AN EXPERIMENTAL ANALYSIS OF THE CURATIVE ACTION OF PENICILLIN IN ACUTE BACTERIAL INFECTIONS

III. The Effect of Suppuration upon the Antibacterial Action of the Drug*

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Plates 24 and 25

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Once established, suppurative lesions caused by pyogenic bacteria are notoriously resistant to penicillin therapy (1, 2). Only in their earliest stages are they regularly cured by the antibiotic alone. Pneumococcal empyema, for example, when fully developed often fails to respond to penicillin treatment, unless the lesion is also drained either by repeated aspiration or by surgical incision (3, 4).[1] In contrast, lobar pneumonia caused by the same strain of pneumococcus may be promptly cured by relatively small amounts of penicillin (3, 4). This striking difference in the response of focal and diffuse lesions to antimicrobial therapy has never been satisfactorily explained. In the following experiments an attempt is made to define the principal factors involved in the refractoriness of established abscesses to treatment with penicillin.

Methods

Experimental pneumonia was produced in white rats by intrabronchial inoculation (6) of either type I (strain A-5 (6)) or type III (strain 8 H.C.C. (7)) pneumococci. Intramuscular penicillin therapy—3,000 units of procaine penicillin in oil (duracillin)—was begun 18 hours after inoculation, and the injections were repeated every 12 hours throughout the period of observation. In each experiment the infected animals were sacrificed at the intervals indicated in Text-fig. 1. Bacterial counts were made from each pneumonic lesion by means of the method described in an earlier study (8).

Subcutaneous abscesses were produced in white rats by a modification of the method of Selye (9). Through a 20 gauge needle 25 ml. of air was injected beneath the skin of the back

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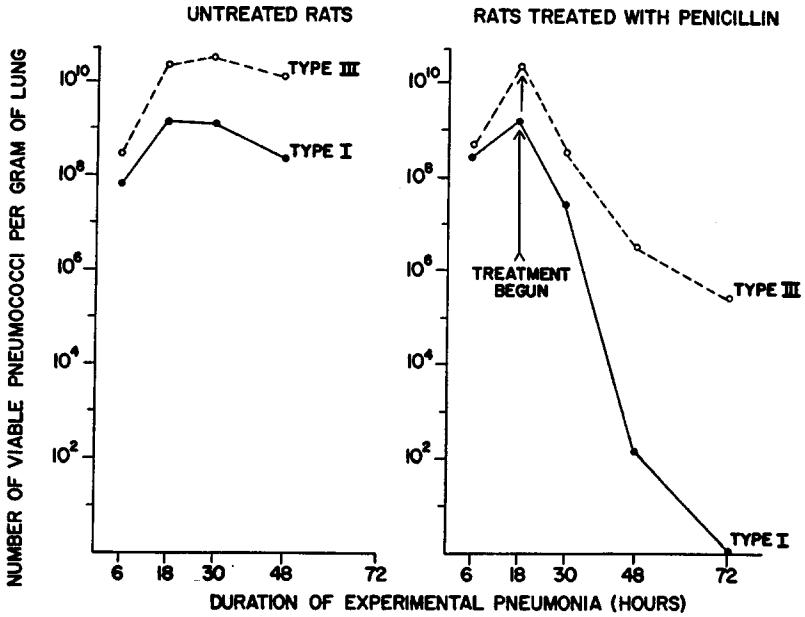
[1] MacLeod (1) in discussing this point cites the clinical observations of Tillett *et al.* (5) as indicating that penicillin alone, when injected intrapleurally in sufficient amounts, is capable of curing empyema. It should be pointed out, however, that in Tillett's series of cases "each injection of penicillin was preceded by removal of as much exudate as possible."



TEXT-FIG. 1. (Left) Growth of type I and type III pneumococci in lungs of rats with experimental pneumococcal pneumonia. (Right) Comparative effects of penicillin therapy upon the survival of type I and type III pneumococci in pneumonic lesions.

TABLE I

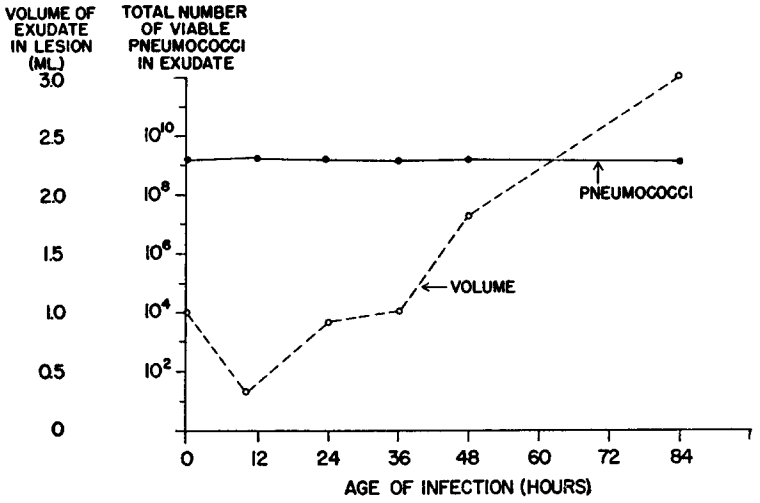
Characteristics of Experimental Pneumococcal Lesion

