nificant erythropoietic response in polycythemic mice, this is an extremely high dose and normal female mice injected with 125 μg daily for 15 days had no increase of RCV (unpublished).

The erythropoietic effect of prolactin does not require the mediation of the testes(2). Estrogens(8), progesterone and growth hormone(2) have not been shown to stimulate erythropoiesis in the endocrinologically intact animal. Of the hormones not investigated, erythropoietin (ESF) cannot be excluded as a possible cause of the observed erythropoietic activity in lactating mice. Since the kidney is the major site of ESF production and prolactin has been shown to increase the glomerular filtration rate and renal plasma flow(9), the interrelationship of prolactin and ESF production is under investigation.

The decrease of RCV in nonlactating mice was more rapid and greater than could be explained by cessation of erythropoiesis alone. The associated increase of the splenic weights of these mice suggests that sequestration of erythrocytes in the spleen and their subsequent destruction early postpartum, may have been responsible in part or in whole for this rapid decrease of RCV.

Summary. RCV and plasma erythropoietic activity were significantly increased throughout lactation in the mouse as compared with normal female mice and with nonlactating postpartum mice. The RCV decreased and plasma erythropoietic activity disappeared rapidly in nonlactating mice. Prolactin stimulated erythropoiesis in postpartum nonlactating mice and polycythemic mice while adrenal steroids and thyroid hormone were ineffective.

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Effect of Sublethal Concentrations of Penicillins on the Lysis of Bacteria by Lysozyme and Trypsin. (30573)

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Previous studies from this laboratory(1-3) demonstrated that when strains of *Staphylococcus aureus* were grown in the presence of sublethal concentrations of nafcillin, the normally lysozyme-resistant *S. aureus* was rendered susceptible to partial lysis by lysozyme. Subsequently it was found that the organisms were almost completely lysed if trypsin and lysozyme were added successively to the cell suspensions(2). The precise relationship between the concentrations of nafcillin required to influence lytic response and to prevent growth has not been examined in detail; however, the results obtained with a number of semisynthetic penicillins suggest significant quantitative differences between the ability of nafcillin and the isoxazole penicillins to render *S. aureus* susceptible to enzymic action. Our observation has prompted speculation that these antibiotics may involve somewhat different mechanisms and/or primary sites of action on cell wall synthesis and that sublethal levels which inhibit synthesis of the mucopeptide polymer may play a role in pathogenesis by rendering the susceptible bacteria vulnerable to enzymic lysis.

Lysozyme, which is present in body fluids and in rather high concentrations in leukocytes, may alter bacterial cells previously exposed to small amounts of specific antibiotics. Thus the enzymes may create a tissue environment in the host that is unfavorable to the parasites.

The present work was undertaken to obtain further evidence on the mode of action of nafcillin on *S. aureus* and to explore lytic response with enzymes as a method for differentiating antibiotics that are otherwise identical in ability to inhibit susceptible organisms. In an effort to determine the relationship between the inhibitory concentration and the inhibition of mucopeptide synthesis in *S. aureus,* particular attention was given to the factors which may influence lysis of bacterial cells previously exposed to various antibiotics. The specificity of the lytic effect was also investigated by determining the influence of nafcillin on the fate of the cell wall of other organisms sensitive to this penicillin. All experiments involved a comparison of the lytic effect on susceptible organisms previously propagated in the presence of concentrations of antibiotic which did not interfere appreciably with growth.

Materials and methods. Bacterial strains. The following organisms were used in this investigation: *S. aureus* strain CHP; *Neisseria catarrhalis* (ATCC No. 8193); *Streptococcus faecalis* (ATCC No. 6057); *Sarcina lutea* (ATCC No. 9341); *Diplococcus pneumoniae* strain 37, type 1; and *Gaffkya tetragena* (ATCC No. 10875).

Media and cultural conditions. In general, the same preparations were used in this study as reported previously(1-3). The bacteria were grown in Blake bottles containing 200 ml of medium. *D. pneumoniae* was cultivated on blood agar base supplemented with 5% defibrinated sheep's blood. All other bacteria were grown on brain-heart infusion agar (Baltimore Biological Laboratories) adjusted to pH 7.6-7.8. Following sterilization, the bottles were seeded with 1.0 ml of a saline suspension of an 18-hr nutrient agar or blood agar culture standardized to contain 1.08×10^9 bacteria per ml and then incubated for 18 hours at 37°C.

