A STUDY ON THE MECHANISM OF ACTION OF PENICILLIN AS SHOWN BY ITS EFFECT ON BACTERIAL MORPHOLOGY

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Although the exact mechanism by which penicillin exerts its antibacterial effect is not clear, some light may be thrown on this fundamental problem by a consideration of the alterations that it produces in the morphology of susceptible bacteria.

Gardner (1940) was the first to point out the striking changes in shape of bacteria after exposure to varying concentrations of penicillin. *Clostridium welchii*, *Eberthella typhosa*, *Vibrio cholerae*, and even *Escherichia coli* all showed elongation and swelling of the bacterial cells. Staphylococci became spherically enlarged, and streptococci showed both enlargement of the cells and increased length of the chains. It was his interpretation that growth proceeds in the bacteria but that there is failure of fission, normal division does not follow, and many cells then autolyze. He observed these changes in bacteria at dilutions of penicillin well above those which were completely inhibitory to their growth. For this reason he felt that some action might be expected of penicillin *in vivo* at concentrations much lower than those needed for complete inhibition.

Since this interesting observation of Gardner, many others have noted similar morphologic changes in bacteria. Fleming (1941), in a discussion of the bacteriostatic effect of penicillin, referred to the fact that in low dilutions it affects the morphology of bacteria and interferes with their division. Smith and Hay (1942) noted a marked increase in size of staphylococci exposed to inhibiting concentrations of penicillin and found that the cells then underwent lysis, leaving behind a granular material in the sediment. This swelling and lysis were apparently only associated with the *active growth* of the staphylococci, suggesting to the authors that penicillin either acts directly on the cellular wall or prevents the assimilation of certain growth factors necessary for actual fission of the growing cell. *Fully grown* suspensions of the organisms did not show these changes, even when high concentrations of penicillin were used. Weiss (1943) noted similar changes in size and shape of staphylococci, streptococci, and *Clostridium welchii* and demonstrated these in electron micrographs. Dubos (1944), in a review of the action of various antimicrobial agents, classifies the action of penicillin as bacteriostatic and feels that its production of giant forms in the inhibited cells is evidence that it may affect some step of cellular division rather than "a catabolic process measured by respiration." The studies of Miller *et al.* (1944) on gonococcal urethritis demonstrate the fact that the same type of reaction seems to take place *in vivo*, as greatly enlarged gonococci were found in phagocytes from urethral smears as early as 2 hours after intramuscular

injection of penicillin. The meningococcus was found to be similarly affected *in vitro* (Miller and Foster, 1944). The observations of many workers support the conclusion that young, rapidly growing organisms are the ones which are the most susceptible to the action of penicillin, and that substances in the media which enhance the growth of the bacteria seem to render them more sensitive to its action (Hobby *et al.*, 1942; Bigger, 1944; Hobby and Dawson, 1944; Lee *et al.*, 1944; Miller and Foster, 1944; Rantz and Kirby, 1944). As a general rule the corollary to this is also true, for if the bacteria are inoculated into a medium which is lacking in some of the growth requirements, the efficiency of the penicillin is definitely lowered. An exception to this general rule has been pointed out by Garrod (1945), for he found that penicillin was more active at a temperature of 42 C—although growth of the bacteria had stopped—than it was at 37 C.

Most of these authors likewise noted the occurrence of lysis in their cultures as a further step in the action of penicillin. This was particularly true in the case of the staphylococcus, though it was found to occur in other organisms also. Todd (1945) observed bacteriolysis in all the penicillin-sensitive organisms tested, including the pneumococcus, viridans streptococcus, hemolytic streptococcus, staphylococcus, and *Clostridium welchii*. The most rapid lysis occurred in organisms at the maximal rate of multiplication.

A report by Chain and Duthie (1945) is of particular interest in this regard. They studied the oxygen uptake of cultures of staphylococci in the presence of penicillin in various phases of growth and found the organisms to be most susceptible during the lag and logarithmic phases of active growth, but not affected even by high concentrations of penicillin in the "resting" phase. It is their opinion that penicillin "appears to interfere with a metabolic function involved in the early stages of bacterial growth."

Our own studies were concerned, first, with observations on the effect of penicillin on the morphology of the staphylococcus and streptococcus *in vitro* and, secondly, with its action on the pneumococcus and its capsule, both *in vitro* and *in vivo*.

STAPHYLOCOCCUS EXPERIMENTS

Methods. Penicillin-sensitive staphylococci were grown in beef infusion broth and tested with penicillin at various periods of their growth, also during the lag phase and after their multiplication had stopped. The penicillin employed in these first experiments was the crude form, prepared from two different strains of *Penicillium*, the usual dilutions in the cultures being approximately 0.5 to 0.125 units per ml. Heavy inocula of staphylococci were used, 0.1 ml of the whole broth culture, in order to ensure adequate organisms for the microscopic and photographic studies. Representative specimens of the penicillin-treated cultures and the controls were spun down in a centrifuge at various time intervals, and a sample of the sediment was spread on a slide and fixed with heat. The films were then stained on a single slide in a very dilute solution of carbol