Time of infection	Exudate in cavity of lesion				
	Volume	Characteristics	Metachromasia of pneumococcal capsules	Phagocytosis	No. of animals with bacteriemia
days	*ml.*				
2	0.5	Viscous, turbid fluid	3+	4+	4/4
5	3.1	Fluid less viscous and turbid, with masses of fibrinous exudate	2+	1+	2/4
10	8.2	Non-viscous, turbid fluid above layer of fibrinous exudate on floor of cavity	1+	1+	0/4
18	0.4	Relatively uniform mass of thick, sticky pus	0	0	0/4

to form a blind pouch into which were introduced approximately 2.0×10^9 viable pneumococci suspended in 1 ml. of beef infusion broth containing 10 per cent sheep serum and 0.2 per cent dextrose. Preliminary trials revealed that well circumscribed abscesses (Figs. 1 and 2) could be produced by type III pneumococci (strain 8 H.C.C.), whereas type I organisms (strain A-5) caused a rapidly spreading cellulitis which resulted in early bacteriemia and death. Accordingly, type III strains were used in all experiments.

The abscesses thus produced gradually matured over a period of about 18 days, at the end of which time they were lined with a definite fibrinous capsule and contained thick sticky pus (Table I). As shown by the data graphed in Text-figs. 2 and 3, the bacterial counts on the pouch fluid remained remarkably constant during the early stages of the infection, but gradually fell off as the abscess matured. It will be noted also (Text-fig. 2) that the volume of fluid in the cavity, measured directly upon opening the lesion, decreased during the first 12 hours (apparently owing to absorption of the injected broth), but thereafter continued to increase for several days.

Necrosis of the skin overlying the abscess was often visible by the 10th to 12th day (Fig. 2) and spontaneous rupture frequently occurred by the end of 2 weeks, if not earlier. Such drainage invariably led to eventual healing of the lesion. Because the ruptured abscesses tended to become secondarily infected, all animals in which spontaneous drainage occurred



TEXT-FIG. 2. Volume of exudate and number of viable pneumococci within cavities of subcutaneous lesions during first 84 hours of infection.

were discarded. In experiments requiring relatively late suppurative lesions (12 to 18 days[2]), a different strain of type III pneumococcus (A-66 (7)) was employed, since on repeated trial it was found to cause less necrosis of the skin. With this strain abscesses were produced which could be studied for as long as 30 days, although in each series of animals infected a significant proportion still had to be discarded because of spontaneous drainage.

Bacterial counts on the exudate from the lesions were done with samples[3] obtained at the

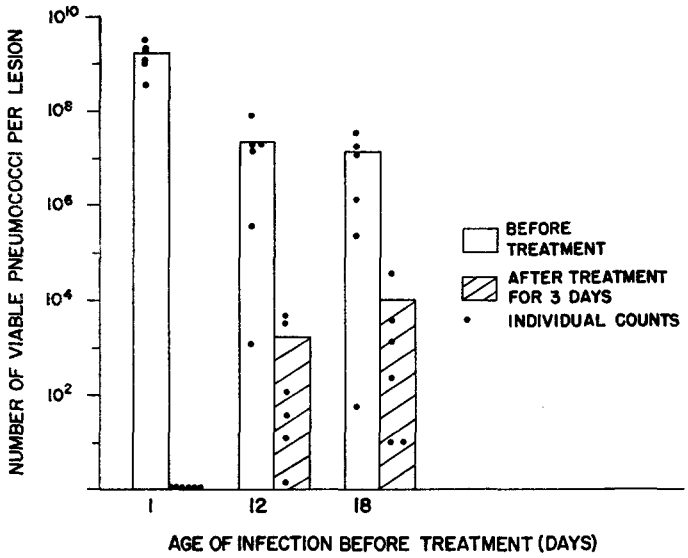
[2] See also *in vitro* experiments of preceding study (10).

[3] Rats were killed by ether and pouches were opened aseptically. During the first 10 days of the infection a 0.1 ml. sample of each exudate was easily obtained by pipette. After the 12th day, however, the pus was too thick to aspirate. Accordingly, thereafter the pus was transferred by spatula to a mortar containing glass beads and was ground with a pestle in order to insure homogeneity. The ground pus was then diluted and mixed with 1 ml. of tryptose phosphate broth and either a 0.1 ml. or a 1 ml. sample was used for the bacterial count. Following penicillin therapy of early infections the pouches frequently were devoid of exudate. When none was present, the pouch was washed with 1 ml. of tryptose phosphate broth which was then used for counting.

intervals indicated in Text-figs. 2, 3, and 4. The counts were made on pour plates of blood agar by means of the standard dilution technique. In each experiment in which penicillin was used, penicillinase was added as previously described (10).

