell number markedly increased after the incubation time was prolonged to 16 hr and slightly decreased after that to 24 hr of incubation. After 4 hr incubation, the viable cell number of one-fourth the MIC-treated culture was significantly lower than the control value ($p < 0.05$). After 16 hr of incubation, the viable cell number of one-fourth the MIC-treated culture was slightly lower than the control value

and after 24 hr of incubation it was approximately equal to the control value. Fig. 4, page 45 showed the growth curves of *P. aeruginosa*. In the presence of one-half the MIC of gentamicin in TSB, the treated cells grew rapidly during the 4 to 16 hr of incubation and this growth rate was higher than the control, after that the treated cells still grew but in slower rate while the control growth rate declined. In the presence of one-fourth the MIC of gentamicin in TSB, the treated cells grew in slower rate than the control during the first 4 hr. From 4 to 16 hr of incubation the growing rate was also slower than the first 4 hr, and the growth curve declined during 16 to 24 hr of incubation with the viable cell number slightly decreased at 24 hr.

Table 7 Numbers of cfu per ml of *Pseudomonas aeruginosa* after incubation in TSB and TSB containing gentamicin.

The number of cfu per ml at starting time for each was 5.42×10^5 .

Incubation time (hr)	Drug-free TSB	TSB containing gentamicin		
		$\frac{1}{4}$ MIC	$\frac{1}{2}$ MIC	1 MIC
4	3.64×10^7	1.42×10^7 ^a	2.06×10^4 ^{a,b}	2.00×10^2 ^{a,b,c}
16	2.65×10^9	2.47×10^9	2.16×10^8 ^{a,b}	1.24×10^2 ^{a,b,c,d}
24	2.18×10^9	2.19×10^9	2.55×10^9	5.90×10^1 ^{a,b,c,e}

- a Significantly different from the drug-free TSB culture at the same incubation time ($p < 0.05$, t-test).
- b Significantly different from the one-fourth the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- c Significantly different from the one-half the MIC-treated culture at the same incubation time ($p < 0.01$, t-test).
- d Significantly different from one MIC-treated culture after incubation for 4 hr ($p < 0.02$, t-test).
- e Significantly different from one MIC-treated culture after incubation for 16 hr ($p < 0.01$, t-test).



2. Effect of antibiotics on microscopic morphology of bacteria.

2.1 *Staphylococcus aureus* and ampicillin

2.1.1 Light microscopy

After incubation in the presence of minimal inhibitory concentration of ampicillin in TSB for 4, 16 and 24 hr, most of *S. aureus* were killed so a little could be observed in Gram stain. These cells turned into large cells, large diplococci and large irregular shape. In the presence of subinhibitory concentrations ($\frac{1}{2}$ and $\frac{1}{4}$ MIC), for the same time as above, they also resulted in large cells, large diplococci, small diplococci, and some large irregular shape in addition to normal cells (Fig. 5, page 50). These large cells retained more crystal violet than control cells after Gram staining. Moreover, both of the ampicillin free culture and ampicillin treated culture of *S. aureus* showed a few of the large irregular shape which retained less crystal violet than control cells after Gram staining and these seem to be lysis further. The number of abnormal cells of one-half the MIC of ampicillin-treated culture reduced in percentages, with statistically significant difference ($p < 0.005$), after prolonged incubation as shown in Table 8, page 51. One-fourth the MIC of ampicillin-treated cells also had the same manner. After 24 hr incubation in the presence of one-half the MIC of ampicillin in TSB, the amount of abnormal cells of *S. aureus* were significantly different from *P. aeruginosa* ($p < 0.005$).