Antibiotics. Nafcillin, oxacillin, cloxacillin, methicillin, cephalothin, novobiocin, vancomycin, chloramphenicol, tetracycline hydrochloride, erythromycin and penicillin G were used. Stock solutions in sterile buffered 0.85% salt solution were stored at —20°C, thawed, and final dilutions prepared and added to previously liquified media immediately before use. Unless otherwise stated, the highest concentrations of each antibiotic which permitted appreciable growth to occur were incorporated into the medium.

Enzyme preparations. Crystalline egg white lysozyme (Armour) and crystalline trypsin (Worthington Laboratories) dissolved in 0.1 M phosphate buffer, pH 7.0, were used.

Determination of enzymic lysis. Unless otherwise noted, the lytic action was studied with the following preparations: (1) untreated bacteria suspended in 0.1 M phosphate buffer, pH 7.0; (2) cell suspensions which had previously been grown in sublethal levels of the various antibiotics. Cells were harvested from the medium with distilled water and centrifuged; the deposits were washed twice with distilled water and suspended in 0.1 M phosphate buffer, pH 7.0.

The Klett-Summerson photoelectric colorimeter with a red filter (No. 66) was used throughout the investigation for measuring turbidities. Suspensions of bacteria were adjusted to final Klett readings of 400 and placed in a water bath at 37°C. To these tubes 1.0 ml of lysozyme or trypsin in 1.0 ml of buffer was added and the contents thoroughly mixed. The tubes were incubated for various time intervals in a 37°C water bath and were then read for changes in optical density. The percentage of lysis was calculated as described by Kohn(4).

Antibiotic sensitivity test. Minimal inhibitory concentrations (MIC) were determined by the conventional agar-diffusion procedure in brain-heart infusion agar on Blake bottles. The same inoculum size and other experimental conditions that were described for evaluating the influence of an antibiotic on the lytic response with lysozyme and trypsin were employed.

Plate counts. Viable count determinations were made by serial dilution in distilled water