Smears stained with methylene blue (7) were also made from the pus at the same intervals as the bacterial counts. Particular attention was paid, not only to the morphologic appearance of the leucocytes and the evidence of phagocytosis, but also to the staining of the pneumococcal capsules. Since metachromatic staining of the capsule of pneumococcus III has been shown in previous studies to occur only during rapid growth of the organism (7), its presence afforded an indirect indication of active multiplication.

Penicillin treatment of the animals with the subcutaneous infections included both local and systemic injections. To insure a maximal therapeutic effect, the drug was given in large



TEXT-FIG. 3. Comparative effects of 72 hours of penicillin therapy when begun on the 1st, 12th, and 18th days of the subcutaneous infection.

doses and at frequent intervals. 100 units of aqueous penicillin[4] (in a volume of 0.1 ml.) were introduced directly into the cavity of each lesion and 4,000 units[5] (also in a volume of 0.1 ml.) were given intramuscularly every 8 hours. Therapy was begun at various stages of the infection, as indicated in Text-fig. 3, and was continued until the end of the experiment (72 hours).

RESULTS

The Inhibitory Effect of Suppuration upon the Antimicrobial Action of Penicillin in Experimental Pneumococcal Pneumonia.—As has been previously stated, fully encapsulated strains of type III pneumococcus when injected intrabronchially in rats produce lung abscesses as well as lobar pneumonia (8).

[4] Penicillin G crystalline-potassium—Eli Lilly & Co., Indianapolis.

[5] Procaine penicillin and buffered crystalline penicillin for aqueous injection (3,000 units procaine, 1,000 units crystalline)—Parke Davis & Co., Detroit.

When type I pneumococci are injected in comparable numbers, suppuration does not develop in the pneumonic lesions (6). Because of this basic difference in the behavior of type I and type III pneumococci, and because the strains of both used in these experiments were equally sensitive to penicillin, it was possible to measure the influence of abscess formation upon the action of the antibiotic by merely comparing the speed with which the bacteria were destroyed during therapy in the two types of lesions. When treatment was begun 18 hours after inoculation in the type I infections, no pneumococci could be recovered by culture after 54 hours of therapy (see Text-fig. 1). At the same stage in the treatment of type III infections, on the other hand, there remained in the suppurating pneumonic lesions approximately 100,000 viable pneumococci per gm. of tissue. Furthermore, cultures taken after more than 2 weeks of intensive penicillin therapy still revealed surviving pneumococci.[6]

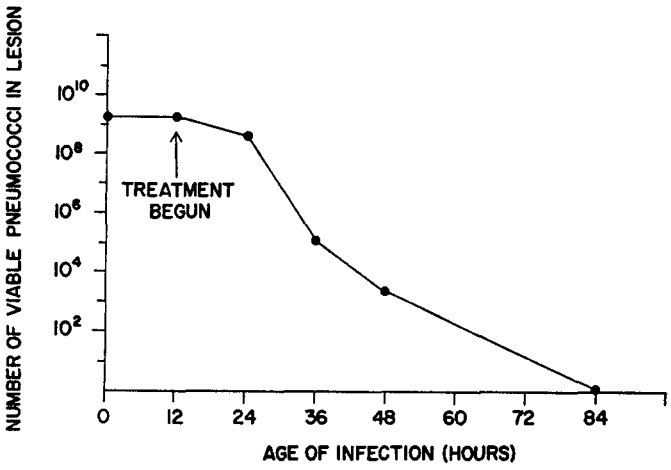
The Effect of Penicillin in the Treatment of Subcutaneous Abscesses.—The principal characteristics of the exudate at various stages in the development of the acute pneumococcal lesions produced subcutaneously in white rats are indicated in Table I. It will be noted that during the first few days after inoculation the exudate was relatively thin. At the same time, however, it contained an appreciable number of leucocytes which were actively phagocytic (Fig. 3). Although the bacterial count was already at its maximum (see Text-figs. 2 and 3), the metachromatic staining of many of the pneumococcal capsules indicated that a significant number of the organisms were in a state of active multiplication. This continued growth, at what seemed to be the maximum population density, was apparently perpetuated by the fact that pneumococci were simultaneously being removed from the serous exudate—both by phagocytosis (*vide supra*, Fig. 3) and by lymphatic drainage (note presence of bacteriemia recorded in Table I). Also the volume of fluid in the pouch, as shown in Text-fig. 2, was on the increase. Under these circumstances the constancy of the total number of viable bacteria in the lesion can be accounted for only on the basis of compensatory multiplication. As might be anticipated, when penicillin was administered at the end of the first 12 hours of infection, a significant bactericidal effect was achieved (Text-fig. 4). It should be noted, however, that the rate at which the bacteria were killed was considerably slower than in early muscle lesions where the bacteria were multiplying more rapidly at the time that treatment was begun (see curves *A* and *B* of Text-fig. 1 and *E* and *F* of Text-fig. 2 in the preceding paper (11)).

