

Comparative Activity of Amoxycillin and Ampicillin in an Experimental Bacterial Infection in Mice

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Against experimental infections in mice with strains of *Escherichia coli* and with *Proteus mirabilis*, amoxycillin was found to be more active than ampicillin both by the oral and the subcutaneous routes of administration. With the bacteria used in these experiments, ampicillin and amoxycillin showed the same minimal inhibitory concentrations and, after subcutaneous administration, the levels of ampicillin and amoxycillin in the plasma and tissue homogenate were also similar. However, counts of the number of viable bacteria present in the infected tissue showed that amoxycillin exerted a more rapid and a more marked bactericidal effect than did ampicillin, and this could be correlated with the difference in therapeutic effect. When given by mouth, amoxycillin was more completely absorbed than ampicillin and gave rise to higher levels of antibiotic in the plasma. This may have accounted in part for the difference in therapeutic activity seen when these penicillins were given by the oral route. However, when appropriate oral dosages of ampicillin and amoxycillin were used so as to achieve similar levels of antibiotic in the plasma and tissue homogenate, amoxycillin was again found to exert a more marked bactericidal effect than did ampicillin, and this was accompanied by greater therapeutic activity. In experiments in vitro, amoxycillin also showed a more rapid bactericidal effect than did ampicillin against the bacteria which were used to produce the experimental infections.

Amoxycillin is a new semisynthetic penicillin with a spectrum and level of antibacterial activity in vitro very similar to that of ampicillin (3). When administered by mouth, amoxycillin is better absorbed than ampicillin in man (3) and in experimental animals (1), and the serum concentrations obtained with amoxycillin after oral dosage are considerably higher than those of ampicillin. This difference in blood levels might account, at least in part, for the superior therapeutic activity which amoxycillin shows in mice compared with ampicillin when the drugs are given by mouth (1). When given by subcutaneous injection, however, amoxycillin has also been shown to be more active than ampicillin against experimental bacterial infections in mice, yet the blood levels of ampicillin and amoxycillin are very similar when the drugs are given by this route (1).

In the work reported here the activity of ampicillin and amoxycillin in vivo has been investigated further by using an intramuscular infection in mice resulting in a lesion which

persists over a period of several days. Numbers of viable bacteria present in the infected tissue were counted and the levels of antibiotic in the blood and tissue homogenate were assayed in order to correlate these data with the therapeutic effect of these penicillins.

MATERIALS AND METHODS

Cultures. The bacteria used in the animal experiments were maintained as suspensions frozen in liquid nitrogen. A 6-h shaken culture in nutrient broth was prepared, 10% glycerol was added slowly, and the suspension was dispensed in 2-ml volumes in plastic ampoules. These were plunged directly into liquid nitrogen and stored for periods of up to 1 year. Very little loss in viability occurred after freezing, and once frozen, no appreciable loss in viability or virulence was found during storage. When required for use, an ampoule was removed and thawed quickly by immersion in a water bath at 45 C. Dilutions were then made in phosphate buffered saline (PBS) pH 7.2, to give the required number of organisms. All cultures were periodically passaged in mice to maintain virulence. The cultures used in the experiments in vitro were maintained on nutrient agar slopes.

Mice. Female CS1 mice from Charles River, weight 16 to 20 g, were used throughout.

Antibiotics. Commercial preparations of ampicillin trihydrate (Penbritin, Beecham Research Laboratories, 815 $\mu\text{g}/\text{mg}$ anhydrous free acid) and amoxycillin (Amoxil, Bencard, 805 $\mu\text{g}/\text{mg}$ anhydrous free acid) were used. Dosages were calculated on the basis of pure anhydrous free acid penicillin. Solutions for subcutaneous administration were prepared by dissolving the free acids of ampicillin or amoxycillin in 20 M sodium carbonate-sodium bicarbonate buffer, pH 9.8. During solution of the penicillin the pH fell to approximately 8.5, and this was then adjusted to 7.2 with 1N HCl. Preliminary experiments showed negligible loss of activity of ampicillin or amoxycillin after 30 min at pH 9.3. Suspensions of ampicillin or amoxycillin for oral administration were prepared by suspending the penicillin free acids, together with a little acacia, in distilled water. Solutions of ampicillin or amoxycillin for in vitro experiments were prepared by dissolving the penicillins in 10 M phosphate buffer, pH 8.0, and then diluting with PBS to give the desired range of concentrations.

Thigh lesion test. Mice were infected intramuscularly in the right hind leg with 0.2 ml of a bacterial suspension. The inoculation was made into the muscles of the back of the thigh as described by Selbie and O'Grady (2). The thigh diameters were measured by using calipers 24 h after inoculation and then daily over 4 to 6 days. A group of 10 noninfected mice was always used as control. Antibiotics were administered orally or subcutaneously to infected mice in groups of 10 and the percentage protection from thigh enlargement was calculated as follows: % protection = $100 \times$ mean daily thigh enlargement of infected controls - mean daily thigh enlargement of test group / mean daily thigh enlargement of infected controls. All of the thigh diameter measurements in each experiment were made by the same person, and to avoid bias the animals were presented for measurement at random.