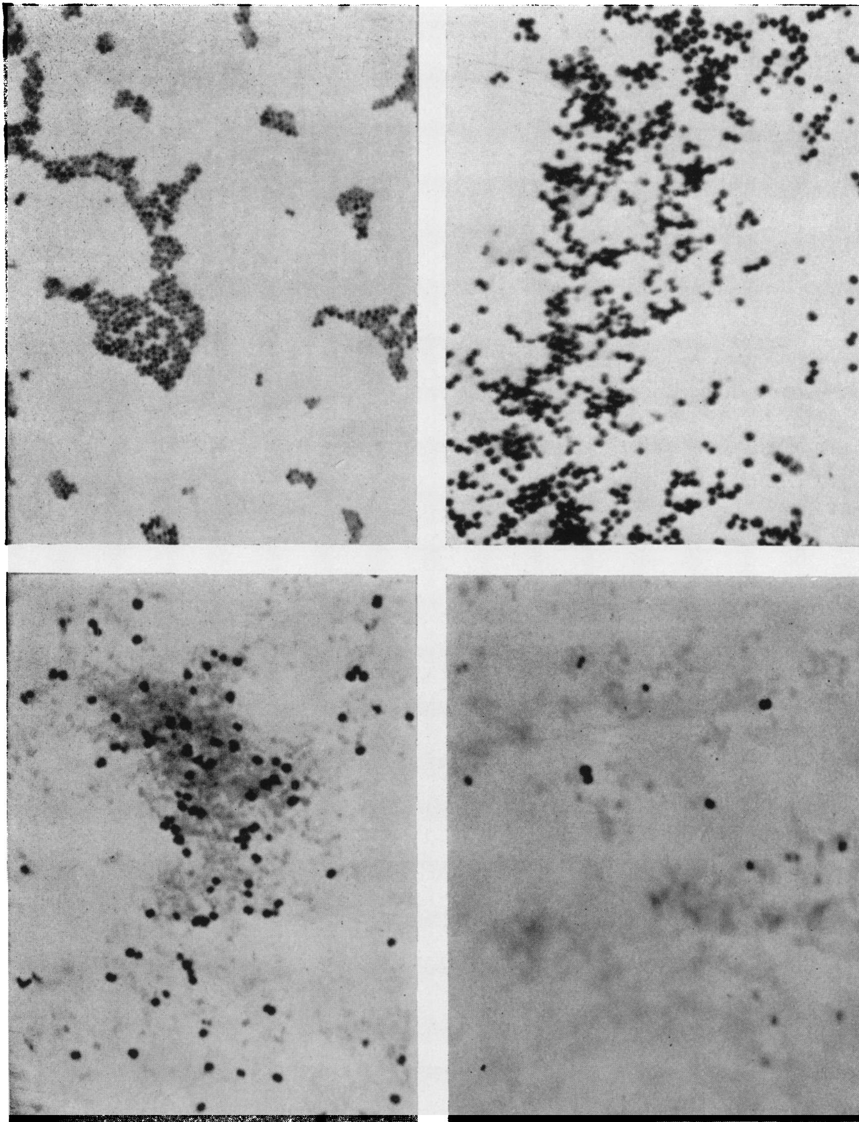

FIG. 1. STAPHYLOCOCCUS SERIES
Mag. × 1,300

Upper left: Control staphylococcus culture at 3 hours.
Upper right: Staphylococcus with penicillin at 3 hours. The organisms are all enlarged and deeply stained.
Lower left: Staphylococcus with penicillin at 7½ hours. Abnormal staphylococci and much granular debris.
Lower right: Same at 10 hours. Very few and markedly altered bacteria are left. Debris of lysed cells. Culture sterile at this stage.

fuchsin for about 30 minutes, and photomicrographs were made from these preparations.[1] The results are illustrated in figure 1.

[1]All the photographs were taken by Mr. Joseph Drane and Mr. John McQuaid in the Department of Anatomy.

It is clearly shown in these pictures that the first effect of penicillin on the staphylococcus which is observable microscopically is the production of marked enlargement of the cell body, with a definite increase in the intensity of its staining properties by dilute carbol fuchsin. This is followed by a dissolution of the bacterial cell, which is quite marked in 7½ hours, and complete, involving practically all the organisms, in 10 hours. The staphylococcus from the control tube at 10 hours had exactly the same appearance as at 3 hours. Subcultures of the centrifuged sediment taken at these same intervals revealed a marked decrease in the number of surviving organisms at each stage, with sterility in 10 hours. Similar results were obtained with several other strains of staphylococci.

This same phenomenon was observed if the inoculum of bacteria was taken very early in the growth period, i.e., at one hour's growth, presumably during the lag phase. Similar results were obtained if the cultures were taken at 2½ hours, i.e., in the early logarithmic phase of growth. On the other hand, no such morphologic changes resulted when penicillin was added to a tube containing an old, fully grown culture of a penicillin-sensitive staphylococcus, this culture having been in the incubator for 10 days previously. Likewise, when penicillin was added to a culture of a sensitive strain in the usual way and then placed at once in the icebox for 18 hours instead of the incubator, no recognizable changes in shape and no evidence of bacteriolysis were observed, and subcultures remained strongly positive. Two strains of penicillin-resistant staphylococci were treated in a similar manner with no change in their morphology.

These findings are in agreement with the conclusions of other workers and show that the action of penicillin on the staphylococcus, as observed microscopically, is one which leads at first to enlargement of the bacterial cell and then to its dissolution. With even higher dilutions of penicillin, though the staphylococci are not killed in the foregoing manner, the appearance of many of them is altered in such a way as to suggest that they represent degenerate forms which would presumably be more easily destroyed by the phagocytes of the body. This evidence of penicillin activity at very high dilutions may help explain the fact that it seems to influence infections at times when it is present in the blood in such small quantities as to be undetectable by the standard methods for its determination.

STREPTOCOCCUS EXPERIMENTS

Methods. These studies in general were similar to those employed for the staphylococci. Heavy inocula of 18-hour cultures of group A beta hemolytic streptococci were added to broth enriched with a small amount of rabbit's blood, and crude penicillin was added to this preparation in final dilutions of approximately 0.5 to 0.25 units per ml. These preparations were then spun down, slowly at first to get rid of most of the blood cells, then rapidly for 15 minutes. Films and cultures were made from the sediment and these films were all stained on the same slide with a dilute solution of carbol fuchsin for approximately 30 minutes. Photomicrographs were then made from these stained films. These results are shown in figure 2.

The photographs demonstrate the marked inhibition in growth of this culture of group A beta hemolytic streptococcus, this being corroborated by the cultures which became nearly sterile at 10 hours and completely so at 24 hours. They also show the formation of the "giant forms" which have been remarked on by others, and there is no evidence of debris to suggest that many of the bacteria have become lysed.

