of an oral penicillin preparation, and indeed a penicillin preparation for parenteral use, will be dependent upon some characteristic of the penicillin itself, or upon its combination with some substance which will protect it against agents in the intestinal tract or body fluids and tissues that destroy or inactivate it.

In the case of oral penicillin preparations, the amount that may be absorbed under the most favorable conditions is dependent upon the protection of penicillin, while in the intestine, from destruction by bacteria or their enzymes. We have demonstrated in the foregoing experiments that protection against the most common of these agents, penicillinase, can be had by a combination with sodium sulfanilate, sodium benzoate or certain other compounds. The mechanism of this protection cannot be explained at this time, since related compounds (chemically) fail to confer this protection.

It has further been observed that certain compounds, such as bromsaligenin, that do not interfere with enzymatic activity may produce a similar result by inhibiting the penicillinase-producing bacteria.

It can be shown that sodium sulfanilate or sodium benzoate protect penicillin to some extent against penicillinase *in vitro*. If breakdown of the penicillin after absorption is accomplished by enzymes in body tissues and fluids (and this seems likely since there is a considerable loss of penicillin even after parenteral administration), it seems possible that this, too, may be reduced by the protective effect of these drugs.

*Conclusions.* 1. Penicillin blood serum levels can be prolonged by the administration of penicillin with sodium sulfanilate, sodium benzoate, bromsaligenin or certain other compounds. 2. Effect of bromsaligenin on penicillin has been demonstrated *in vitro* to be due to inhibition of growth of *Esch. coli* and other Gram-negative intestinal bacteria. 3. The effect of sodium benzoate or sodium sulfanilate on penicillin has been shown *in vitro* to be due to inhibition, at least temporarily, of the enzyme penicillinase. 4. That such drugs are useful and effective in prolonging penicillin levels in the blood and so increasing the efficiency of penicillin has been shown by *in vivo* tests in mice and rabbits. 5. A list of compounds that do not possess this quality has been given, as well as some chemicals that give questionable results.

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## Action of Penicillin on Staphylococcus. Effect of Size of Inoculum on the Test for Sensitivity.*

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In some of the early reports[1,2] on the antibacterial effect of penicillin, it is stated that the action of this agent appears to be unrelated to the number of bacteria present. Perhaps as a result of this, the commonly used tests[3,4] for penicillin susceptibility of organisms vary widely from laboratory to laboratory in respect to the size of the inoculum which is used. In the course of certain other work

* This work was supported in part by The Upjohn Company.

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **241**, 177.

2 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.

3 Rammelkamp, C. H., and Maxon, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

4 Spink, W. W., and Ferris, V., *Science*, 1945, **102**, 221.

TABLE I.
Penicillin Inhibition of Staphylococcus.

| Penicillin per ml | "Susceptibility Ratio" (see text) | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| 64. | | 1 | 1 | | | | | | | | | | | 2 |
| 32. | | | | | | | | | | | | | | |
| 16. | | | | | | | | | | | | | | |
| 8. | | | | | | | | | | | | | | |
| 4. | | 3 | | | | | | | | | | | | 3 |
| 2. | | | | | | | | | | | | | | |
| 1. | | | | | | | | | | | | | | |
| .50 | | | | | | | 1 | | 10 | 5 | | | | 16 |
| .25 | | | 1 | 1 | | 1 | | 1 | 2 | 2 | 1 | 2 | | 11 |
| .12 | 3 | 2 | 1 | 1 | | | | | 1 | | | | 2 | 10 |
| .06 | 5 | 40 | 14 | | 3 | | | 1 | | | | | | 63 |
| .03 | 13 | 13 | 12 | 1 | | | | | | | | | | 39 |
| .016 | | 8 | 6 | 1 | 2 | | | | | | | | | 17 |
| .008 | | | 1 | 1 | | | | | | | | | | 2 |
| Total | 21 | 67 | 36 | 5 | 5 | 1 | 1 | 2 | 13 | 7 | 1 | 2 | 2 | 163 |