With *Escherichia coli* strain 9, injection of 10^7 or more viable bacteria per thigh was required to produce a consistent and large thigh lesion (Table 1, Fig. 1). With this infection the thigh diameter increased from the normal value of approximately 3.7 mm to

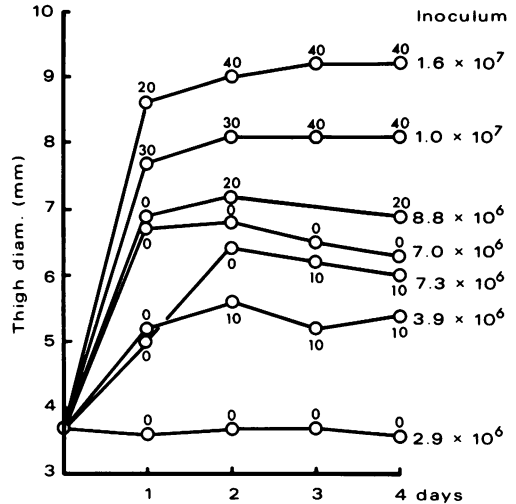


FIG. 1. Thigh lesions in mice in relation to the size of inoculum using *E. coli* strain 9. The numbers of viable bacteria injected per thigh are shown together with the percentage mortality at 24-h intervals. Thigh diameter measurements are the mean of groups of 10 mice.

over 7 mm by 24 h, and the number of viable bacteria per thigh increased to over 10^9 in the same period. Mortality after 24 h varied from 0 to 30%, and approximately 50% mortality occurred over a period of several days. Inoculation with 4×10^6 to 8×10^6 viable bacteria per thigh resulted in a smaller and variable thigh enlargement though bacterial numbers increased considerably over 24 h. With an infection of 2×10^6 viable bacteria or less per thigh, bacterial numbers remained fairly constant or fell slightly over 24 h, and no thigh lesion developed. With *E. coli* strain 8 and *Proteus mirabilis* strain 13, 5×10^7 to 10^8 viable bacteria were required to be injected per thigh in order to produce a large and consistent thigh lesion.

Bactericidal activity in vivo. Groups of 30 to 40 mice were infected intramuscularly as described for the thigh lesion test, and ampicillin or amoxycillin was administered orally or by subcutaneous injection. A similar group of untreated mice was used as control. Animals were sacrificed at intervals and the infected thighs were homogenized as described below. The numbers of viable bacteria present in the homogenate were counted, and in certain experiments the levels of antibiotic were also assayed in the homogenate and in blood samples taken from the same animal.

Counts of viable bacteria in infected mouse thigh. Mice were killed by dislocation of the neck. The outer skin, foot, and lower leg were removed from the infected thigh which was then weighed, chopped coarsely, and homogenized for 10 s at room temperature in 5 ml of PBS by using an Ultra-Turrax homogenizer (Janke and Kunkel KG). This homogenization procedure did not bring about any detectable loss of viability in broth suspensions of the bacteria used. Tenfold dilutions of the thigh homogenate were prepared in PBS, and 0.02-ml drops were placed on

TABLE 1. Thigh lesion in mice in relation to size of inoculum using *E. coli* strain 9^a

No. of viable bacteria injected/thigh	No. of viable bacteria recovered	Recovery (%)	No. of viable bacteria after 24 h	Mean thigh diam (mm) after 24 h
1.1×10^7	7.5×10^6	68	4.0×10^9	8.6
8.4×10^6	6.5×10^6	77	1.4×10^9	6.7
5.6×10^6	4.3×10^6	77	1.4×10^9	5.2
3.8×10^6	3.5×10^6	92	1.1×10^8	4.2
2.4×10^6	1.6×10^6	67	3.6×10^6	3.9
2.1×10^6	1.4×10^6	67	5.3×10^5	3.7
4.2×10^5	2.3×10^5	55	4.7×10^4	3.7
				3.7 ^b

^a Results are the mean of three animals.

^b Uninfected animals.

dried nutrient agar petri dishes. A minimum of eight drops was used per dilution, and numbers of colonies were counted after overnight incubation at 30 C. Recovery of bacteria in thigh homogenate preparations, immediately after infection, ranged from 50 to 90% of the numbers injected (Table 1).

Antibiotic assays. Blood was collected from the axillary region of mice into heparinized tubes, and the majority of these samples were assayed undiluted against ampicillin or amoxycillin standard solutions prepared in whole horse blood. Preliminary experiments showed that the inhibition zone diameters of ampicillin and amoxycillin standards in horse blood did not differ appreciably from those in mouse blood. Certain blood samples containing high concentrations of ampicillin or amoxycillin were diluted as required with PBS and assayed against standards prepared in corresponding dilutions of horse blood in PBS. Samples for assay were placed in holes in nutrient agar plates (14 by 16 in, approximately 35 by 40 cm) seeded with either *Bacillus subtilis* ATCC 6633 or *Sarcina lutea* ATCC 9341. *B. subtilis* was used for the majority of the samples, but those anticipated to be of low potency were assayed using *Sarcina*. Plasma levels were calculated from the assays of whole blood by allowing for a packed red cell volume of 40%.

Tissue homogenates were diluted as required with PBS and assayed against standards similarly prepared in PBS., prior experiments having shown that the inhibition zone diameters of ampicillin and amoxycillin standard solutions in PBS did not differ significantly from those prepared in thigh homogenate.