It seems, from this experiment at least, that the group A streptococcus is not killed in the same manner as the staphylococcus by penicillin. Only a moderate number of enlarged forms are to be found, and though these probably do become broken up, the majority of the organisms apparently do not undergo complete dissolution. This is in agreement with findings reported by Hobby *et al.* (1942), these authors having found that penicillin did not cause lysis of streptococci, though this reaction was observed in some later experiments (Hobby and Dawson, 1944).

PNEUMOCOCCUS EXPERIMENTS

In the studies of the effect of penicillin on the pneumococcus we were interested in two reactions, (1) the effect on the organism itself and (2) the effect on its capsule.

In Vitro Tests

Methods. The medium employed was beef infusion broth enriched either with human serum, rabbit's blood, or ascitic fluid. Crude or purified penicillin was added to this in amounts usually approximating 1 to 0.1 unit per ml, and heavy inocula of 18-hour cultures of pneumococci (0.3 to 0.5 ml) were added to this, and then placed in the incubator. Cultures and microscopic preparations were then made at various intervals after centrifuging. The bacterial stains employed in these experiments were the gram stain and the dilute carbol fuchsin. The capsule stain was a modification of that described by Butt *et al.* (1936). These authors recommended mixing a loopful of the material to be stained with an equal amount of 6 per cent glucose solution, then with india ink. This was spread on a microscopic slide, fixed with methyl alcohol, and counterstained with methylene blue. A counterstain of safranine proved much more satisfactory in our hands than methylene blue. This was at first applied for a few minutes, but stains of longer duration gave much better definition of the bacteria in the capsules; the best preparation resulting from a stain of 1 to 2 hours. It is, of course, absolutely necessary that the control and penicillin-treated specimens be prepared in exactly the same manner and stained for an equal length of time. The results of these *in vitro* studies are shown in figures 3 and 4.

The following observations were made on the gross and microscopic appearance of the pneumococcus cultures. In a typical experiment penicillin dilutions of 5, 1, 0.1, and 0.01 units per ml and a control culture were used. The first three of these tubes remained clear throughout; they were nearly sterile at 6 hours and completely so at 18 hours. The tube containing 0.01 unit penicillin per ml was almost as cloudy as the control at 6 hours, but completely clear and nearly sterile at 18 hours. This indicated slight inhibition at this highest dilution in

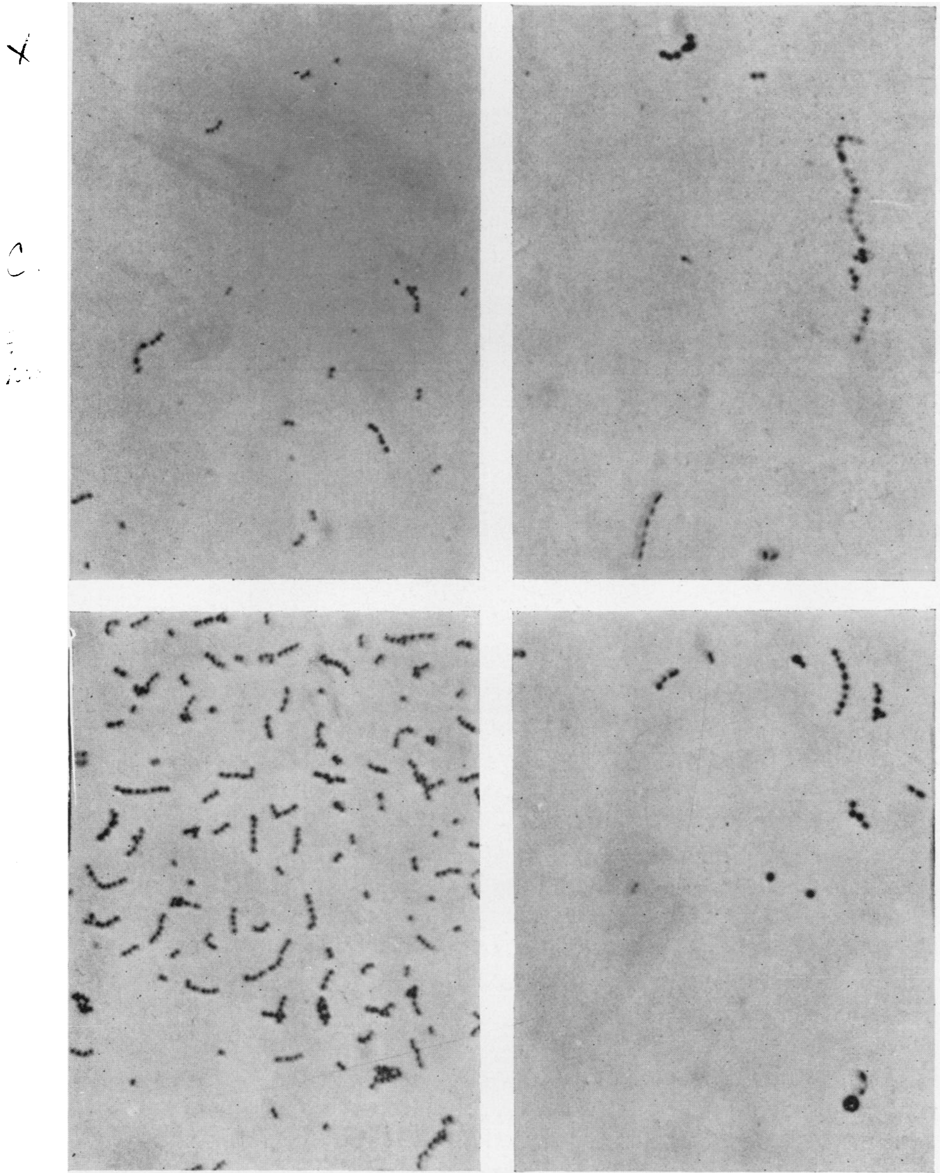

FIG. 2. STREPTOCOCCUS SERIES
Mag. × 1,300

Upper left: Streptococcus control at 3 hours.
Upper right: Streptococcus with penicillin at 3 hours. Early changes in morphology and staining may be noted.
Lower left: Control at 7½ hours.
Lower right: Streptococcus with penicillin at 7½ hours. Note the appearance of giant forms and marked reduction in number of organisms.