Conditions within the abscess cavities after 12 days, on the other hand, were quite different. By now the exudate had become relatively thick. Most of the leucocytes in the cavity's center had degenerated and were non-phagocytic

[6] Since at this late stage the pulmonary abscesses were often secondarily infected with other bacteria, positive cultures for the originally inoculated pneumococcus were reported only when the organisms were definitely identified by the "quellung" reaction.

(Fig. 4). The number of viable bacteria present was significantly less than during the 1st day of the infection (Text-fig. 3). The absence of metachromatically stained capsules on the majority of the remaining pneumococcal cells also suggested that they were not multiplying rapidly. When penicillin treatment was instituted at this stage, the bactericidal effect of the antibiotic was less pronounced than when given on the first day of the infection (see Text-fig. 3).

After 18 days the average bacterial count on the exudate was slightly lower than at 12 days (Text-fig. 3). The few organisms that were seen on smear failed to stain metachromatically. The cavities of the abscesses had by now become



TEXT-FIG. 4. Antimicrobial effect of penicillin on subcutaneous pneumococcal lesion when treatment is begun 12 hours after inoculation.

lined with fibrous capsules (Figs. 5 to 7), and the exudate was so thick that it could not be drawn through even a large needle. Morphologically intact phagocytes were present only at the very periphery of each cavity (Fig. 7); at the center most of the cells were completely necrotic (Fig. 8). When at this stage penicillin was administered locally as well as systemically, the remaining viable bacteria were killed even more slowly than in the 12 day lesions.

Attempts to study still later lesions were met with difficulties, the most important one being that caused by the continued decrease in the number of viable pneumococci in the exudate. At the end of 24 days the bacterial counts from untreated lesions were both relatively low and inconstant; after 30 days the majority of the exudates were sterile. This spontaneous and progressive decline in the bacterial population of such abscesses made the treatment of more chronic lesions impractical.

DISCUSSION

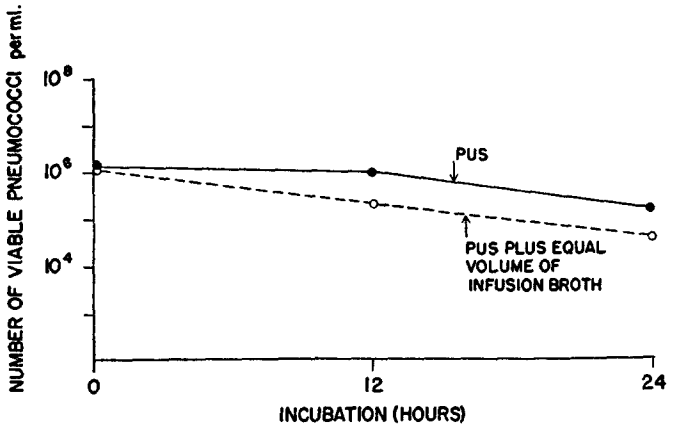
The results of these studies reveal at least two important reasons for the relative ineffectiveness of penicillin in the treatment of pneumococcal infections complicated by frank suppuration. First, as indicated by the earlier *in vitro* experiments (10), pneumococci grow poorly in pus. Its bacteriostatic properties are evident also *in vivo*. The comparatively low bacterial counts obtained in established pneumococcal abscesses and the failure of the type III capsule to continue to stain metachromatically (7) both indicate a significant suppression of bacterial growth. Their metabolism thus slowed, the surviving pneumococci remain relatively resistant to the bactericidal effect of penicillin (10). Secondly, histologic examination of the pus from the centers of the abscesses shows the majority of cells to be necrotic. They are obviously in no condition to provide an effective phagocytic defense to supplement the action of the penicillin. For these two reasons alone it is not surprising that pneumococci continue to survive in purulent foci in spite of intensive penicillin therapy.

A third reason often given (1, 12) for the failure of penicillin to cure bacterial abscesses relates to a postulated failure of the drug to penetrate the lesions. In the present experiments this factor was eliminated as far as possible by direct and repeated injections of the antibiotic into the cavities of the abscesses. It should be borne in mind, however, that the lesions studied were not as mature as those encountered in such conditions as chronic pneumococcal empyema. The possibility cannot be excluded, therefore, that the failure of penicillin to penetrate the entire lesion may contribute to the inability of the drug to eradicate certain chronic suppurative infections, but the thesis that this factor alone accounts for the resistance of suppurative lesions to penicillin therapy appears no longer to be tenable.