The lowest level of ampicillin or amoxycillin detectable in undiluted blood samples using *S. lutea* varied from 0.01 to 0.03 $\mu\text{g}/\text{ml}$. Because of the dilution involved in the homogenization of the thighs, the lowest level of antibiotic detectable in the tissue was 0.3 to 0.5 $\mu\text{g}/\text{ml}$.

Determination of minimal inhibitory concentration and bactericidal activity in vitro. A 1:1,000 dilution of an overnight broth culture of the test organism was prepared in nutrient broth (Oxoid, no. 2), and 9-ml volumes were dispensed in tubes. To each tube, 1 ml of appropriate solutions of ampicillin or amoxycillin were added to give a range of twofold dilutions. Tubes were incubated at 37 C, and 0.5 ml was removed at intervals of time and the number of viable bacteria were counted. This was carried out by preparing 10-fold dilutions of the samples in PBS, and 0.02-ml drops of these dilutions were then placed on dried nutrient agar petri dishes. A minimum of eight replicate drops was used and colonies were counted after incubation overnight at 30 C. Turbidity of the tubes was recorded after overnight incubation, and the minimal inhibitory concentration (MIC) was expressed as the minimal concentration of antibiotic which prevented visible growth.

RESULTS

Subcutaneous dosage: infection with *E. coli* strain 9. Results are shown in Fig. 2 for a number of experiments in which the activity of

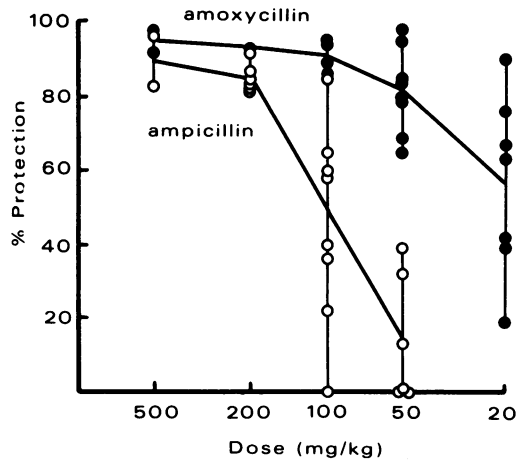


FIG. 2. Activity of ampicillin and amoxycillin in the thigh lesion test by using *E. coli* strain 9. Antibiotics were administered subcutaneously as a single dose at the time of infection to groups of 10 mice. Points represent the results of individual experiments.

ampicillin and amoxycillin was determined in the thigh lesion test using *E. coli* strain 9 as the infecting organism. The antibiotics were administered subcutaneously as a single dose at the time of infection and the therapeutic effect, expressed as percentage protection from thigh enlargement, is plotted against antibiotic dosage. In in vitro tests, no difference could be found in the MIC for ampicillin and amoxycillin with this strain of *E. coli*, the value for both antibiotics being 5 $\mu\text{g}/\text{ml}$. However, in the thigh lesion test using this strain of *E. coli* amoxycillin was considerably more active than ampicillin when given by subcutaneous injection (Fig. 2). At a dose of 50 mg/kg, amoxycillin resulted in 80% protection, and a mean value of over 50% protection was obtained with a dose as low as 20 mg/kg. Ampicillin, however, at a dose of 100 mg/kg was only comparable in activity with amoxycillin at a dose of 20 mg/kg, and a dose of 200 mg/kg was required with ampicillin to give a similar degree of protection to that achieved with amoxycillin at 50 mg/kg.

Results are shown in Fig. 3 for the plasma levels obtained in mice infected with *E. coli* strain 9 after single subcutaneous dosage of ampicillin or amoxycillin at 25, 50, and 100 mg/kg. It will be seen that the plasma levels of the two antibiotics were very similar, particularly at a dosage of 25 and 50 mg/kg. At 100 mg/kg the levels of amoxycillin appeared to be slightly higher than those of ampicillin, but this difference was not great. At no time was the plasma level of amoxycillin at a dose of 50

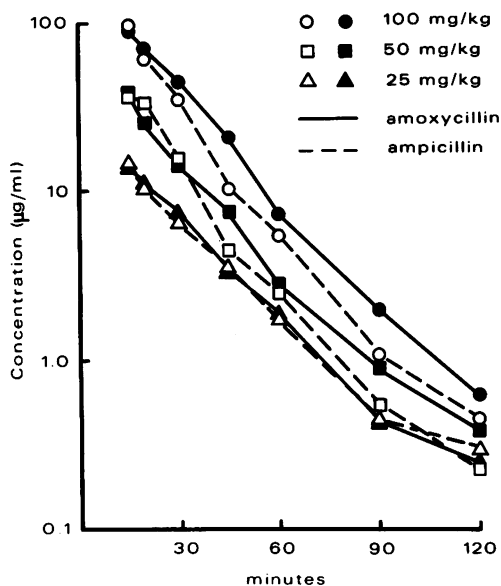


FIG. 3. Plasma levels of ampicillin and amoxycillin in mice infected intramuscularly with *E. coli* strain 9. Antibiotics were administered subcutaneously at the time of infection. Results are the mean of four experiments involving 10 to 23 animals at each time interval.

mg/kg as high as that obtained with ampicillin at 100 mg/kg, whereas the results of the thigh lesion tests in Fig. 2 show amoxycillin to be considerably more active than ampicillin at these respective doses.