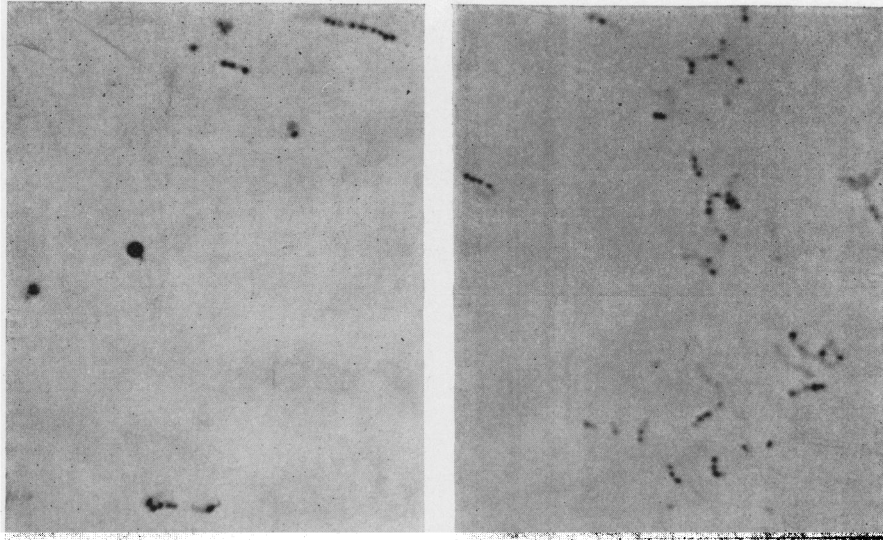

FIG. 2—Continued
Left: Penicillin-streptococcus preparation at 10 hours.
Right: Same at 24 hours. Most of the bacteria appear dead. Culture sterile at this point. Note persistence of chain forms and visible bacterial cells.

the early stages, followed by lysis. Thus the penicillin could be said either to cause lysis of the pneumococci or to accelerate the natural tendency of these organisms to autolyze. Gram stains of the centrifuged sediment of the first three tubes showed a few organisms almost all of which were gram-negative, usually occurring singly, a few diplococci, and almost no chains. Stains from the tube showing slight inhibition showed a picture very much like the control, except that most of the bacteria were gram-negative. There were chains just as in the control and only an occasional form that seemed somewhat larger than normal.

As for the studies on the pneumococcus capsule, in these experiments and many similar ones it was established that by 3 hours there were both a marked reduction in the total number of capsules to be found in a preparation and a consistent tendency for the majority of them to appear entirely *empty* or to contain a small amount of irregularly shaped bacterial substance. The capsules themselves were not perceptibly altered. Similar but less advanced changes were noted by 2 hours. Though no very high dilutions of penicillin were employed, it was found that, when its strength was 0.1 or 0.5 unit per ml, this capsule phenomenon was more easily demonstrated than when 1 unit per ml was used. In the latter case the most striking finding was a great reduction in the number of organisms—even in 3 hours both by culture and by film—making it difficult to find more than a few scattered capsules, though very heavy inocula had been employed.