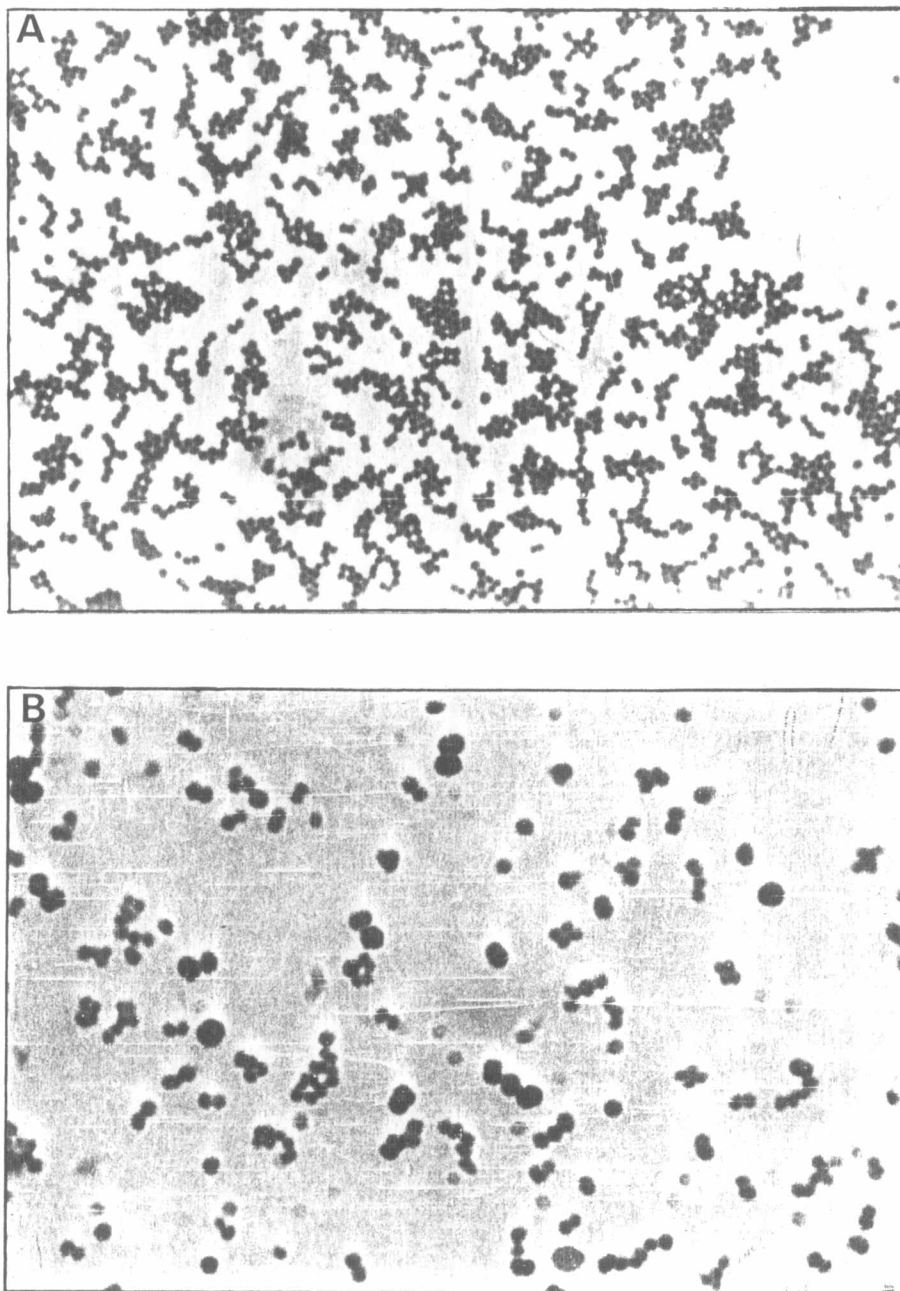


Fig. 5 *Staphylococcus aureus* grown for 16 hr (A) in drug-free TSB and (B) in the presence of one-half the MIC of ampicillin in TSB. Gram stain. X3,300.

Table 8 Percentages of abnormal cells of *Staphylococcus aureus* and *Pseudomonas aeruginosa* after incubation in TSB in the presence of one-half the MIC of ampicillin.

Incubation time (hr)	Percentages of abnormal cells*		p**
	<i>S. aureus</i>	<i>P. aeruginosa</i>	
4	96.0	90.9	>0.1
16	76.7	72.6	>0.25
24	60.3	20.6	<0.005
p**	<0.005	<0.005	

* Counted in total 1,000 cells.

** Chi-square test.

2.1.2 Electron microscopy

Negatively stained preparation of one-half the MIC of ampicillin treated cells after 16 hr incubation revealed cells with large irregular shape and rather thinner cell wall than the controls. Some had irregular thick cross wall with electron dense appearance. Some had very broad cross wall with no central dense layer. Moreover, some collapsed cells could also be observed. The cytoplasm of these abnormal cells had the appearance differed from the controls (Fig. 6, page 52).