Results are shown in Fig. 4 for the antibiotic levels present in the homogenized thigh of mice infected with *E. coli* strain 9 after subcutaneous dosage of ampicillin or amoxycillin. It will be seen that these tissue levels of ampicillin and amoxycillin were very similar indeed, particularly when the antibiotics were given at a dose of 100 mg/kg. At a dose of 50 mg/kg, from 45 min onwards, the levels of amoxycillin were slightly higher than those of ampicillin, but the difference was less than a factor of two. As in the case of the plasma levels, the homogenate levels obtained with amoxycillin at 50 mg/kg were not as high as those obtained with ampicillin at 100 mg/kg, whereas at these dosages amoxycillin was considerably more active therapeutically.

The numbers of viable bacteria present in the thigh of mice infected with *E. coli* strain 9 after subcutaneous dosage with either ampicillin or amoxycillin are shown in Fig. 5. Over the first hour after a single dose of amoxycillin at 50 mg/kg, a marked fall occurred in the number of viable bacteria present in the thigh (Fig. 5A). Between 1 and 2.5 h the viable count remained relatively constant and then increased slowly.

In contrast, the same dosage of ampicillin resulted in only a slight fall in the viable count 1 h after dosage, and thereafter the number of viable bacteria increased at the same rate as seen in the untreated control animals. The plasma concentrations of ampicillin and amoxycillin in the animals in this experiment were very similar, but at 24 h a marked difference in therapeutic effect was seen. In the amoxycillin-treated animals a slight thigh enlargement was present after 24 h, corresponding to 78% protection, whereas in the ampicillin group a much larger thigh size was present, corresponding to only 46% protection. This difference in therapeutic activity was maintained, the protection over the period of 1 to 4 days being 82 and 40%, respectively.

Figure 5B shows the results obtained by using a dose of 100 mg/kg. At this dosage both antibiotics brought about a more marked and a more sustained bactericidal effect than was obtained with a dose of 50 mg/kg. In the amoxycillin group, the viable count continued to fall over the first 2 h, then remained relatively constant until 4 h, and thereafter increased slowly to give a count at 24 h only slightly higher than the number present at the time of infection. In contrast, in the ampicillin-treated group, the viable count fell over the first

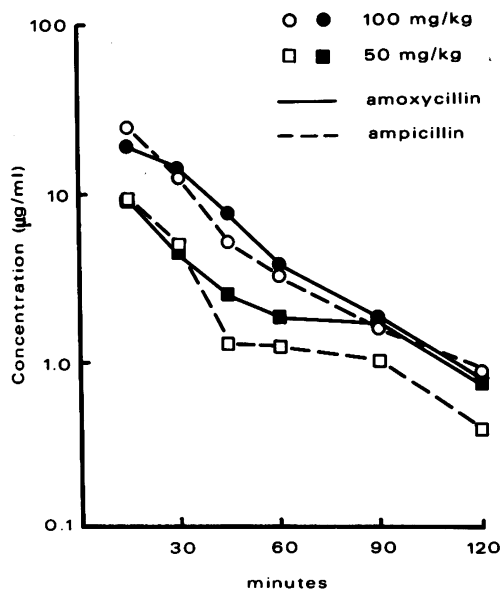


FIG. 4. Levels of ampicillin and amoxycillin in thigh homogenates of mice infected intramuscularly with *E. coli* strain 9. Antibiotics were administered subcutaneously at the time of infection. Results are the mean of four experiments involving 10 to 23 animals at each time interval.