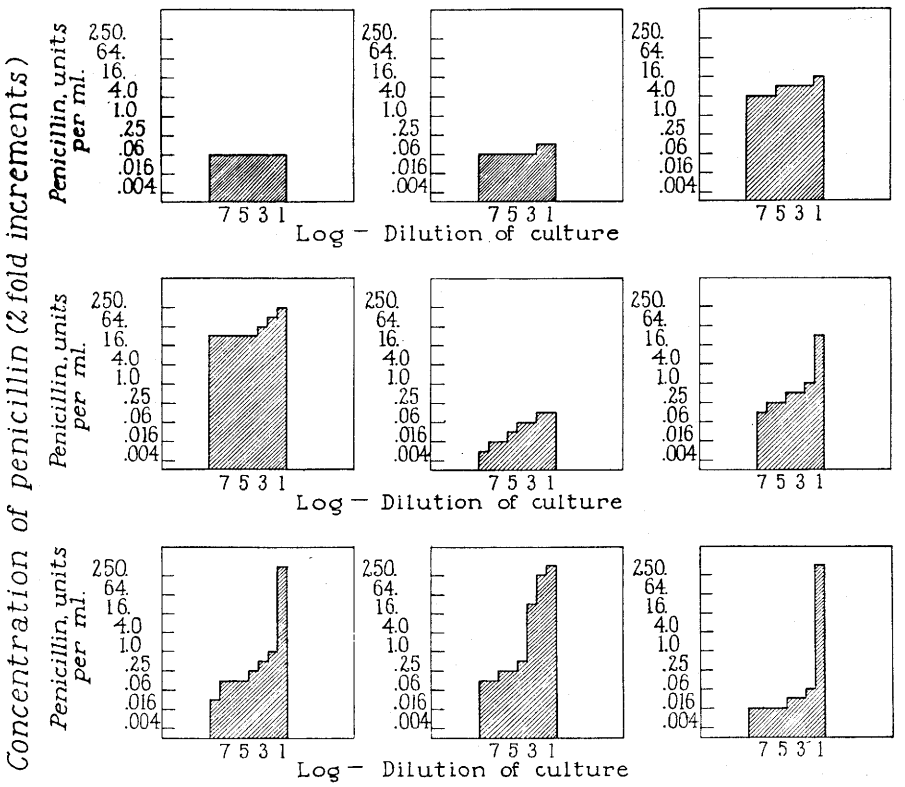
in this laboratory on the action of penicillin on Staphylococcus it was observed that the antibacterial action of penicillin was not always independent of the density of the bacterial population, but that the results of penicillin sensitivity tests might be profoundly influenced by the size of the inoculum. Because of the great practical as well as theoretical importance of the question it was decided to explore the problem systematically.

One hundred and sixty-three strains of *Staphylococcus aureus*, isolated in the routine diagnostic laboratory serving Lakeside Hospital, were subjected to tests of penicillin susceptibility as follows. A series of culture tubes was prepared, having in each 0.5 ml of tryptose phosphate broth, and containing penicillin in concentrations from 1000 to 0.002 units per ml. Two such series were used for each organism tested, one series being inoculated with 0.5 ml of a $10^{-2}$ dilution of a 48-hour culture in tryptose broth and one with a $10^{-6}$ dilution. They were incubated overnight, and the tube in each series containing the minimal inhibitory concentration of penicillin was identified. The viable cell count of the 48-hour broth cultures used for inoculation was Log 8.85 (S.D. 0.65) per ml, the inoculum therefore Log 2.85 and Log 6.85 on the average. The concentration of penicillin giving complete inhibition of growth in the small-inoculum series was arbitrarily taken as the "susceptibility" of the organism. The discrepancy, if any, between the 2 tests was recorded as the number of tubes (*i.e.* 2-fold increments of penicillin concentration) by which the end-points differed. Thus an organism, growth of which was prevented by 0.06 units/ml when using a small and 0.25 units/ml when using the large inoculum was recorded as having a penicillin susceptibility of 0.06-2. The results of these tests are collected in Table I.

It is seen from the totals given in the last column of Table I that the distribution of the organisms with regard to penicillin susceptibility approaches normality, although there is a definite suggestion that 2 populations are involved. Of these, the one might have a peak at susceptibility to 0.06 unit per ml of penicillin, the other at susceptibility to 0.50 unit per ml.

In the bottom row of Table I will be found the distribution of organisms according to the "susceptibility ratio." This is the ratio between the results of the 2 tests of susceptibility, expressed as the number of tubes in the series which separate the end-points of the determinations. Here there are evidently 2 distinct populations. In one, the outcome of the test of penicillin sensitivity is influenced little if at all by the number of organisms inoculated; in the other, the influence is striking. The separateness of the 2 groups of organisms is revealed also in

Fig. 1.




the correlation table itself, for there are evidently 2 centers around which the data tend to cluster. In one of these the organisms have low resistance, and a low ratio between the results of high- and low-inoculum tests; in the other definitely greater resistance to penicillin is associated with a high ratio.