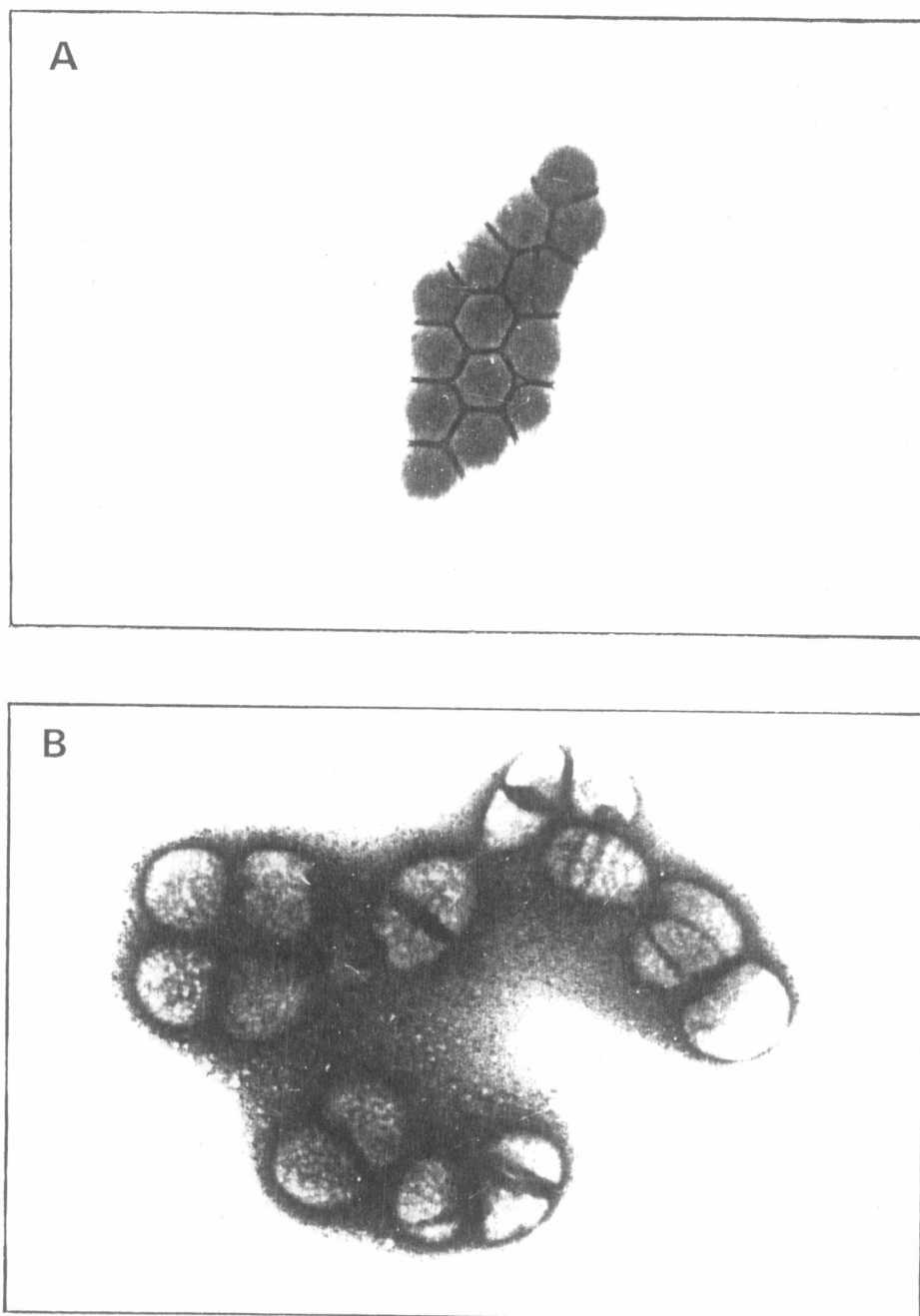


Fig. 6 *Staphylococcus aureus* grown for 16 hr (A) in drug-free TSB and (B) in the presence of one-half the MIC of ampicillin in TSB. Negatively stain. x 13,200. Noted that the cells were irregular in shape, large in size, and the cross wall was much wider than the controls.

2.2 *Staphylococcus aureus* and gentamicin

2.2.1 Light microscopy

After incubation in the presence of minimal inhibitory concentration of gentamicin in TSB for 4, 16 and 24 hr, most of *S. aureus* were killed so a little could be observed in Gram stain. Most of the remaining cells were small diplococci with concave attached side. Some were larger than the control cells. Large diplococci and large irregular shape of bacterial cells which retained less crystal violet was also seen in a few of the control cells and these finally seemed to be lysed. After incubation with subinhibitory concentrations of gentamicin in TSB, for the various times as mentioned before, the results were as such as described earlier. In addition, the rather round cells and normal cells were also found (Fig. 7, page 54). However, the abnormal shape of them could not markedly differentiate from the controls so that it was not possible to enumerate for statistical difference.

2.2.2 Electron microscopy

Negatively stained preparation of one-half the MIC of gentamicin-treated cells in TSB for 16 hr confirmed the light microscopic observation. The electron micrograph showed rather round cells and cells with the wide convex cross wall which had no central dense layer (Fig. 8, page 55). However, few cells from antibiotic free culture of *S. aureus* also showed the wide convex cross wall, but their cell walls were clear

(Fig 6 A, page 52) which could not be observed clearly in the gentamicin-treated cells.

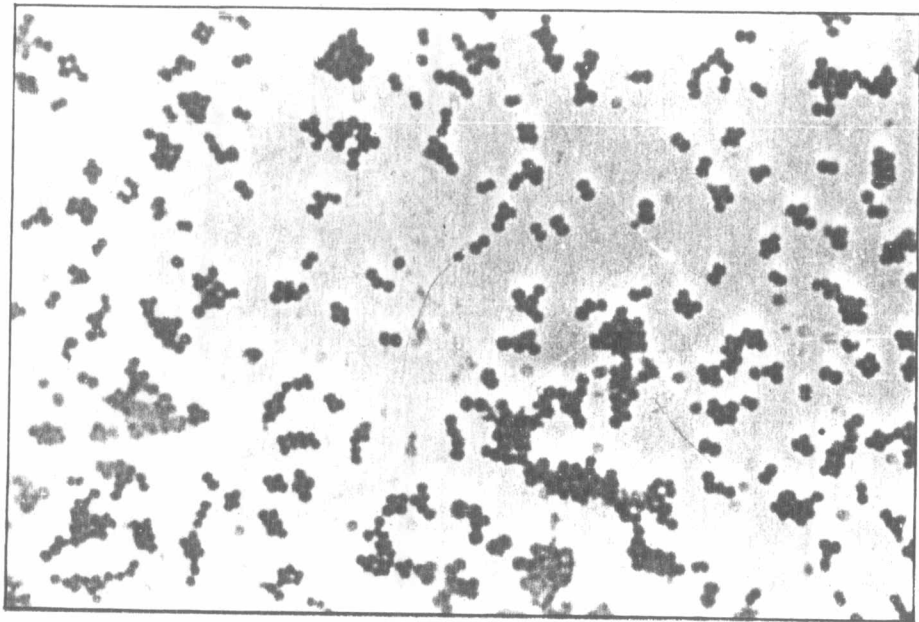


Fig. 7 *Staphylococcus aureus* grown in the presence of one-half the MIC of gentamicin in TSB for 16 hr. Gram stain. x 3,300. (The control culture was shown in Fig. 5 A, page 50).