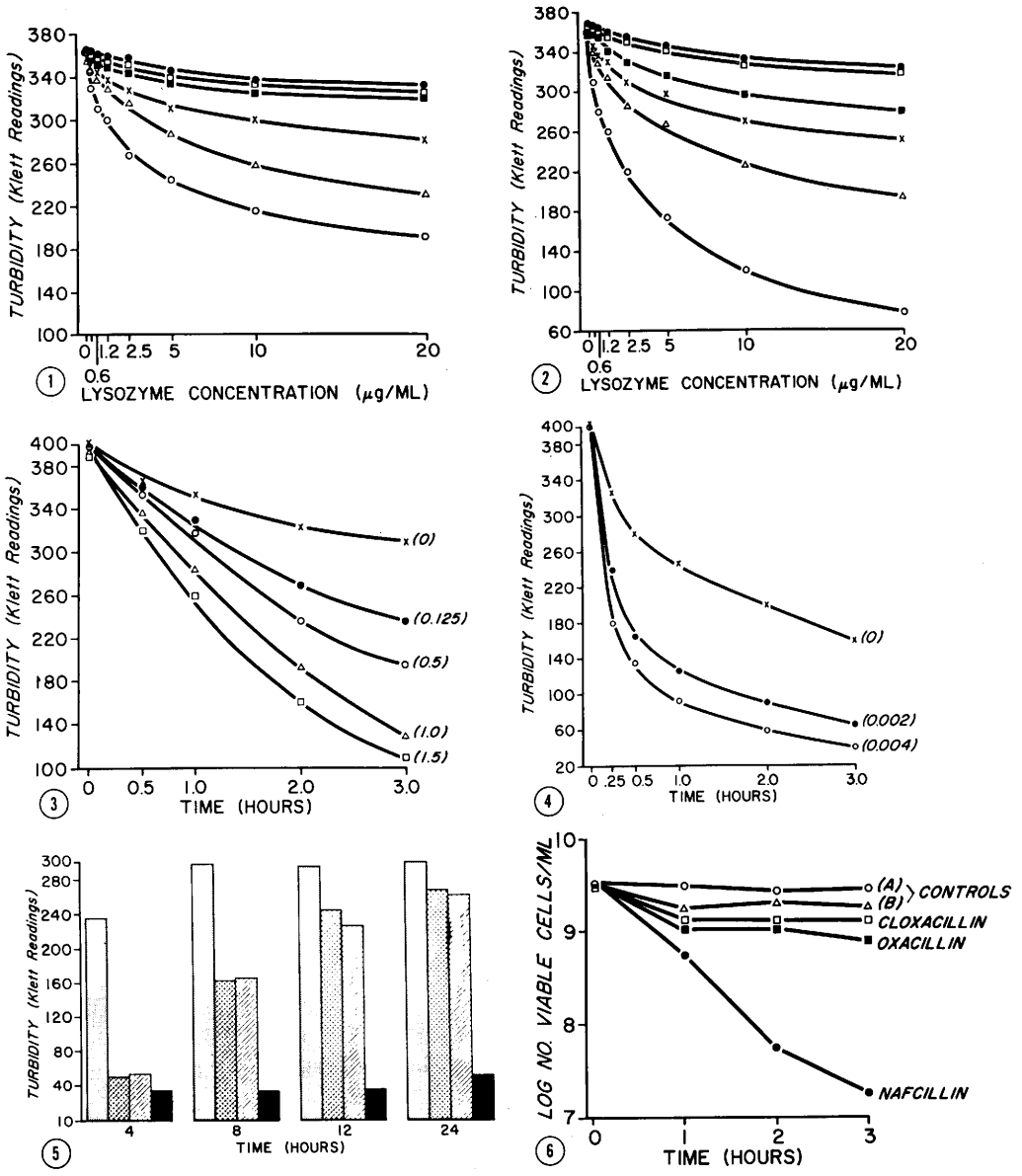

FIG. 1. Lysis of *S. aureus* strain CHP grown in medium containing sublethal concentrations (0.1 μg/ml) of antibiotics. ●—●, control; □—□, cephalothin; ■—■, cloxacillin; ×—×, oxacillin; △—△, methicillin; ○—○, nafcillin. Cell suspensions + enzyme incubated 3 hr at 37°C.

FIG. 2. Lysis of *S. aureus* strain CHP grown in medium containing sublethal concentrations (0.1 μg/ml) of antibiotics. Fixed concentration of trypsin (10 μg/ml) added to each system. ●—●, control; □—□, cephalothin; ■—■, cloxacillin; ×—×, oxacillin; △—△, methicillin; ○—○, nafcillin. Cell suspensions + enzymes incubated 3 hr at 37°C.

FIG. 3. Lysis of nafcillin-treated suspensions of *Strep. faecalis* Strain 6057 by lysozyme (20 μg/ml) and trypsin (10 μg/ml). Figures in parentheses denote concentration of nafcillin in μg/ml. Cell suspensions + enzymes incubated 3 hr at 37°C.

FIG. 4. Lysis of nafcillin-treated suspensions of *D. pneumoniae* Type 1 Strain 37 by lysozyme (20 μg/ml) and trypsin (10 μg/ml). Figures in parentheses denote concentrations of nafcillin in μg/ml. Cell suspensions + enzymes incubated 3 hr at 37°C.

FIG. 5. Effect of age of penicillin-treated *S. aureus* strain CHP in relation to lytic response with lysozyme (20 μg/ml) and trypsin (10 μg/ml). Stippled, control; criss-crossed,

TABLE I. Sensitivity to Antibiotics of a Penicillinase-Producing Strain of *Staphylococcus aureus* (CHP).

Antibiotic	Minimal inhibitory conc,* μg/ml
Tetracycline HCl	2.0
Erythromycin	.8
Vancomycin	4.0
Novobiocin	.64
Chloramphenicol	8.0
Cephalothin	.8
Nafcillin	.8
Cloxacillin	.8
Oxacillin	.8
Methicillin	6.4
Penicillin G	>100.0

* Estimated on brain-heart infusion agar with 1.08×10^9 bacteria.

and plating in brain-heart infusion agar. Colonies were counted 24 hours after inoculation.

Results. Effect of antibiotics on growth of S. aureus. Extensive tests in our laboratory have fairly well established that lysis of nafcillin-treated suspensions of staphylococcal cells by lysozyme and trypsin increases progressively with the concentration of the penicillin short of the MIC. The lytic effect was determined on staphylococci previously propagated in the presence of antibiotic concentrations not interfering appreciably with growth. It was therefore considered of interest to determine the MIC values for the antibiotics studied in the lytic system and to ascertain whether there was a relationship between the concentration of the antibiotic which interfered with growth and the sublethal concentration that rendered the cells susceptible.