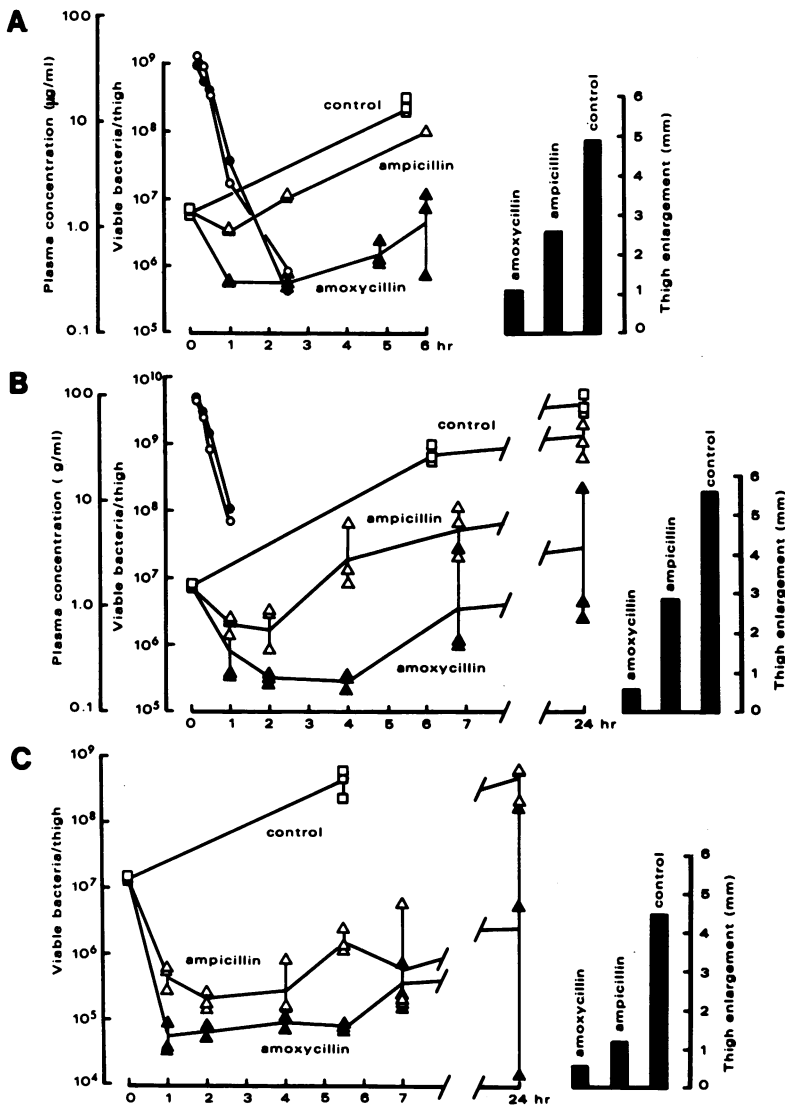


FIG. 5. Thigh enlargement, plasma levels, and numbers of viable bacteria present in the thigh of mice infected with *E. coli* strain 9 after subcutaneous dosage with ampicillin or amoxycillin. Antibiotics were administered at the time of infection at the dosage indicated. Viable counts were made using groups of three mice; the lines show the geometric mean and the points indicate the values for the individual animals. Thigh enlargements are the mean values at 24 h for the group of animals as a whole. Plasma concentration: O, ampicillin; ●, amoxycillin. Viable bacteria per thigh: Δ, ampicillin; ▲, amoxycillin. A, Dosage at 50 mg/kg; B, dosage at 100 mg/kg; C, dosage at 200 mg/kg.

hour, though not so markedly as in the amoxycillin group, and then remained constant up to 2 h, after which time the bacterial numbers increased to give a count at 24 h approaching that in the untreated control animals. The difference in bactericidal effect of ampicillin and amoxycillin was again accompanied by a marked difference in the thigh enlargement. In

the amoxycillin group, after 24 h only a very slight thigh lesion occurred, corresponding to 89% protection, whereas in the ampicillin group a considerable enlargement occurred, corresponding to only 48% protection. Over the period of 1 to 4 days the percentage protection was 93 and 40% for amoxycillin and ampicillin, respectively.

Increase in antibiotic dosage to 200 mg/kg (Fig. 5C) resulted in almost complete prevention of thigh enlargement in the amoxycillin-treated group and only a slight enlargement in the ampicillin group. The percentage protection at 24 h was 92 and 74% for amoxycillin and ampicillin, respectively, and over the period of 1 to 4 days the values were 93 and 82%. A marked bactericidal effect also occurred in both groups, but it will be seen that the rate and the extent of this bactericidal action was more marked with amoxycillin than with ampicillin. This difference in bactericidal effect was apparent not only over the first few hours after dosage but also 24 h later, when the mean viable count in the amoxycillin-treated group was still below that present at the time of infection, whereas in the ampicillin group the mean viable count at 24 h had risen to nearly 10^9 bacteria per thigh.

In the experiments shown in Fig. 2 and 5, the antibiotics were administered as a single dose at the time of infection. Results of thigh lesion tests involving repeated subcutaneous dosage of ampicillin or amoxycillin are shown in Table 2. *E. coli* strain 9 was used as the infecting organism and the antibiotics were administered at the time of infection and again at 2 and 4 h after infection. It will be seen that amoxycillin again showed greater therapeutic activity than did ampicillin.

Infection with *E. coli* strain 8. The MIC of ampicillin and amoxycillin for *E. coli* strain 8 was found to be 2.5 μ g/ml, and repeated serial dilution tests failed to indicate any difference in sensitivity to these two antibiotics. The results of a thigh lesion experiment carried out using this strain of *E. coli* are shown in Fig. 6. It will be seen that a single subcutaneous dose of

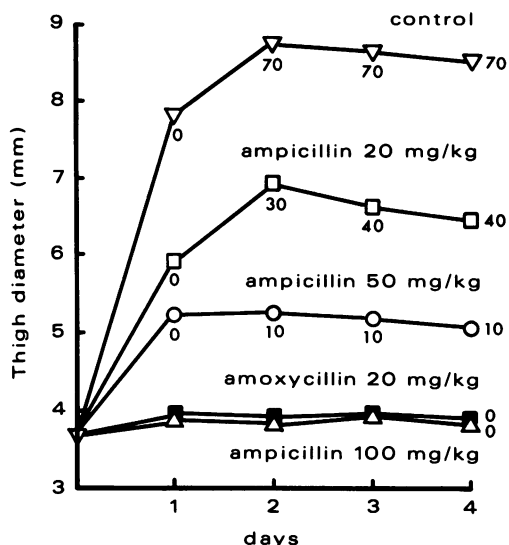


FIG. 6. Therapeutic effect of ampicillin and amoxycillin in the thigh lesion test using *E. coli* strain 8. Antibiotics were administered subcutaneously at the time of infection. Thigh diameter is shown together with percentage mortality at 24-h intervals.

amoxycillin at 20 mg/kg completely prevented the formation of any thigh enlargement, whereas with ampicillin, a dose of 100 mg/kg was required to bring about a comparable effect. The numbers of viable cells of *E. coli* strain 8 in the thigh of mice after subcutaneous dosage with ampicillin or amoxycillin at 50 mg/kg are shown in Fig. 7. As in the experiments with *E. coli* strain 9, amoxycillin exerted a more rapid and a more marked bactericidal effect than did ampicillin, and this was accompanied by a corresponding difference in therapeutic effect in terms of thigh enlargement.