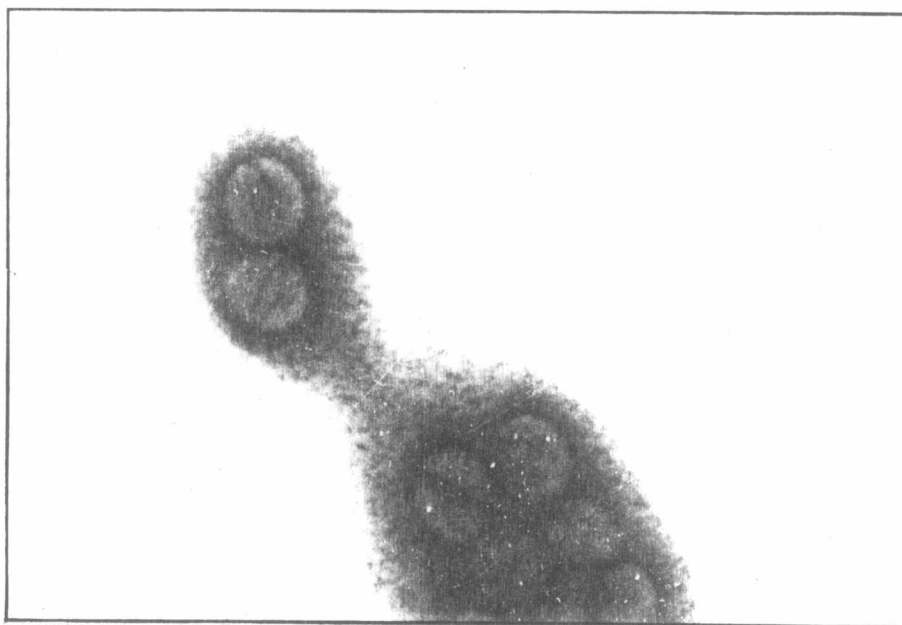


Fig. 8 *Staphylococcus aureus* grown in the presence of one-half the MIC of gentamicin in TSB for 16 hr. Negatively stain. x 13,200. (The control culture was shown in Fig. 6 A, page 52).

2.3 *Pseudomonas aeruginosa* and ampicillin

2.3.1 Light microscopy

After incubation in the presence of minimal inhibitory concentration of ampicillin in TSB for 4, 16 and 24 hr, *P. aeruginosa* turned to be Gram-positive with long filamentous appearance. Some filaments were branched, some were swell, some retained less crystal violet and seemed to lyse further, and some had club shape at one end. In the presence of one-half and one-fourth the MIC of ampicillin in TSB, for the same time as above, Gram staining appeared to be positive, some were long filaments, branched

filaments, long bacilli and few remained in normal shape (Fig. 9, page 57). Some filaments had granular appearance, some were swell and seemed to lyse, some retained less crystal violet, some were thin and also retained less crystal violet, and some bacilli had bipolar appearance. The number of abnormal cells that were affected by treatment with one-half the MIC of ampicillin reduced in percentage after prolong incubation as shown in Table 8, page 51. Cells which were affected by one-fourth the MIC of ampicillin treatment also gave the same result. As the incubation time prolonged, the amount of abnormal cells reduced by significant difference ($p < 0.005$). As 24 hr incubation in the presence of one-half the MIC of ampicillin in TSB, the amount of abnormal cells of *P. aeruginosa* was significantly different from *S. aureus* ($p < 0.005$) as shown in Table 8, page 51.

2.3.2 Electron microscopy

Negatively stained preparation of 16 hr one-half the MIC of ampicillin-treated cells confirmed the light microscopic observation. An electron micrograph revealed long filament with failure of septum formation and long bacilli. The filamentous forms contained disrupted cell wall which distinctly separated from the cell membrane by a large space. Some filaments contained electron dense material in the cytoplasm as shown in Fig. 11 A, page 59, and in some filaments, disruption of the cell wall caused loss of