Table I shows the MIC values of nafcillin, oxacillin, cloxacillin, methicillin, cephalothin, novobiocin, vancomycin, chloramphenicol, tetracycline hydrochloride, erythromycin and penicillin G required for a penicillin-resistant strain of *S. aureus* (CHP). It can be noted that the staphylococcal strain was inhibited by essentially the same quantity of nafcillin, oxacillin, cloxacillin and cephalothin. Although novobiocin and erythromycin showed the same order of activity as the semisynthetic penicillins and cephalothin, the other antibiotics required greater concentrations to inhibit this organism.

Action of lysozyme on antibiotic-treated suspensions. The action of lysozyme on *S. aureus* grown in the presence of sublethal concentrations of a number of penicillins and cephalothin is shown in Fig. 1. Turbidity readings of enzyme-treated control suspensions showed no significant differences. The addition of increasing concentrations of lysozyme to nafcillin-treated cells of *S. aureus* caused a progressive increase in lysis. It may be noted that incubation of suspensions of methicillin- and oxacillin-treated cells resulted in smaller but consistent lysis. By contrast, when lysozyme was added to cloxacillin- and cephalothin-treated suspensions, no appreciable turbidity decrease was observed.

Action of lysozyme and trypsin on antibiotic-treated suspensions. The course of lysis of *S. aureus* after treatment with various antibiotics and incubation with lysozyme and trypsin is illustrated in Fig. 2. The enzyme mixture brought about a rapid decrease in turbidity of nafcillin-treated cells; no such change occurred in control cells exposed to similar concentrations of enzymes. Repeated attempts to detect lysis of antibiotic-treated cells during extended periods of incubation with trypsin alone were unsuccessful. Thus the simultaneous action of the enzymes resulted in an enhanced response. This response was much greater with nafcillin than with comparable concentrations of methicillin, oxacillin, cloxacillin or cephalothin.

Effects of nafcillin and other antibiotics on enzymic lysis. Since nafcillin, other penicillins and cephalothin rendered staphylococcal cells susceptible in varying degrees to enzymic lysis, the question arose as to whether or not other antibiotics whose action results in impairment of the structure or synthesis of the bacterial cell wall of *S. aureus* likewise alters the integrity of the cell wall of this organism and renders such cells vulnerable to lysis by lysozyme and trypsin. In addition

oxacillin; diagonal, cloxacillin; solid, nafcillin.

FIG. 6. Changes in viable population of penicillin-treated *S. aureus* strain CHP incubated 3 hr at 37°C with lysozyme (20 μg/ml) and trypsin (10 μg/ml). Control (A), cells in 0.1 M phosphate buffer alone; control (B), cells incubated with lysozyme and trypsin.

TABLE II. Effect of Antibiotic Exposure on Enzymic Lysis of *S. aureus* Strain CHP.

Antibiotic	Conc (μg/ml)	% Lysis* Enzyme reaction time 3 hr	24 hr
Erythromycin	.1	24	55
Chloramphenicol	4.0	20	38
Tetracycline HCl	.5	27	35
Vancomycin	1.0	25	60
Cephalothin	.1	25	57
Novobiocin	.1	31	77
Methicillin	.1	53	78
Nafcillin	.1	83	96
Oxacillin	.1	40	67
Cloxacillin	.1	38	65
Control	—	25	38

* Buffered cell suspensions incubated with lysozyme (20 μg/ml) and trypsin (10 μg/ml). Control cells incubated with similar amounts of each enzyme.

the specificity of the antibiotic effect on cell wall synthesis was determined by the investigation of other antibiotics whose primary effect is inhibition of protein synthesis, *e.g.*, tetracycline hydrochloride, chloramphenicol, erythromycin. In particular, it was of interest to determine whether or not sublethal concentrations of these antibiotics could produce an alteration in the integrity of the cell, thereby making such cells accessible to the action of the enzymes. To determine whether prolonged incubation of the bacterial suspensions with the enzyme could influence the lytic response, the reaction mixtures were incubated for both 3 and 24 hours at 37°C. Since *S. aureus* strain CHP was inhibited by the same quantities of the semisynthetic penicillins, cephalothin, novobiocin and erythromycin, it was considered expedient for comparative purposes to use a fixed concentration (0.1 μg/ml) of these antibiotics in this study.