All of the strains studied were subjected to the duplicate test of penicillin sensitivity. In addition, a certain number were subjected to a more elaborate one. In this test, replicate determinations of penicillin sensitivity were performed, using as inoculum serial 10-fold dilutions of culture from $10^{-1}$ to $10^{-8}$. For each inoculum the minimal inhibitory concentration of penicillin was recorded. A representative group of the results are collected in Fig. 1, inspection of which reveals that it is possible to arrange the histographs in a fairly regular sequence.

At one extreme are found those strains in which the result has no relation to the size of the inoculum. At the other extreme are those in which stepwise increase in size of inoculum results in a stepwise increase in the apparent resistance of the organism to penicillin, with progress from one extreme to the other being made gradually.

While this study was in progress, Luria[5] called attention to the fact that the results of the dilution test of sensitivity to penicillin might be profoundly influenced by the number of organisms used in the inoculum. The technic which he proposed for testing penicillin sensitivity is essentially the one used here, and these results may therefore be interpreted as an extension of his, and confirmation of his prediction that the phenomenon was not rare. In Luria's opinion those organisms which showed a marked discrepancy in the results of the 2 tests were those which were able to elaborate penicil-

[5] Luria, S. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 46.

linase. The data reported here give no direct information on this point, but the variety of curves obtained when resistance to penicillin is plotted against regular increases in the number of cells in the inoculum suggests that the phenomenon may have a complex basis. That this rule may not always hold is suggested by the fact that one organism, resistant to penicillin and capable of producing penicillinase showed only a slight difference in apparent sensitivity in the 2 tests. Further work on this line is now in progress.

On the other hand, that in regard to this character there are 2 sorts of staphylococci seems apparent. It is of considerable importance clinically that the great bulk of the organisms fall in the group against which the antibacterial action of penicillin is exerted with little regard to the actual density of the bacterial population.

*Conclusions.* 1. The action of penicillin *in vitro* against most strains of Staphylococcus is related only in a minor degree to the number of bacteria present. 2. In a not inconsiderable group of strains, somewhat more resistant to penicillin than the majority, the outcome of tests for penicillin sensitivity is profoundly influenced by the number of cells present.

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## Clinical Trial of Gamma Globulin in the Prevention of Common Respiratory Diseases.

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Serum gamma globulin, separated and concentrated by chemical fractionation of normal human blood, was administered at monthly intervals to one-half of a group of 70 students throughout the respiratory disease season from October, 1945, to May, 1946. The incidence of acute respiratory disease, including whatever the individual considered as a "cold" of any degree of severity was carefully tabulated in the entire group.

The chemical fractionation of human blood plasma has made available certain fractions of globulin which appear to contain many specific neutralizing antibodies. Its value in the prophylaxis of measles[1,2] is now common knowledge in spite of the fact that it is still impossible to measure the concentration of measles protective substance directly. The potency of Fraction II (largely gamma globulin) was shown by Enders[3] in the case of neutralizing antibody for influenza A virus to approximate that of sera taken during early convalescence from the disease. Its protective value in clinical trial studies has not been demonstrated to our knowledge. The subjects in our study passed through an influenza epidemic but the numbers in both groups contracting the disease in a clinically recognizable form were so small in each that no conclusions can be drawn as to its prophylactic value in this instance. Enders[3] found Fraction II to contain antibodies reacting with diphtheria toxin, streptococcal erythrogenic toxin, influenza A virus, mumps virus, and the H antigen of *E. typhosa*. These antibodies he found to be concentrated from 15 to 30 times in the fraction as compared to pooled plasma. From pools of plasma obtained from several areas throughout the United States, a satisfactory degree of uniformity between the various preparations was demonstrated.[3] The gamma globulin used

[1] Ordman, C. W., Jennings, C. G., and Janeway, C. A., *J. Clin. Invest.*, 1944, **23**, 541.

[2] Stokes, J., Jr., Maris, E. P., and Gellis, S. S., *J. Clin. Invest.*, 1944, **23**, 531.

[3] Enders, John F., *J. Clin. Invest.*, 1944, **23**, 510.