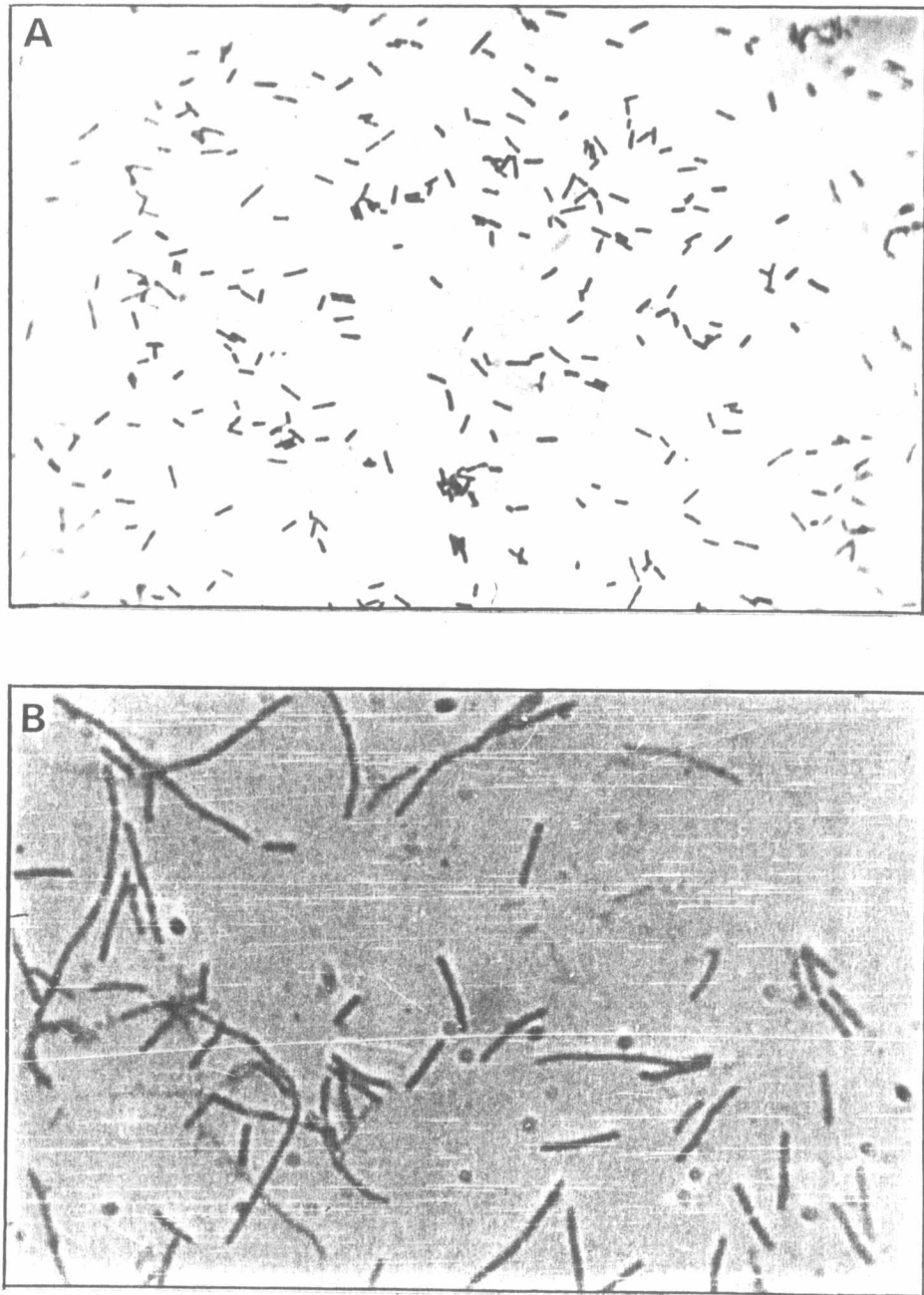


Fig. 9 *Pseudomonas aeruginosa* grown for 16 hr (A) in drug-free TSB and (B) in the presence of one-half the MIC of ampicillin in TSB. Gram stain. x 3,300.

cytoplasmic material. The long bacilli contained a large number of small vacuoles and electron dense material dispersion in the cytoplasm, and some parts of the cell wall was disrupted as shown in Fig. 11 B, page 59.

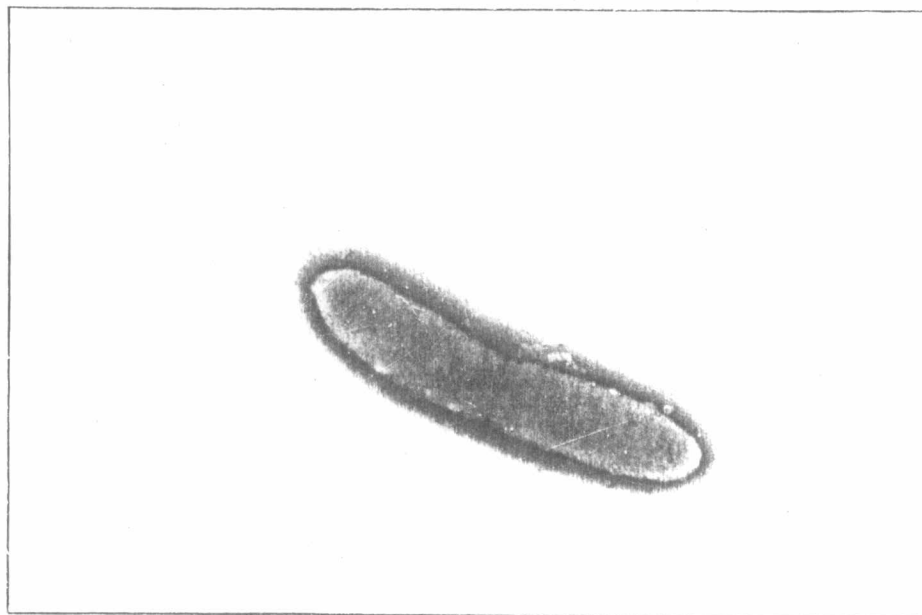


Fig. 10 *Pseudomonas aeruginosa* grown in drug-free TSB for 16 hr.
Negatively stain. x 13,200.