The comparative effects of sublethal concentrations of various antibiotics on the lytic response of *S. aureus* are presented in Table II. Of the antibiotics that have been reported to interfere with cell wall synthesis of susceptible bacteria, with the 3-hour incubation, only the penicillins were found to influence lysis of cell suspensions. For methicillin and the isoxazole penicillins the lytic response ranged between 38 and 53%. A comparable concentration of nafcillin resulted in a percentage lysis of 83%. Attempts to render *S. aureus* susceptible to enzymic lysis by growth in the presence of chloramphenicol, tetracycline hydrochloride and erythromycin were unsuccessful.

Incubation of antibiotic-treated suspensions of *S. aureus* with enzymes for 24 hours resulted in an enhancement of the lytic response for the majority of the antibiotics studied. Thus, pretreatment with novobiocin, vancomycin, cephalothin, methicillin, oxacillin and cloxacillin, which had supported either no lysis or only limited lysis with the 3-hour incubation, now gave significant lysis. The difference in relative concentrations of methicillin needed for lysis and inhibition was interesting. For inhibiting *S. aureus,* methicillin required 8 times the concentration of oxacillin or cloxacillin on a weight for weight basis, whereas for influencing lysis it was effective at concentrations similar to those of the isoxazole penicillins. Of the antibiotics which inhibit protein synthesis, only erythromycin was able to activate enzymic lysis, and then only following a prolonged incubation period with the enzymes.

Action of lysozyme and trypsin on other bacteria. Since nafcillin is bactericidal to a number of Gram-positive bacteria other than *S. aureus,* several of these organisms were included in this study. It appeared likely that the growth of these organisms in the presence of sublethal concentrations of nafcillin might also result in extensive lysis following the addition of lysozyme and trypsin. Fig. 3 and 4 present the results of typical experiments with *Streptococcus faecalis* and *Diplococcus pneumoniae,* respectively. *Str. faecalis* and *D. pneumoniae* both showed appreciable enzymic lysis when cells were grown in the presence of nafcillin, and this lysis appeared to be dose dependent. However, *D. pneumoniae* also showed appreciable lysis with lysozyme and trypsin even when nafcillin was omitted from the culture medium.

Additional organisms, sensitive to nafcillin in concentrations of 0.015 to 2.0 μg/ml, (*e.g., Neisseria catarrhalis, Sarcina lutea* and *Gaffkya tetragena*) were studied under conditions similar to those used with the other organisms. However, no lysis was detected on

TABLE III. Effect of pH on Enzymic Lysis of *S. aureus* Strain CHP.

	pH of reaction mixture			
	5.0	6.0	7.0	8.0
Antibiotic*	% Lysis			
Nafcillin	37	90	86	78
Methicillin	41	80	45	31
Oxacillin	40	65	38	22
Cloxacillin	38	55	24	27
Cephalothin	39	42	23	15
Vancomycin	39	36	22	12
Novobiocin	40	42	25	18
Control	29	39	18	12

* Cells grown for 18 hr in presence of antibiotic at 0.1 μg/ml. Suspensions washed as described in *Materials and methods*. Buffered suspensions incubated with lysozyme (20 μg/ml) and trypsin (10 μg/ml) for 3 hr at 37°C.

incubation with the enzymes. Nafcillin-treated cells of *G. tetragena* did show a rapid decrease in turbidity, in comparison with the control suspensions, after 3-hour incubation with the enzymes and change of the ionic strength of the buffer system to .01 M. *S. lutea* and *N. catarrhalis* continued to be refractory to lysis, and only after addition of alkali (pH 10) did the cell suspension clear.

Effect of age of culture. In previous work (1-3) the cells used for preparing bacterial suspensions to study enzymic lysis were obtained from 18-24-hour cultures. In this series of experiments the enzymic lysis of cells of *S. aureus* was measured during the logarithmic and decelerating growth phases. When cultures were incubated with either oxacillin, cloxacillin or nafcillin for 4 hours, significant lysis of cells exposed to lysozyme and trypsin was observed. However, when the incubation period was extended, the sensitivity of the oxacillin- and cloxacillin-treated cells to the enzymes progressively decreased. On the other hand, cultures incubated with a comparable concentration of nafcillin lysed to the same degree regardless of time of incubation (Fig. 5).