The rationale for combining drainage with penicillin therapy in treating walled-off suppurative lesions becomes self-evident from the results of the present experiments. Removal of the necrotic pus, by repeated aspiration, by surgical means or by enzymatic debridement (13), eliminates not only many of the infecting bacteria, but also much of the bacteriostatic exudate in which surviving organisms remain resistant to the bactericidal effect of the antibiotic. Furthermore, evacuation of the pus leads to the formation of fresh serous exudate (13) in which the bacteria grow more readily and thus become susceptible to the killing action of the penicillin. The newly formed exudate also provides numerous viable leucocytes (13) which contribute further to the destruction of the bacteria. The combined effect of all of these factors usually results in the prompt cure of abscesses previously resistant to the most intensive penicillin therapy.

The antimicrobial properties of the pus itself are also of considerable interest. The failure of the necrotic exudate to support rapid multiplication of pneumo-

cocci might be assumed to have been due merely to a depletion of constituents essential for growth. That such was not the case, however, is indicated by the fact that addition of an equal volume of nutrient broth failed to reverse the bacteriostatic properties of the exudate (Text-fig. 5). This finding suggests the presence of antibacterial substances in the pus. Such substances have been described by numerous investigators in the past, and although they have never been precisely defined, they appear to be derived from leucocytes and have consequently been referred to as "leucins" (14). Their possible importance was further indicated in the present studies by the observation that incubation of pneumococci in certain samples of pus from older abscesses (24 to 32 days) resulted in definite killing of organisms, the number of viable



TEXT-FIG. 5. Failure of added beef infusion broth to increase growth of pneumococci in thick pus from 23 day lesion.

bacteria decreasing more than a hundredfold during a period of 24 hours of incubation. Since the pH of such preparations, even at the end of incubation, was never found to be below 7.4, it seems unlikely that the observed decrease in bacterial count was due solely to pneumococcal autolysis (15). Rather it would appear more probable that the demonstrable antibacterial action of the pus was caused by the above mentioned leucins. Through slow but persistent action they may likewise have contributed to the eventual self-sterilization of the pneumococcal abscesses which were subjected to prolonged study.

SUMMARY

In contrast to the dramatic antibacterial action in non-suppurating lesions of pneumococcal pneumonia (type I), penicillin therapy failed to effectuate prompt sterilization of analogous pneumonic lesions (pneumococcus III) complicated by abscess formation. Whereas the non-suppurative lesions were free

of pneumococci in less than 54 hours of the start of treatment, the infecting organisms were demonstrable in the suppurative lesions for more than 2 weeks.

Subcutaneous abscesses produced with type III pneumococci were also shown to be relatively refractory to penicillin therapy. As the lesions matured with time, the rate at which the bacteria were destroyed during treatment diminished. The decreased rate of killing of the bacteria was shown to be due to: (*a*) necrosis and death of the leucocytes in the purulent exudate—resulting in less destruction of pneumococci by phagocytosis, and (*b*) a deceleration of bacterial multiplication which resulted from changes in the growth-promoting properties of the exudate and caused the infecting organisms to be less susceptible to the bactericidal action of penicillin.

The possible effect of the failure of penicillin to penetrate to all parts of the suppurative lesions was minimized by repeated injections of the antibiotic directly into the abscesses. A definitive evaluation of this factor, however, could not be made because the experimental lesions tended to be self-limited and therefore may be considered not to have been analogous to the most chronic forms of pyogenic infection.

GENERAL CONCLUSIONS

The results of the experimental analysis reported in this and the two preceding papers (10, 11) indicate that in murine pneumococcal infections penicillin *per se* destroys the invading organisms only in those parts of the lesions where the bacteria are multiplying rapidly and are thus maximally susceptible to the bactericidal action of the drug. In areas where the bacterial growth rate is slowed, either because the pneumococci have reached a maximum population density, or because the accumulated exudate affords a relatively poor medium for rapid growth, the destructive effect of the antibiotic is greatly diminished. In such portions of the lesions the cellular defenses of the host are observed to play a major role in eliminating the bacteria. In sites where frank suppuration has developed, however, even the combined actions of the penicillin and the cellular defenses of the host are relatively ineffective in ridding the tissues of bacteria. Here, because of the poor medium provided by the pus, the pneumococci remain metabolically sluggish and therefore are not killed rapidly by the penicillin. At the same time the leucocytes in the necrotic exudate have deteriorated to the point where they cannot effectively perform their phagocytic functions. As a result, bacteria persist in such lesions for many days in spite of the most intensive penicillin treatment administered both locally and systemically.