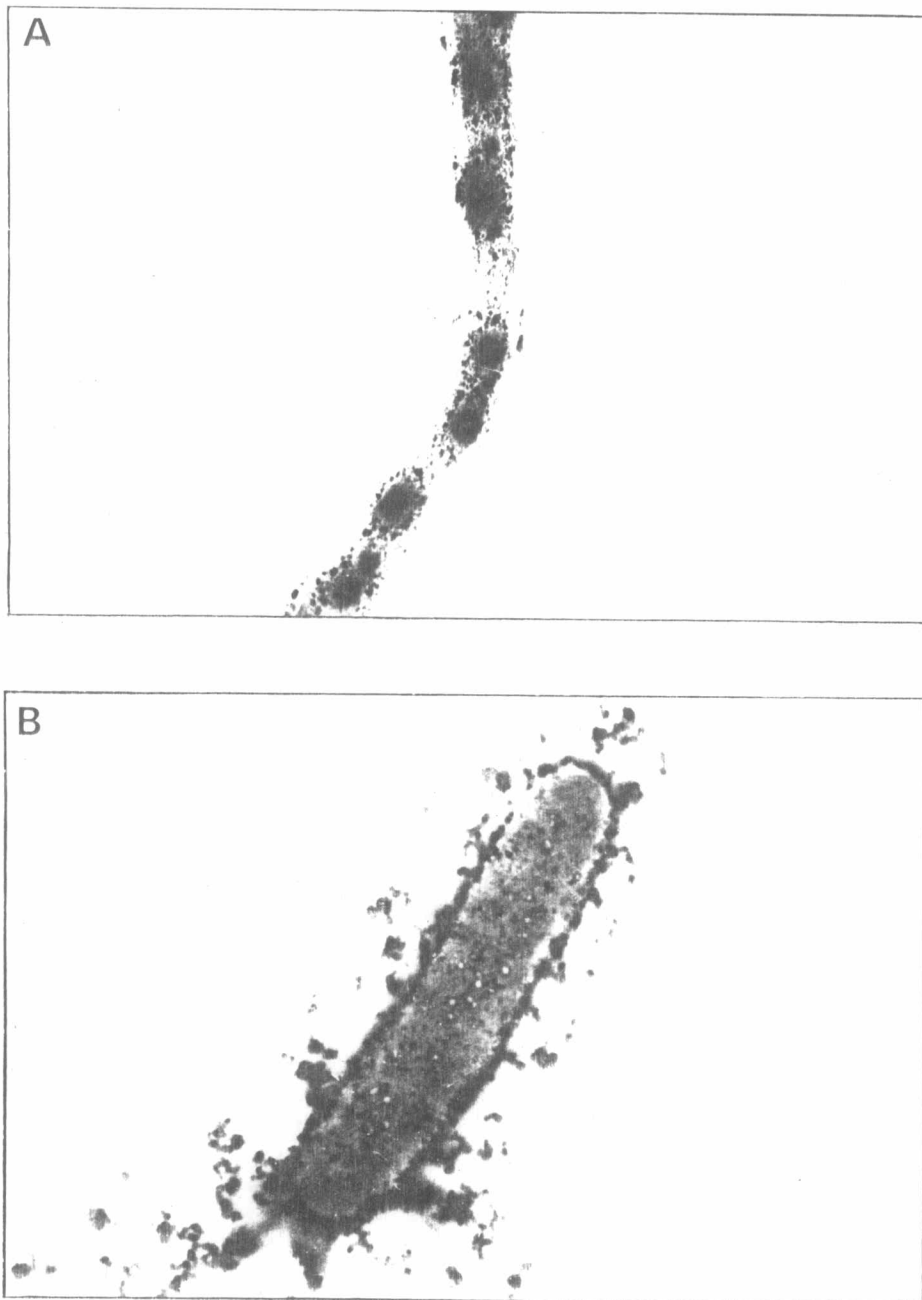


Fig. 11 (A and B) *Pseudomonas aeruginosa* grown in the presence of one-half the MIC of ampicillin in TSB for 16 hr. Negatively stain. A x 13,200, B x 15,400. (The control culture was shown in Fig. 10, page 58).

2.4 *Pseudomonas aeruginosa* and gentamicin

2.4.1 Light microscopy

After incubation in the presence of minimal inhibitory concentration of gentamicin in TSB for 4, 16 and 24 hr, most of *P. aeruginosa* were killed, the few remaining cells turned to be Gram-positive smaller bacilli, a little some turned to be long bacilli and filamentous form. After incubation in the presence of one-half the MIC of gentamicin in TSB for 4, 16 and 24 hr, they also turned to be Gram-positive bacilli with varying cell length, some were bipolar, some were swollen, some were disrupted and a little some were filamentous as shown in Fig. 12, page 61. However, the abnormal morphology of them were not markedly differentiated from the controls so that they could not be enumerated for statistically difference. In the presence of one-fourth the MIC of gentamicin, cells still retained Gram-negative staining, most of all were rather larger than the controls, some were swollen, some were disrupted, and some were bipolar. However, incubation in the absence of antibiotic, a little some of them showed swollen and disrupted cells.

2.4.2 Electron microscopy

Negatively stained preparation of culture which grew in the presence of one-half the MIC of gentamicin for 16 hr confirmed the light microscopic observation. It showed cells with high density of ribosome at two ends, and some

were coccobacilli. The cytoplasm of these cells contained a number of small vacuole, and some cells showed extruded cytoplasm as shown in Fig. 13 A, page 62. Moreover, the cell wall was irregularly undulated with loose and disrupted appearance as shown in Fig. 13 B, page 62.

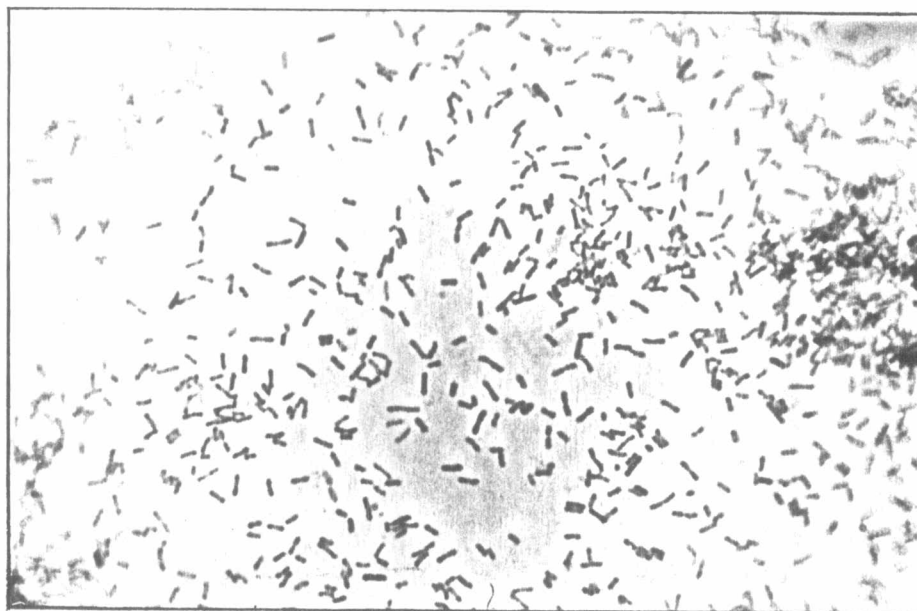


Fig. 12 *Pseudomonas aeruginosa* grown in the presence of one-half the MIC of gentamicin in TSB for 16 hr. Gram stain. x 3,300.

(The control culture was shown in Fig. 9 A, page 57).