Optimal pH for enzymic lysis. A uniform, washed suspension of antibiotic-treated cells of *S. aureus* strain CHP was buffered to various pH values with 0.1 M acetate (pH 5-6) or 0.1 M phosphate (pH 7-8). The lytic values are shown in Table III. At pH 5.0 little or no difference in lytic response as compared with the control suspensions was demonstrated for the antibiotics studied. The greatest lytic response (86-90%) for nafcillin-treated cells occurred between pH 6.0 and 7.0, and this response decreased to 78% at pH 8.0. Lytic activity for methicillin and the isoxazole penicillin-treated cells was found to be maximal at pH 6.0 (55-80%), but when the pH was increased above 6.0, the lytic response decreased considerably. However, the lytic response of *S. aureus* grown in the presence of similar concentrations of cephalothin, vancomycin and novobiocin was little different from control. Control suspensions which were not exposed to antibiotic but were incubated with comparable concentrations of lysozyme and trypsin also showed peak lytic activity at pH 6.0. No significant clearing of antibiotic-treated suspensions occurred when such suspensions were incubated without the enzymes.

Effect of lysozyme and trypsin on viability. Since some bacteria are killed but not lysed by lysozyme(5), viable counts of *S. aureus* were made of cells exposed to sublethal doses of oxacillin, cloxacillin and nafcillin and treated with lysozyme and trypsin. Fig. 6 depicts the number of viable organisms determined at various intervals during the same period in which the optical measurements were recorded (Fig. 2). A distinct difference was noted between the isoxazole penicillins and nafcillin in influencing the enzymic lysis of cell suspensions. A remarkable effect of lysozyme and trypsin was evident from the rapid drop in viable count, which continued to decrease with time and reached a low level after a 3-hour incubation. In contrast, the viable count remained quite high for the isoxazole penicillin-treated cells and only a small drop in viable count occurred. In general, the viable count correlated well with the turbidity readings of enzyme-treated cell suspensions.

Discussion. The results of the present work confirm earlier reports(1-3) that sublethal amounts of a penicillin may have profound effects on the *S. aureus* cell wall. These effects, which include the loss of resistance to lysis with lysozyme and trypsin, can be explained in terms of an alteration or disor-

ganization of the cell wall by antibiotic concentrations inadequate to interfere appreciably with the growth of the organism, followed by a consequently increased sensitivity of the cell to enzymic lysis.

A study of the effects of a number of penicillins and other antibiotics on the phenomenon observed revealed that the lytic response of bacterial cells is not conditioned by the *in vitro* activity of the antibiotics. Thus, novobiocin, cephalothin, oxacillin, cloxacillin and nafcillin on a weight-for-weight basis possessed the same *in vitro* antibacterial activity against a penicillin-resistant strain of *S. aureus*. But in comparable concentrations these antibiotics failed to influence *S. aureus* sensitivity to lysis with lysozyme and trypsin to the same extent.

The superiority of nafcillin over isoxazole penicillins in facilitating lytic response suggests that this agent exerts a pronounced effect on the integrity of the mucopeptide that is synthesized and that the structural configuration may play an important role in the penicillin's ability to interfere with cell wall synthesis. The experiments reported here also revealed that different penicillins may produce their antibacterial effects by somewhat different mechanisms and/or primary sites of action and that the action on cell wall synthesis may be independent. Thus methicillin, which on a weight basis required 8 times the concentration of the isoxazole penicillins to inhibit *S. aureus* strain CHP, activated a better enzymic response than the isoxazole penicillins when similar levels of each penicillin were used.

The prolonged incubation of antibiotic-treated cells of *S. aureus* with lysozyme and trypsin was found to produce an enhanced lytic response. It was particularly interesting to find that antibiotics (erythromycin, vancomycin, cephalothin, novobiocin) which did not significantly influence lysis after a 3-hour incubation period achieved an appreciable lysis following a 24-hour period. There is apparently a direct relationship between the extent to which the antibiotic produces a physical disorganization of the bacterial cell and the accessibility of the substrate(s) to lysozyme and trypsin, thereby permitting different thresholds of lysis. Regardless of the mechanism, the experiments demonstrate that enzymic lysis of bacterial cells exposed to sublethal amounts of an antibiotic is a phenomenon secondary to the alteration of the cell wall.