A strict analogy cannot be drawn between the action of penicillin upon specific pneumococcal lesions produced in the laboratory and its effect upon acute bacterial infections in man. Host-parasite relationships in acute bacterial infections are determined not only by the strain of parasite and the specific

host involved, but also by the site in the body at which the infection occurs (16). Nevertheless, in spite of the number of variables involved, it may be possible, by means of selected laboratory models, to illustrate general principles of infection which in all probability apply to human disease. Bearing in mind the limitations of the methods employed in the present experiments, it would appear justifiable to draw the following conclusions concerning the clinical use of penicillin in *acute* infections caused by *penicillin-sensitive bacteria.*

The earlier that treatment is begun the more likely is penicillin to effectuate a rapid cure. When therapy is started before the bacteria have reached a maximum population density in any part of the lesion, and before a cellular exudate is formed, the great majority of the infecting organisms will be in a state of active multiplication and thus will be killed promptly by the bactericidal action of the drug.

If, on the other hand, treatment is delayed until the bacterial growth has attained its maximum in older parts of the lesion, and the inflammatory reaction has become well advanced, the resultant slowing of bacterial metabolism will so interfere with the bactericidal action of the penicillin that ultimate destruction of many of the bacteria will have to depend upon the slower clearing effect of the phagocytic cells. In such instances of delayed therapy specific antibody, which is formed relatively slowly, may play an important role in recovery (6). If relapse is to be avoided, however, penicillin therapy must often be continued longer in well established infections than in those treated at a very early stage.

Still further delay in treating infections which are prone to cause tissue destruction and suppuration, may lead to the establishment of abscesses. Fully developed abscesses often will not respond to chemotherapy alone; they will ultimately require drainage. As shown by the present murine experiments, the relative ineffectiveness of penicillin under these circumstances is due not only to the failure of the drug to kill the metabolically sluggish bacteria surviving in the pus, but also to the ineffectiveness of the phagocytic cells, most of which are non-motile or dead. Even if specific antibody gains access to such purulent foci, many of the bacteria will continue to survive because of the degenerated state of the leucocytes. It is evident, therefore, that the *stage of the infection* at which penicillin treatment is begun is often crucial.

Equally critical may be the *location* of the infection. Bacterial lesions in different sites of the body vary greatly in their responses to penicillin therapy. This inconstancy of therapeutic effectiveness is due primarily to the participation of host factors of defense which differ widely in various tissues and at the same time play a major role in the curative action of the antibiotic. In cases of pneumococcal pneumonia, for example, in which each milliliter of the patient's blood contains more than 1000 pneumococci, blood cultures may become negative in a matter of minutes after the start of intensive treatment (17). The remarkable promptness with which penicillin therapy controls such

acute bacteriemia is due, first, to its suppressive effect upon the primary infection in the lungs and regional lymph nodes from which the bacteria are being poured into the blood stream (16) and, secondly, to its synergistic action with the cellular defenses of the circulation. The latter are known to be extraordinarily efficient, perhaps more so than in any other tissue of the body (18). Assisting them in destroying the circulating bacteria is the penicillin's own bactericidal effect, which operates rapidly upon the metabolically active organisms in the plasma. Rarely, if ever, as they often do in other tissues of the body (10), do bacteria in the bloodstream reach such numbers, or do inflammatory cells accumulate intravascularly to such an extent, as to create metabolic conditions which depress the bactericidal actions of the antibiotic.

In contrast, more prolonged and extensive penicillin therapy is needed to cure pneumococcal endocarditis (19), meningitis (19, 20), or infections of the serous cavities (3, 4). The cellular defenses of the heart valves and of the "open" fluid-containing cavities of the body are relatively inefficient as compared to those that operate in the bloodstream and in tissues with tightly knit architectures such as the lungs and lymph nodes (16). In endocarditis relatively few phagocytic cells ever reach the site of the offending bacteria (21), and in infections of fluid-containing cavities, the phagocytic efficiency of the mobilized leucocytes is seriously interfered with by the "dilution effect" of the fluid (22, 23). Accordingly, final destruction of the bacteria must depend primarily upon the bactericidal effect of the antibiotic itself, since little assistance is provided by phagocytosis. It is no wonder, therefore, that such infections, as compared to bacteriemia, are relatively refractory to penicillin therapy.

Certainly penicillin, in spite of its remarkable therapeutic properties, falls far short of being a therapia sterilans magna (24). Its effectiveness does not depend solely upon the inherent susceptibility of the infecting agent to its antimicrobial action. How readily it will cure a given infection is determined also by the state of growth of the bacteria in the various zones of the lesions, the influence of the purulent exudate upon the bactericidal action of the drug, and the destructive effect of the inflammatory phagocytes upon the invading bacteria. Optimal use of penicillin as a therapeutic agent requires due consideration of all of these factors.