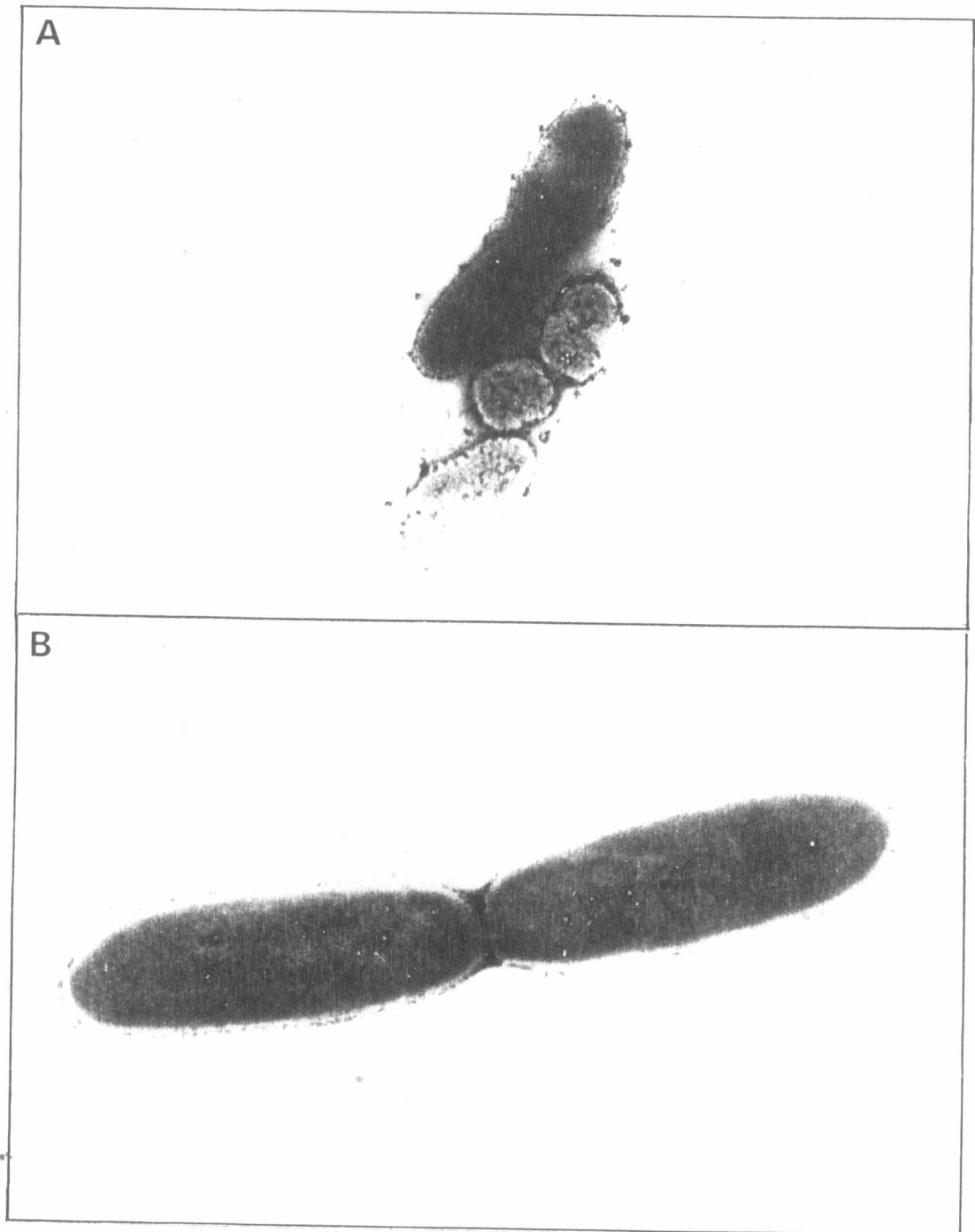


Fig. 13 (A and B) *Pseudomonas aeruginosa* grown in the presence of one-half the MIC of gentamicin for 16 hr. Negatively stain. A x 10,780, B x 18,700. (The control culture was shown in Fig. 10, page 58).

3. Morphology and MICs redetermination for microorganisms pre-exposed to various concentrations of antibiotics.

3.1 *Staphylococcus aureus* pre-exposed to ampicillin.

The *S. aureus* cells, which had been exposed to minimal inhibitory concentration of ampicillin and then transferred to drug-free TSB for 18 hr, still exhibited large abnormal shape and small diplococci could be observed in addition to normal cells. But most of the cells which had been pre-exposed to subinhibitory concentrations of ampicillin were normal, with some cells still exhibited large abnormal shape in addition to small diplococci.

MICs of ampicillin and gentamicin on *S. aureus* that had been pre-exposed to various concentrations of ampicillin were shown in Table 9, page 64. The MICs of ampicillin were higher than the controls and most of them increased progressively as the ampicillin-treated time prolonged, excepted the MIC on the 24 hr-minimal inhibitory concentration-treated cells which was lower than the MIC on 16 hr-treated cells. No significant change in the MICs of gentamicin redetermined from those cells which had been pre-exposed to various concentration of ampicillin before, only little changes could be observed from cell which had been pretreated with one-half the MIC of ampicillin and 24 hr-one-fourth the MIC of ampicillin.

Table 9 MICs of ampicillin and gentamicin redetermined on ampicillin pretreated *Staphylococcus aureus* that subsequently grown in drug-free TSB for 18 hr.