Infection with *P. mirabilis* strain 13. The activity of ampicillin and amoxycillin in the thigh lesion test using a strain of *P. mirabilis* is shown in Table 3. With this strain of *P. mirabilis*, a heavy infection was required to produce a consistent thigh lesion, and single dosage with ampicillin or amoxycillin at the time of infection gave rise to variable results. More reproducible results were obtained with repeated dosage, and in the experiment shown in Table 3 the antibiotics were administered subcutaneously at the time of infection and again at 2 and 4 h after infection. As in the experiments with *E. coli*, amoxycillin was more active than ampicillin in terms of prevention of thigh enlargement. Mortality was also lower in the amoxycillin-treated animals than in the ampicillin group. In *in vitro* tests with this strain of *P. mirabilis*, the

TABLE 2. Activity of ampicillin and amoxycillin by repeated subcutaneous dosage in the thigh lesion test using *E. coli* strain 9^a

Expt	Dosage (mg/kg)						
	Ampicillin			Amoxycillin			
	100	50	25	100	50	25	10
1	66			92			
2		63	18			85	39
3		77	46			85	46
4			30			95	
Mean	66	70	31	92		88	42

^a Antibiotics were administered to groups of 10 mice at the time of infection and at 2 and 4 h later at the dosage indicated. Results indicate percentage protection.

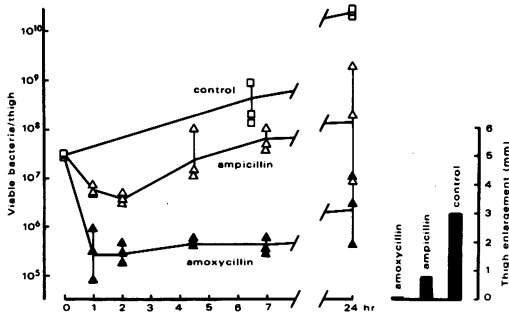


FIG. 7. Thigh enlargement and number of viable bacteria present in the thighs of mice infected with *E. coli* strain 8 after subcutaneous dosage with ampicillin or amoxycillin. Antibiotics were administered at a dose of 50 mg/kg at the time of infection. Viable counts were made using groups of three mice; the lines show the geometric mean and the points indicate the values for the individual animals. Thigh enlargements are the mean values at 24 h for the group of animals as a whole.

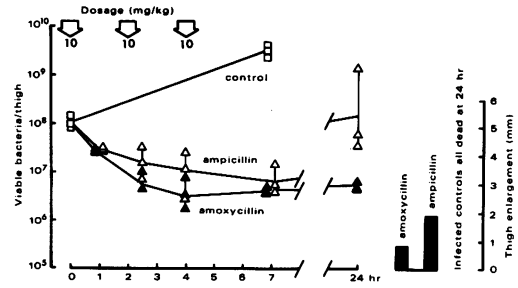


FIG. 8. Thigh enlargement and number of viable bacteria present in the thighs of mice infected with *P. mirabilis* strain 13, after subcutaneous dosage with ampicillin or amoxycillin. Antibiotics were administered at a dose of 10 mg/kg at the time of infection and also at 2 and 4 h after infection. Viable counts were made by using groups of three mice; the lines show the geometric mean and the points indicate the values for the individual animals. Thigh enlargements are the mean values at 24 h for the group of animals as a whole.

TABLE 3. Thigh lesion test in mice using *P. mirabilis* strain 13^a

Description	Dosage (mg/kg)							
	Ampicillin				Amoxycillin			
	20	10	5	2	20	10	5	2
Protection (%)	88	83	40	8	96	91	72	26
No. of mice dead after 48 h	1	1	6	9	0	0	2	7

^a Ampicillin or amoxycillin was administered subcutaneously to groups of 10 mice at time of infection and at 2 and 4 h after infection at the dosage indicated.

MIC of both ampicillin and amoxycillin was 1 µg/ml.

Viable counts of *P. mirabilis* in the infected thigh of mice after subcutaneous dosage with ampicillin or amoxycillin are shown in Fig. 8. The antibiotics were administered at a dose of 10 mg/kg at the time of infection and at 2 and 4 h subsequently. It will be seen that the numbers of viable bacteria were lower in the amoxycillin-treated group, and this was particularly marked at 24 h. At this time the count in the amoxycillin-treated animals had remained low, but in the ampicillin group considerable bacterial multiplication had occurred and this was accompanied by a marked difference in the thigh enlargement.