Finally, it should be emphasized that the conclusions drawn from this experimental analysis cannot be applied to antibiotic therapy in general. They pertain only to the action of *penicillin* in acute infections caused by *penicillin-sensitive bacteria* which act in the host as *extracellular parasites* (16). The most common human infections included in this category are those caused by pneumococci and Group A beta hemolytic streptococci.[7] Whether they apply

[7] In the case of beta hemolytic streptococci, Wilson has described occasional egestion of phagocyted organisms by leucocytes *in vitro* (25). Whether this phenomenon occurs *in vivo* is not known. Studies on experimental streptococcal pneumonia, however, indicate that, once phagocyted, beta hemolytic streptococci are usually destroyed (26).

also to infections due to penicillin-sensitive staphylococci may be questioned because of recent evidence that certain pathogenic strains will survive phagocytosis (27). In diseases such as tuberculosis, brucellosis, and typhoid fever, which are treated with antibiotics having properties different from those of penicillin (28) and which are caused by bacteria capable of intracellular parasitism (28), factors other than those considered in the present analysis must certainly be involved in the curative effect of antimicrobial therapy.

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EXPLANATION OF PLATES

PLATE 24

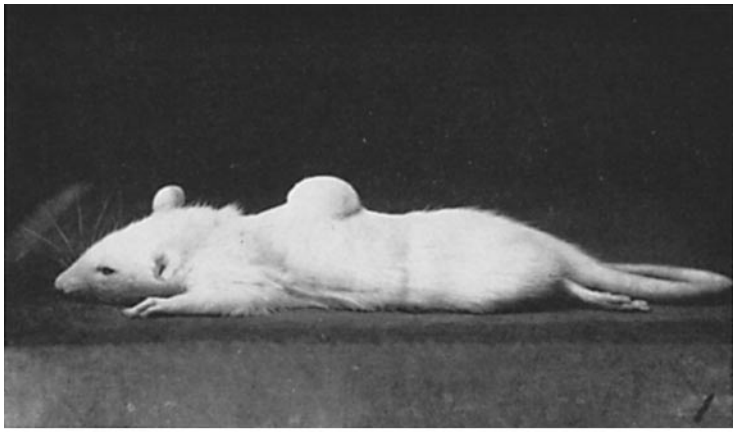
All sections fixed in Zenker-formol solution and stained by a modification of the Gram-Weigert technique (6). The smears of exudate shown in Figs. 3 and 4 were stained with methylene blue.

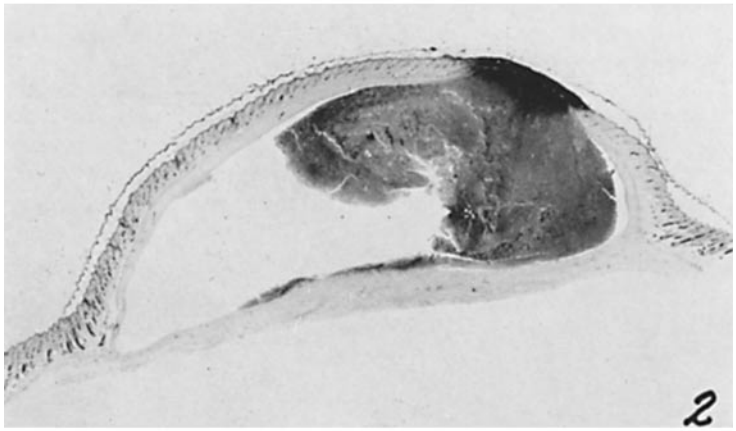
FIG. 1. Subcutaneous abscess on back of rat 23 days after inoculation. × ⅓.

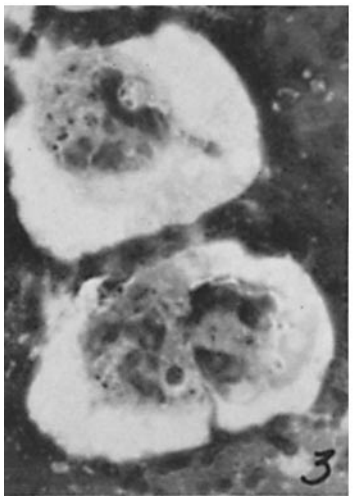
FIG. 2. Cross-section of abscess cavity 18 days after inoculation. × 3.

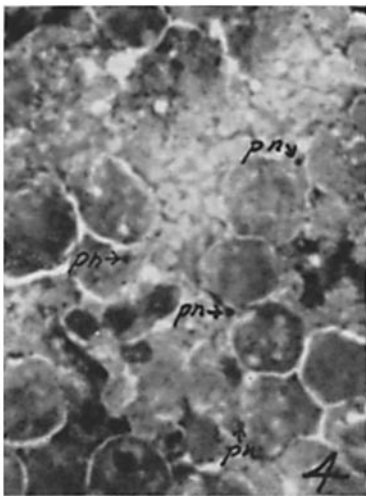
FIG. 3. Phagocytosis of pneumococci in exudate of subcutaneous lesion 24 hours after inoculation. Note that majority of leucocytes are morphologically intact. Numerous unphagocyted pneumococci are visible in serous fluid of exudate. × 1300.