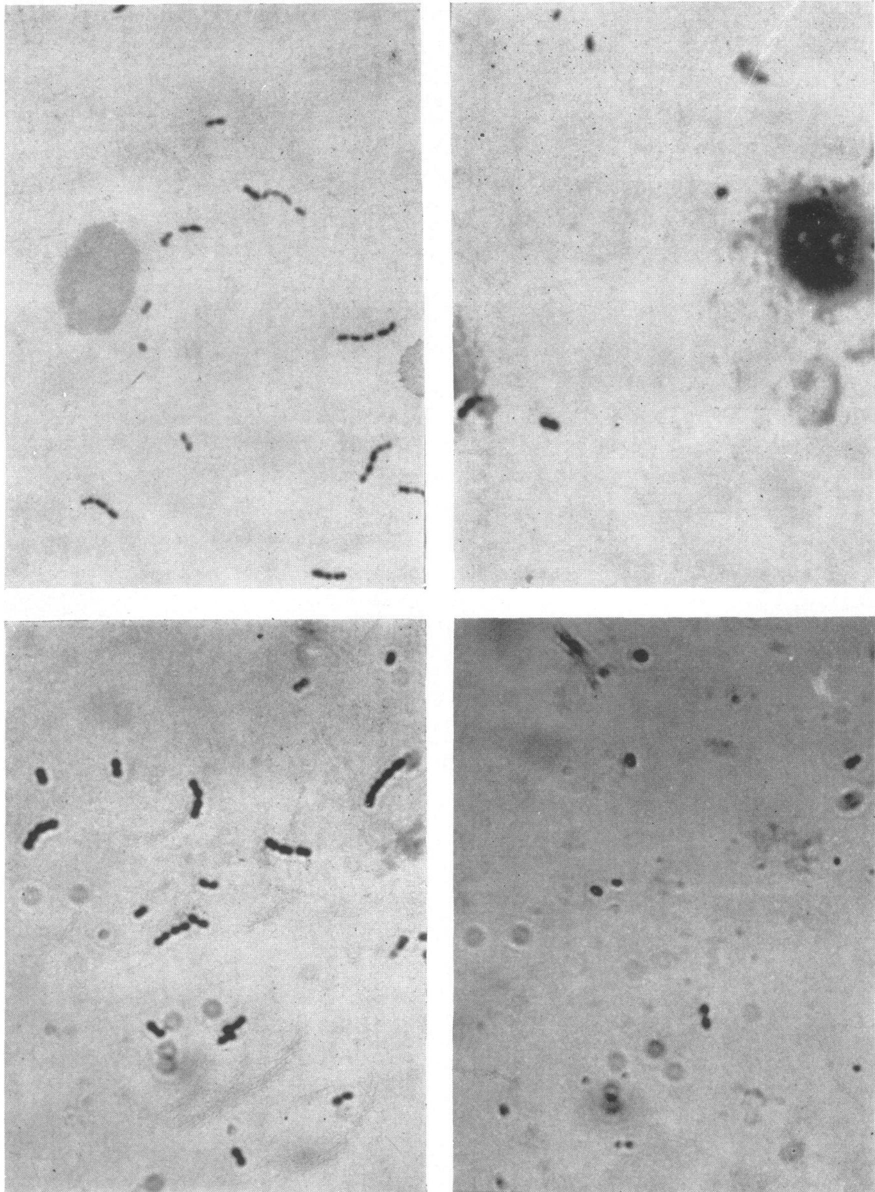

FIG. 3. PNEUMOCOCCUS SERIES
Mag. × 1,250

Upper left: Pneumococcus type I control at 3 hours. Note tendency to formation of short chains. (Carbol fuchsin stain.)

Upper right: Pneumococcus type I with penicillin. One unit per ml at 3 hours. Organisms reduced in number and deeply stained.

Lower left: Pneumococcus type III control at 6½ hours. (Gram stain.)

Lower right: Pneumococcus type III with penicillin 0.1 unit per ml at 6½ hours.

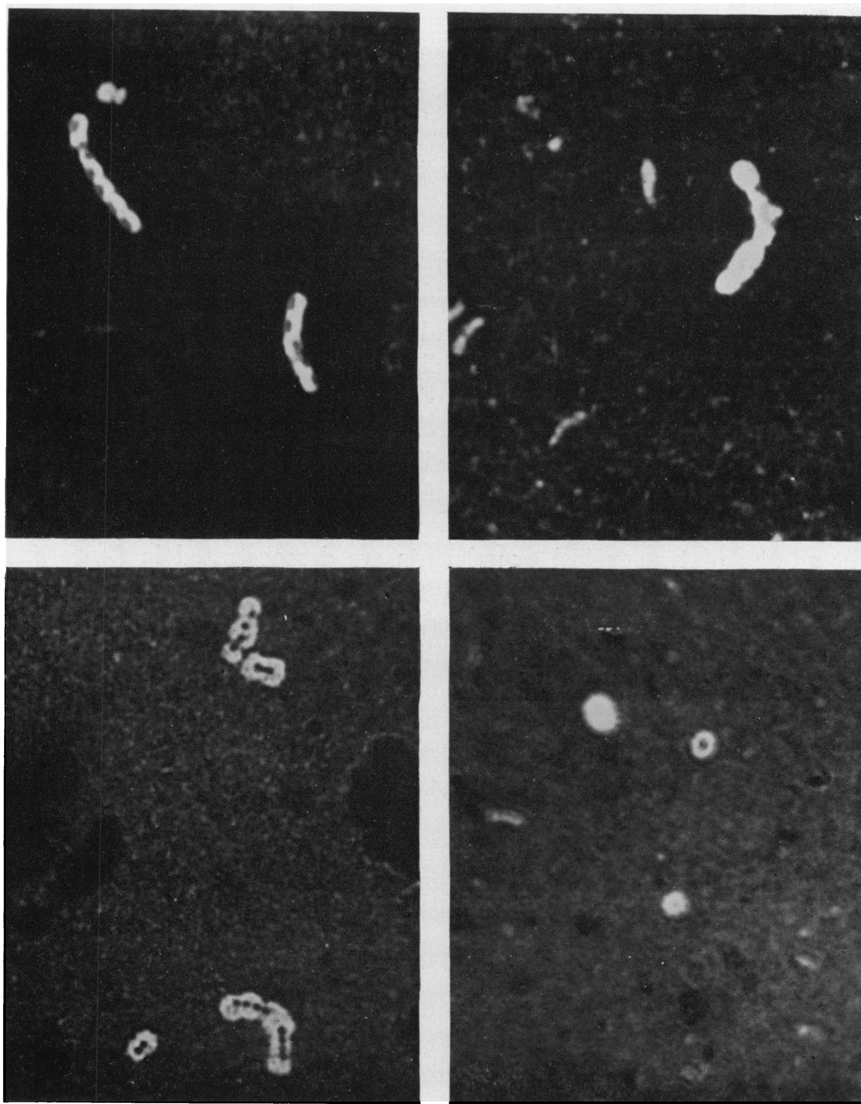

FIG. 4. PNEUMOCOCCUS CAPSULE SERIES

Upper left: Pneumococcus type I control at 3 hours. (Same strain with chain formation as shown in figure 3, upper left.)

Upper right: Pneumococcus type I with penicillin. 1 unit per ml, at 3 hours. Note almost complete absence of stained bacteria in the single capsule. (Other organisms in the background are contaminants in the india ink.)

Lower left: Pneumococcus type III control at 6½ hours.

Lower right: Pneumococcus type III with penicillin 0.1 unit per ml at 6½ hours.