Oral dosage: infection with *E. coli* strain 9. Results in Fig. 9 show the activity of ampicillin and amoxycillin in the thigh lesion test

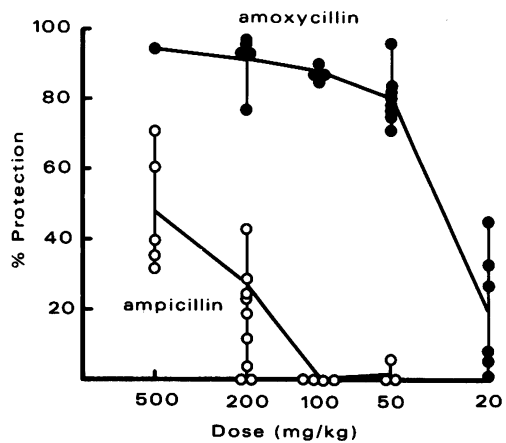


FIG. 9. Activity of ampicillin and amoxycillin in the thigh lesion test using *E. coli* strain 9. Antibiotics were administered orally at the time of infection to groups of 10 mice. Points represent the results of individual experiments.

when the antibiotics were administered by mouth. *E. coli* strain 9 was used as the infecting organism and the antibiotics were administered at the time of infection. It will be seen that amoxycillin was considerably more active than ampicillin. At a dose of 50 mg/kg, amoxycillin resulted in 80% protection from thigh enlargement and showed a slight but measurable therapeutic effect even at a dose as low as 20 mg/kg. With ampicillin, on the other hand, a dose of 50 or 100 mg/kg was without effect on the thigh enlargement and a dose of 200 mg/kg showed only a slight therapeutic effect, compa-

rable with that obtained with amoxycillin at a dose of 20 mg/kg.

When given by mouth, amoxycillin was better absorbed than ampicillin, and the plasma levels obtained in mice after single oral dosage are shown in Fig. 10. It will be seen that ampicillin and amoxycillin showed a similar plasma half-life, but the levels obtained with amoxycillin were considerably higher than those obtained with ampicillin. However, although amoxycillin was certainly better absorbed than ampicillin, the difference in the plasma levels obtained was not as great as the difference in therapeutic effect. For example, the plasma levels obtained with amoxycillin at a dose of 50 mg/kg were similar to those obtained with ampicillin at 200 mg/kg, but at these respective dosages amoxycillin was considerably more effective therapeutically (Fig. 9).

Results are shown in Fig. 11 and 12 in which the effect of oral dosage of ampicillin at 250 mg/kg was compared with oral dosage of amoxycillin at 50 mg/kg. These dosages were chosen in an attempt to achieve similar levels of antibiotic in the blood. It will be seen from Fig. 12,

however, that the levels of amoxycillin both in the plasma and in homogenized tissue were slightly lower than those of ampicillin. Nevertheless, it will be seen from Fig. 11 that a more rapid bactericidal effect occurred in the amoxycillin-treated animals and a corresponding difference was also seen in the therapeutic effect. In the amoxycillin group almost complete protection from thigh enlargement was achieved, whereas in the ampicillin-treated animals after 24 h a considerable thigh lesion occurred, corresponding to only 20% protection.

Bactericidal activity of ampicillin and amoxycillin in vitro. The bactericidal effect of ampicillin and amoxycillin in vitro against *E. coli* strain 8 is shown in Fig. 13. It will be seen that amoxycillin resulted in a more rapid fall in the numbers of viable cells than did ampicillin, the difference in the count at 1 h with 5 and 10 $\mu\text{g/ml}$ being approximately 10-fold. Similar results were obtained with *E. coli* strain 9. With *P. mirabilis* strain 13, amoxycillin was also found to exert a more rapid bactericidal effect, though with this organism the difference between the two antibiotics was not as marked as it was with the strains of *E. coli*.

DISCUSSION

The experiments reported here using an intramuscular infection in mice show amoxycillin to be more active than ampicillin both by oral and by subcutaneous administration. In these experiments, the infecting organisms used showed the same sensitivity to ampicillin and amoxycillin in tests carried out to determine the

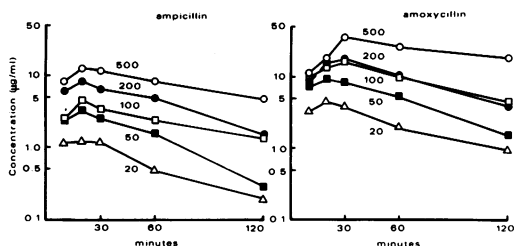


FIG. 10. Plasma levels of ampicillin and amoxycillin after oral dosage in mice. Antibiotics were administered at the dosages indicated (mg/kg). Results are the mean of 10 to 40 animals at each time interval.

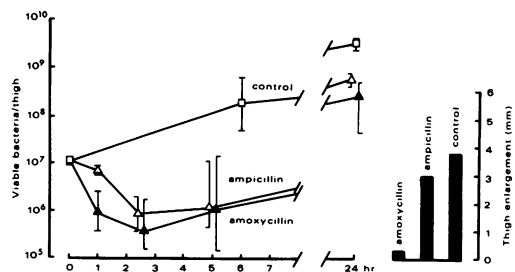


FIG. 11. Thigh enlargement and number of viable bacteria present in the thighs of mice infected with *E. coli* strain 9 after oral dosage of ampicillin at 250 mg/kg or amoxycillin at 50 mg/kg. Antibiotics were administered at the time of infection. Mean viable counts and range of values are shown for groups of 10 mice. Thigh enlargements are the mean values at 24 h for groups of 20 mice.