		Minimal inhibitory concentration ($\mu\text{g/ml}$)					
		Ampicillin			Gentamicin		
Conc ⁿ of ampicillin pre-exposed (MIC)	Exposure time (hr)	4	16	24	4	16	24
	0		0.06	0.06	0.06	1.0	1.0
$\frac{1}{4}$		0.08	0.18	0.24	1.0	1.0	1.4
$\frac{1}{2}$		0.10	0.18	0.40	1.2	1.4	1.4
1		0.12	0.18	0.14	1.0	1.0	1.0

3.2 *Staphylococcus aureus* pre-exposed to gentamicin

After growing for 18 hr in drug-free TSB of *S. aureus*, which had been pre-exposed to various concentrations of gentamicin before, most of the cells appeared in normal morphology as the control cells. Only those that had been pre-exposed to the minimal inhibitory concentration of gentamicin for 16 and 24 hr showed small diplococci in addition to the normal cells, and some were larger than the control cells.

MICs of ampicillin and gentamicin on *S. aureus* that had been pre-exposed to various concentrations of gentamicin were shown in Table 10, page 66. Cells that had been pretreated with one-half the MIC for 16 hr and one-fourth the MIC for 4 and 24 hr resulted in higher MIC values of ampicillin after redetermination. The MIC of gentamicin on cells that had been previously treated with the minimal inhibitory concentration of gentamicin for 4 hr was lower than the control, whereas the MICs on cells that had been pretreated with this drug in the same concentration for 16 and 24 hr were higher than the control. Cells pre-exposed to one-half and one-fourth the MIC of gentamicin for 16 hr exhibited higher MIC values after redetermination.

Table 10 MICs of ampicillin and gentamicin re-determined on gentamicin pretreated *Staphylococcus aureus* that subsequently grown in drug-free TSB for 18 hr.

		Minimal inhibitory concentration ($\mu\text{g/ml}$)					
		Ampicillin			Gentamicin		
Conc ⁿ of gentamicin pre-exposed (MIC)	Exposure time (hr)	4	16	24	4	16	24
	0		0.06	0.06	0.06	1.0	1.0
$\frac{1}{4}$		0.10	0.06	0.14	1.0	1.2	1.0
$\frac{1}{2}$		0.06	0.24	0.06	1.0	2.0	1.0
1		0.06	0.06	0.06	0.6	1.4	1.2

3.3 *Pseudomonas aeruginosa* pre-exposed to ampicillin

After growing for 18 hr in drug-free TSB of *P. aeruginosa*, which had been previously treated with the minimal inhibitory concentration of ampicillin for 4, 16 and 24 hr, many cells exhibited filamentous form in addition to bipolar staining cells and these were Gram-negative. Some of those that grew from cells which had been pre-exposed to various subinhibitory concentrations of ampicillin showed filamentous form and many cells were bipolar staining, but no filament was observed from the cultures which had been pretreated for 24 hr in various subinhibitory concentrations

of this drug.

MICs of ampicillin and gentamicin on *P. aeruginosa* that had been pre-exposed to various concentrations of ampicillin were shown in Table 11, page 68. The MICs of ampicillin were markedly elevated in the cultures that had been pretreated with one MIC for 16 hr, one-half the MIC for 16 and 24 hr, and one-fourth the MIC for 24 hr. The MICs of gentamicin were slightly increased in the cultures that had been pretreated with one-fourth the MIC for 16 hr and one-half the MIC for 16 and 24 hr. The MIC was markedly elevated in culture that had been previously treated with one MIC for 16 hr.

Table 11 MICs of ampicillin and gentamicin redetermined on ampicillin pretreated *Pseudomonas aeruginosa* that subsequently grown in drug-free TSB for 18 hr.

		Minimal inhibitory concentration					
		Ampicillin (mg/ml)			Gentamicin (μ g/ml)		
Conc ⁿ of ampicillin pre-exposed (MIC)	Exposure time (hr)	4	16	24	4	16	24
	0		1.8	1.8	1.8	2.0	2.0
$\frac{1}{4}$		1.8	1.8	2.6	2.0	2.6	2.0
$\frac{1}{2}$		1.8	2.8	2.6	2.0	2.6	2.4
1		1.8	2.8	1.8	2.0	3.6	2.0

3.4 *Pseudomonas aeruginosa* pre-exposed to gentamicin

After growing for 18 hr in drug-free TSB of *P. aeruginosa*, which had been pre-exposed to various concentrations of gentamicin for 4, 16 and 24 hr, many cells appeared Gram-negative bacilli but rather larger than normal and varied in cell length, some were Gram-negative bipolar staining bacilli.

MICs of ampicillin and gentamicin on *P. aeruginosa* that had been pre-exposed to various concentrations of gentamicin were shown in Table 12, page 69. MICs of ampicillin were slightly

elevated in the cultures which had been pretreated with one-fourth the MIC and one-half the MIC of gentamicin for 4 hr. The MICs of gentamicin were markedly increased in the cultures which had been pretreated with one MIC for 4 and 16 hr, and one-half the MIC for 4, 16 and 24 hr. The MIC of gentamicin were also slightly elevated in the cultures which had been pre-exposed to one MIC for 24 hr and to one-fourth the MIC for 4 and 24 hr.

Table 12 MICs of ampicillin and gentamicin redetermined on gentamicin pretreated *Pseudomonas aeruginosa* that subsequently grown in drug-free TSB for 18 hr.

		Minimal inhibitory concentration					
		Ampicillin (mg/ml)			Gentamicin (μ g/ml)		
Conc ⁿ of gentamicin pre-exposed (MIC)	Exposure time (hr)	4	16	24	4	16	24
	0		1.8	1.8	1.8	2.0	2.0
$\frac{1}{4}$		2.0	1.8	1.8	2.4	2.0	2.4
$\frac{1}{2}$		2.0	1.8	1.8	3.0	2.8	3.2
1		1.8	1.8	1.8	4.0	5.0	2.4