In Vivo Tests

Mouse experiments. It next seemed of interest to determine whether or not this capsular phenomenon could be reproduced *in vivo*. Mice were used in these

tests, and cultures of a type III pneumococcus and penicillin were injected intraperitoneally. Two types of inocula were employed, one being a heavy suspension of the pneumococci obtained in saline from the peritoneal cavity of mice injected with a culture of pneumococci a few hours previously and the other an 18-hour culture in blood broth or serum broth. Heavy inocula were used of approximately 1 ml, which were injected intraperitoneally and followed in about 2 hours by intraperitoneal injections of crude or purified penicillin in strengths of 100 to 250 units. The mice were killed at intervals of 4 to 8 hours, and the contents of the peritoneal cavities were washed out with approximately 1 ml of normal saline or broth. This was centrifuged, slowly at first to throw down most of the cellular elements and then rapidly to obtain the bacteria. The sediment was washed again with saline or broth in order to eliminate most of the serum—as this causes clumping of the india ink particles, thus resulting in an unsatisfactory background for the capsule stain. Preparations with india ink were then made from these sediments, fixed, and stained in the usual way. Control preparations were made for each test and treated in exactly the same way as those in which penicillin was used.

The results of two typical experiments are demonstrated in figure 5. In these the capsules are very large, no doubt because a type III pneumococcus was used and grown in the mouse's peritoneum. The findings were essentially the same whether crude or purified penicillin was used. These and many other similar experiments made it clear that the phenomena noted *in vitro* could be reproduced *in vivo*. The capsules seem to be normal in structure and the pneumococci appear to have been either completely destroyed, leaving no trace behind or leaving remnants that stain very faintly. These remnants at times appeared as definite small diplococci, and at other times there was only a small amount of granular material to be seen. A few normal-looking pneumococci could nearly always be found in the same preparation. Subcultures taken at the time these preparations were made invariably showed a marked reduction in the number of viable organisms present, and an occasional culture would be sterile.

Human experiments. A good many observations on patients with pneumococcus infections under treatment with penicillin indicate that this "empty-capsule" phenomenon is a very common, if not a regular, occurrence under such circumstances. Miss Kathleen Foley and others in the bacteriological laboratory noticed, on setting up a *quellung* preparation on some pus from an empyema which had been treated locally with penicillin, that capsules were present which "quelled" normally but that either no pneumococci or only very faintly stained ones were visible within the capsules. We confirmed this finding on several other cases under similar treatment.

It was found that the same reaction could be demonstrated in the sputum of patients with lobar pneumonia. The characteristics of the sputum made it difficult to obtain good preparations with the india ink. This could be done, however, by shaking the specimen up with sterile broth, filtering through sterile filter paper, and then centrifuging. Preparations were then made from the

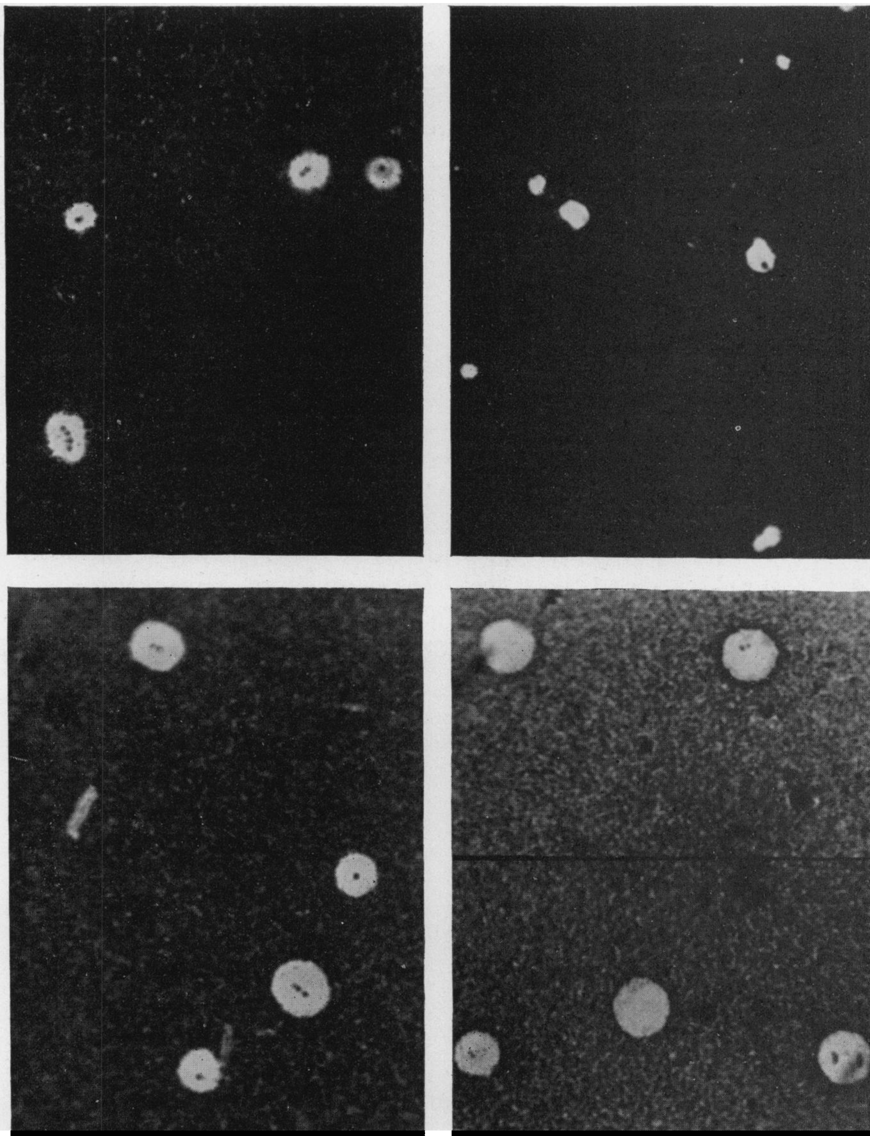

FIG. 5. PNEUMOCOCCUS SERIES, MOUSE EXPERIMENTS
Mag. × 1,200

Upper left: Pneumococcus type III control. From mouse peritoneum after 1½ hours.
Upper right: Pneumococcus type III with sodium penicillin, 100 units. After 4½ hours in mouse peritoneum. (Capsules appear smaller because of very dense background.)
Lower left: Pneumococcus type III control from mouse after 4 hours.
Lower right: Pneumococcus type III from mouse after 4 hours. Top—With crude penicillin, 100 units. Bottom—Same with sodium penicillin, 250 units.