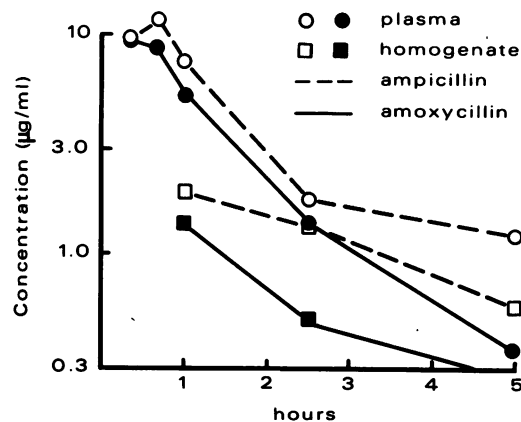


FIG. 12. Ampicillin and amoxycillin levels in plasma and homogenized thighs of the same mice used in the experiment shown in Fig. 11. Ampicillin was administered orally at a dose of 250 mg/kg and amoxycillin at 50 mg/kg. Values shown are the mean of 10 animals.

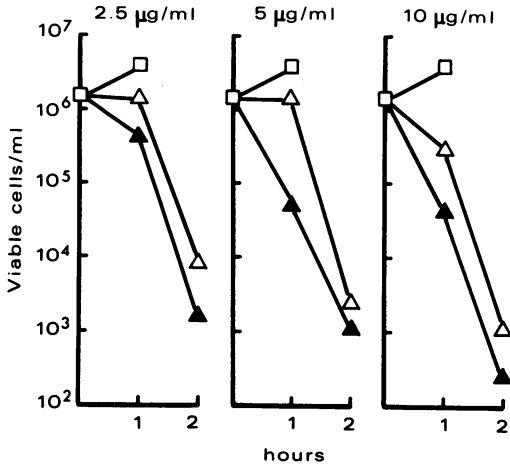


FIG. 13. Bactericidal activity of ampicillin and amoxycillin *in vitro* using *E. coli* strain 8. Viable counts were carried out at 37 C in nutrient broth containing antibiotic at the concentration shown. Symbols: □, control; △, ampicillin; ▲, amoxycillin.

MIC. The difference in activity observed *in vivo*, therefore, cannot be explained on the basis of a difference in sensitivity as measured by using conventional serial dilution tests. Similarly, the superior therapeutic activity of amoxycillin compared with ampicillin in these experiments cannot be accounted for on the basis of higher levels of antibiotic in the animal body. By subcutaneous administration, the peak levels of ampicillin and amoxycillin both in the blood and in the tissue homogenate were very similar indeed, and although the levels of amoxycillin tended to fall rather more slowly than those of ampicillin, this difference was slight and would seem unlikely to account for the difference in therapeutic activity. For example, although the plasma and tissue levels obtained with amoxycillin at a dose of 50 mg/kg were slightly higher than those obtained with the same dose of ampicillin, the levels were clearly lower than those obtained with ampicillin at a dose of 100 mg/kg, but in the thigh lesion tests the therapeutic effect of ampicillin at 100 mg/kg was considerably less than that of amoxycillin at a dose of 50 mg/kg. Furthermore, in experiments in which direct comparisons were made of subcutaneous dosage of amoxycillin at 25 or 50 mg/kg with ampicillin dosage at 50 or 100 mg/kg, greater therapeutic activity and a more marked bactericidal effect was seen with amoxycillin despite the lower dosage.

From the results of the counts of the number of viable organisms present in the infected

tissue, it would appear that the difference in therapeutic activity between these two penicillins is related to the bactericidal action of the drugs *in vivo*, that of amoxycillin being more rapid and more marked than that of ampicillin against the organisms used in these experiments.

When administered by the oral route, amoxycillin is absorbed more completely than ampicillin, resulting in substantially higher levels of antibiotic in the blood, and this may account in part for the greater therapeutic effect obtained with amoxycillin when the antibiotics are given by mouth. However, when appropriate oral dosages of ampicillin and amoxycillin were used which resulted in similar levels of antibiotic in the blood and tissue, amoxycillin was again found to be therapeutically more effective than ampicillin. These results after oral dosage are in agreement with those obtained with subcutaneous administration and show that despite similar levels of antibiotic in the body, amoxycillin exerted a more rapid bactericidal effect than did ampicillin, and it is suggested that this difference in bactericidal effect is responsible for the superior therapeutic activity obtained.

A difference in the rate of bactericidal action between ampicillin and amoxycillin could also be seen in experiments carried out *in vitro*. Whether this difference *in vitro* accounts entirely for the superior therapeutic activity of amoxycillin *in vivo* is not certain.

The experiments reported here all involve infection with *E. coli* or *P. mirabilis*. Work is in progress using other bacteria as infecting organisms. Preliminary experiments with a strain of *Staphylococcus aureus* in the thigh lesion test indicate amoxycillin to be slightly more active than ampicillin when administered subcutaneously, though the difference is not as marked as it is with *E. coli* or *P. mirabilis*.

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