FIG. 4. Necrotic leucocytes and unphagocyted pneumococci (*pn*) in pus from subcutaneous abscess 12 days after inoculation. × 1300.









(Smith and Wood: Curative action of penicillin. III)

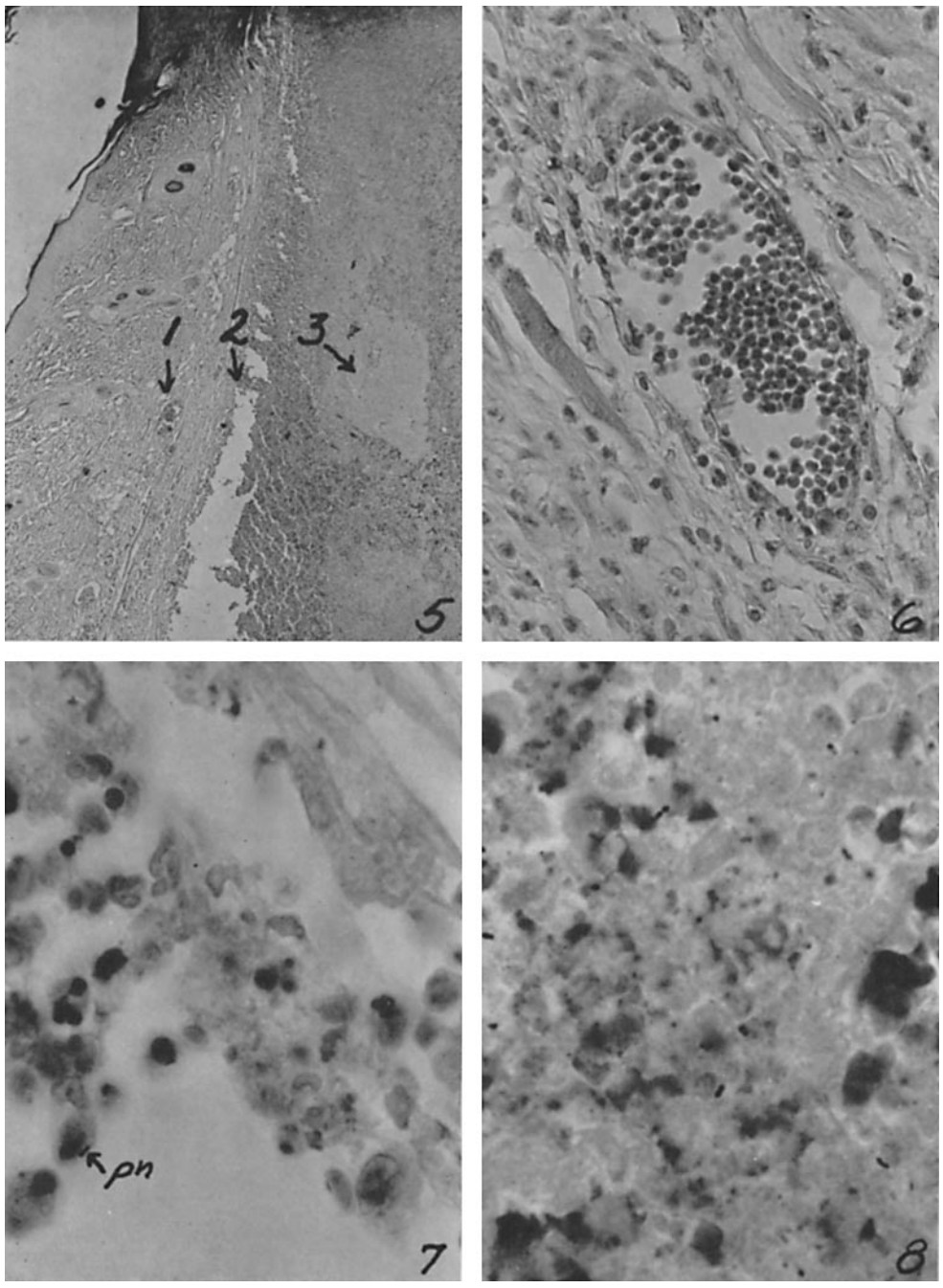
PLATE 25

FIG. 5. Wall of abscess (after 18 days). Note that surface of skin is necrotic at upper border of photograph. × 40. Areas *1*, *2*, and *3* are shown at higher magnification in Figs. 6, 7 and 8 respectively.

FIG. 6. Small vessel in subcutaneous tissue bordering abscess. It is from such vessels that leucocytes have migrated to form exudate in cavity of lesion. × 500.

FIG. 7. Morphologically intact leucocytes at margin of lesion. Note phagocyted pneumococcus (*pn*). × 1200.

FIG. 8. Leucocytic debris and unphagocyted pneumococci in necrotic pus contained within core of abscess. × 1200.



(Smith and Wood: Curative action of penicillin. III)