sediment and stained for capsules. Cultures from the sediment before penicillin therapy were nearly pure for pneumococci and after treatment were often nearly sterile. In three cases satisfactory preparations were obtained in this way

before, and the day following, institution of intramuscular therapy with penicillin in doses of 30,000 units every 3 hours. Most of the capsules which were found

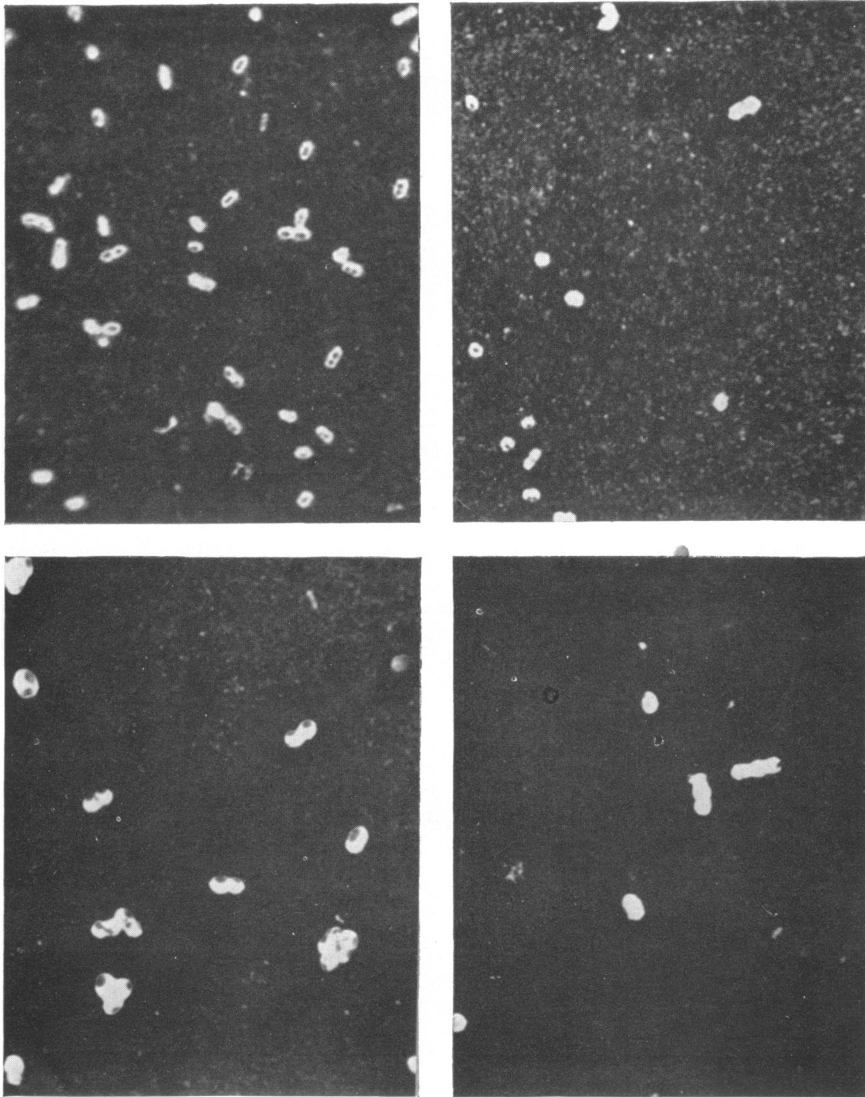

FIG. 6. PNEUMOCOCCUS SERIES, HUMAN INFECTIONS
Mag. × 1,200
Upper left: Pneumococcus type XII meningitis before penicillin therapy.
Upper right: Same 4 hours after 20,000 units penicillin intrathecally.
Lower left: Pneumococcus type II meningitis before penicillin therapy.
Lower right: Same 20 hours after 20,000 units penicillin intrathecally.

after therapy appeared empty, whereas those before treatment showed pneumococci which stained normally.

It was interesting to note also that two of these patients had been taking one

of the sulfonamides at the time of admission and just before the control preparation was made. This suggests that the pneumococcus does not exhibit this type of reaction when exposed to sulfonamides. *In vitro* experiments performed later with strongly and weakly inhibiting concentrations of sulfadiazine and sulfamerazine bore this out, as the pneumococci under such circumstances were found to stain normally in their capsules.

Material was obtained from two patients with pneumococcus meningitis. The results of the studies on their spinal fluids are illustrated in the photographs in figure 6. The first patient had an overwhelming bacteremia and meningitis due to a type XII pneumococcus and died 4 hours after the diagnosis was made. The second specimen in this case was taken by spinal puncture immediately after death—approximately 4 hours after 20,000 units of penicillin had been injected intraspinally. In this preparation it was found by actual count that 50 per cent of the capsules appeared to be empty, whereas only one in five had this appearance before the therapy. The other patient had a type II pneumococcus in the spinal fluid and was given 20,000 units intraspinally. The second sample of spinal fluid was taken 20 hours after this injection, and nearly all the capsules were empty or showed pneumococci which stained very faintly. The bacteriologist doing the routine *quellung* on this material remarked on the peculiar microscopic appearance of the preparation, for the organisms themselves had failed to take the methylene blue stain, and many capsules were left which in some ways resembled red blood cells.

DISCUSSION

It seems clear from this study that though penicillin may have one fundamental effect on bacterial metabolism which holds true for all susceptible organisms, it has varying effects on the cellular structure of the bacteria, depending on the type of organism under consideration. This holds true at least for the three types of bacteria dealt with in this investigation. It is also clear from this, and many other observations, that the bacteria are actually *killed* by penicillin and without the aid of the defensive mechanisms of the body, rather than simply being *inhibited* in their growth. This bactericidal action does not take place immediately—as it does with many antibacterial agents—but subcultures show that during the first 3 hours great numbers of bacteria die if exposed to penicillin in sufficient concentration, and by 10 hours the cultures are usually sterile. These cultural changes may be correlated with actual alterations in the morphology of the various bacteria, as demonstrated by the microscopic studies illustrated in this report.