Significant lysis of oxacillin- and cloxacillin-treated cells of *S. aureus* occurred with lysozyme and trypsin only during the phase of logarithmic multiplication; thereafter, the lytic response decreased rapidly. In contrast, virtually no change in the lytic response was observed for nafcillin-treated cells throughout the 24-hour incubation period. These results suggest that sublethal concentrations of the isoxazole penicillins render the bacterial surface less permeable to the enzymes during the later phases of the growth curve, possibly through a rapid repair or recovery of the integrity of the cell wall. Since differences in the bactericidal and bacteriostatic properties of nafcillin and the isoxazole penicillins against *S. aureus* were ruled out by previous studies(2,3), the lesser influence of the isoxazole penicillins on enzymic lysis can be explained only by assuming they selectively affect some vulnerable components of the bacterial cell not necessarily associated with cell wall synthesis.

The observations concerning the influence of sublethal concentrations of penicillins on enzymic lysis may have potential clinical implications that require consideration. Organisms grown in sublethal concentrations of various antibacterial agents are frequently phagocytosed more efficiently than organisms grown in normal media, presumably because they are not able to synthesize surface components which counteract phagocytosis(6). For example, it has been shown that sublethal concentrations of penicillin markedly reduced precipitable A and M protein in group A streptococci and induced changes in virulence(7). It is reasonable to conclude, therefore, that the action of a penicillin in disorganizing the structures of the bacterial cell wall results in a host environment favorable to phagocytosis. Moreover, since lysozyme is found in rather high concentration in leukocytes, tissues and body fluids, it is conceivable that it may alter bacterial cells

previously exposed to small amounts of the antibiotic and render them more susceptible to the host response. The fact that nafcillin, of the penicillins studied, showed the highest index of activity in disorganizing the cell walls of susceptible cells and subsequently rendering them vulnerable to lysis by lysozyme and trypsin, might account for its superior therapeutic activity against experimental infections in mice(8). To what extent, if any, this property of nafcillin contributes to its therapeutic efficacy in clinical situations remains to be clarified.

Summary. Resistance of *Staphylococcus aureus* and other bacteria to lysis by lysozyme and trypsin is abolished in cultures grown in sublethal concentrations of antibiotics. Antibiotics which inhibit synthesis of the mucopeptide polymer of bacterial cell walls differ widely in their ability to influence the lysis of cell suspensions. Such differences are revealed by both the speed of reduction of turbidity and final level of turbidity reduction. Under the influence of nafcillin, cells of *Staphylococcus aureus, Streptococcus faecalis* and *Diplococcus pneumoniae* are readily lysed but *Neisseria catarrhalis, Sarcina lutea* and *Gaffkya tetragena* are resistant, suggesting that bacterial cells differ in resistance or susceptibility to enzymic lysis. Lysis is not conditioned by the *in vitro* antibacterial activity of the antibiotics; nafcillin, which has the same activity against *Staphylococcus aureus* as oxacillin, cloxacillin, cephalothin, novobiocin and vancomycin, gives a greater rate and extent of lysis than the other antibiotics when used in comparable concentrations. Of the antibiotics interfering with protein synthesis, only erythromycin gives a significant lytic response. The results suggest that sublethal levels of antibiotics may mediate a favorable interplay between the host tissue environment and the parasite.

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Dimethyl Sulfoxide (DMSO) as a Solvent in Acute Toxicity Determinations. (30574)

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Reports relating to various pharmacological effects of dimethyl sulfoxide (DMSO) have appeared recently in the literature. A review by Block(1) cites references for both physiological and chemical properties of this compound.

Some of the studies showed DMSO to influence permeability and absorption of drugs. For this reason it was decided to investigate the effects of this compound when used as a solvent for toxicity (LD_{50}) determinations. Some data in this area have already been presented(2,3).

Ten quaternary ammonium salts were selected for test because as a class they are poorly absorbed by the oral route. Further selection was made on the basis of physiological activity: 2 ganglionic blocking agents, 2 muscle relaxants, 2 "antispasmodics," 2 parasympathomimetics and 2 cationic germicides were employed.

Methods. Both male and female albino