Though the degree and type of the structural changes in the different bacteria vary considerably, one effect of penicillin on the bacterial cell body results in enlargement of the cell. This reaction is most marked at the higher effective dilutions of penicillin. It is interesting to compare this phase of the reaction with the lag period of normal bacterial growth. This period corresponds roughly to the first 1½ hours of life of a fresh culture, and during this time there is little or no evidence of multiplication of the bacteria. They are not in a resting

stage, however, but show normal metabolic activity, which results in *growth* and actual enlargement of bacterial cells (Topley and Wilson, 1938). The normal stimulus to division then takes place, and the bacteria are started on the phase of "active growth." The reasoning which has been advanced that penicillin interferes with this normal stimulus to division seems to offer the most logical explanation for this primary inhibiting effect of the drug. It seems to produce at first an exaggerated lag phase, which in our experiments was much more marked for the staphylococcus than for the streptococcus, and least for the pneumococcus.

In the case of the staphylococcus this phase in which there is inhibition of division and swelling of the bacteria is followed by dissolution of the cells until little remains of the culture but granular debris. These morphologic changes in the staphylococci were noted only in the case of the growing organisms; they were not produced at icebox temperature nor in the old cultures in the resting phase. Resistant strains did not show any such structural alteration when exposed to penicillin. Sulfathiazole failed to induce this reaction when tested with staphylococci either in weakly or strongly inhibiting concentrations.

Definite evidence of complete dissolution of cellular structure was not observed in the cultures of streptococci; and though a good many giant forms were seen during the phase of inhibition—these presumably becoming lysed—the majority of the organisms did not exhibit these alterations, and some chains were still apparent though the cultures became sterile.

It may be mentioned here that sulfonamides have also been found capable of changing the morphology of streptococci, for Gay and Clark (1937), Lockwood (1938), and Tunnicliff (1939) noted enlargement of the individual bacteria and elongation of their chains when exposed to sulfanilamide. Such organisms were found to be more susceptible to phagocytosis than were the controls (Tunnicliff, 1939).

The effect of penicillin on the morphology of the pneumococcus as shown by the stain employed in this work was one of enlargement of a few of the cells, but usually, except for a marked diminution in the number of bacteria, there would be little change from the control preparation. Most of the remaining bacteria, however, did become gram-negative. The changes noted relative to the *capsules* were unexpected and very interesting. The capsule being intimately associated not only with the type specificity but with the virulence of this species, we suspected that it would have to be destroyed before the pneumococcus could be killed by the penicillin. It was interesting, therefore, to find that after the organism itself had evidently been killed and failed to take the safranine stain, the capsular substance remained intact. In this condition it was still capable of reacting in the usual way when exposed to type-specific serum. It appears that the penicillin either passes through the capsule by diffusion and then destroys the organism, or is actively absorbed by the growing pneumococcus and passed through its capsule during the process of bacterial metabolism. That the latter explanation seems the more likely is indicated by the fact that the phenomenon is not observed at icebox temperature; which suggests that it

occurs only when the experiments are performed under conditions favorable to active growth of the bacteria. This is not merely an *in vitro* phenomenon, for it was observed in mice and in several types of pneumococcal infections in human beings. The fact that it was found in the sputum of patients with lobar pneumonia during penicillin therapy indicates that it may take place in high dilutions of the antibiotic. This may be a reaction which is unique for penicillin; at least there has been found no evidence for its occurrence with sulfonamides either *in vitro* or in patients with pneumonia.

It is thus shown that the pneumococcus capsule is easily penetrated by penicillin and does not offer the organism any protection against it. It seems reasonable to presume that the way in which the bacterial cell itself is destroyed in this reaction is by the penicillin's producing enough injury to promote an acceleration of the pneumococcus' natural tendency to autolysis.

Another feature worth emphasizing again for its clinical significance is the production by penicillin of slight inhibiting and morphologic changes in bacteria at higher dilutions than those which are observed in the gross *in vitro* tests of an organism's penicillin sensitivity. Bacteria which are only slightly affected in such a manner are almost certainly altered enough to render them more susceptible to the normal immune mechanisms of the host. Such observations may help explain the finding that penicillin is frequently effective in bacterial infections despite the fact that it is present in the blood and tissue fluids in too low a concentration to enable its detection by the routine methods that are now employed.

All the observations reported in this study support the general belief, expressed by many workers in this field, that penicillin is most effective against young, rapidly growing bacteria. This is one of the features of its action that is of the greatest interest and importance from the clinical standpoint. The fact that penicillin apparently does not affect the bacteria in the resting stage—as shown by its failure to produce morphologic or cultural changes in very old cultures or at icebox temperature—proves that this is not merely a physical or chemical effect on the body of the cell, but one that influences some phase of the organism's metabolic and reproductive activity.

SUMMARY

The *in vitro* action of penicillin on staphylococci causes enlargement of the bacterial cells followed by lysis. This is apparently due to interference with their normal stimulus to division and multiplication rather than to complete inhibition of growth.

The same effect is apparent to a lesser degree on cultures of beta hemolytic streptococci and pneumococci. In the case of the pneumococcus at least, the capsule remains intact for some time after the cell body is destroyed.

This effect on the pneumococcus is apparently a biologic rather than a physical or chemical reaction, since it occurs only under conditions in which the organism is capable of growth.

These various morphologic changes are noted to some extent in the staph-

ylococci within the first 2 hours of their exposure to penicillin, and in the streptococci and pneumococci in 3 hours.

The clinical significance of these findings is discussed.

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