income over this amount to a maximum of £50,000 a year. Dr. F. C. Toye, director of the institute, said that it was proposed to set up an engineering department the results from which would have considerable influence on the design and development of textile machinery.

THE central editorial offices of *The American Journal of Psychology* have been removed from Cornell University to the California Institute of Technology at Pasadena. Professor Madison Bentley continues as responsible editor. The business office remains at Morrill Hall, Ithaca, N. Y.

THE American Standards Association has published a new Safety Color Code for Marking Physical Hazards developed by one of its war committees. The purpose of this code is to unify on a national scale the colored markings used to warn employees of certain physical dangers to be avoided, to mark the location of safety equipment and to identify other protective equipment. The standard is a codification of certain already recognized concepts regarding use of color for safety purposes. Much of the standard deals with suggested applications for these colors such as the marking of safety cans, fire sirens, posts, hand rails, unguarded edges of platforms, location of gas masks, stretchers, etc. The investigation was undertaken at the specific request of the War Department, but it also has a wide application in industry. Neither the War Department nor industry intends to use color markings as a substitute for adequate guards or other safety measures, but to supplement them. The American Standards Association will welcome comments on: (1) difficulties that arise in using the standard, and (2) exceptionally good results from its use.

SPECIAL ARTICLES

QUANTITATIVE ACTION OF PENICILLIN INHIBITOR FROM PENICILLIN-RESISTANT STRAINS OF STAPHYLOCOCCI[1,2]

A POTENT inhibitor of penicillin has been obtained by Kirby[3,4] from coagulase-positive strains of staphylococci which were naturally resistant to penicillin. We have extended this observation and have shown that strains of staphylococci made resistant to penicillin *in vitro* do not produce a penicillin inhibitor, but strains which have become resistant in the human body following the administration of penicillin yield a potent inhibitor of penicillin.[5] This suggested to us a similarity between the development of resistance of staphylococci to the sulfonamides and to penicillin, though the underlying mechanism may differ.[6] The purpose of this preliminary report is to show that the degree of resistance which a group of selected strains of staphylococci has for penicillin, as measured *in vitro*, is quantitatively related to the penicillin inhibitor produced by these strains; that is, the greater the resistance, the more potent is the inhibitor. Furthermore, crude inhibitor prepared from resistant strains of staphylococci will inactivate varying concentrations of penicillin over a period of time in a quantitative manner, the time required for the inactivation being dependent upon the amounts of inhibitor and concentrations of penicillin which are used.

METHODS

Source of Strains. Seven strains of coagulase-positive staphylococci isolated from human patients were studied. Five of these strains had apparently become resistant in the body as a result of therapy with penicillin, since organisms obtained from the same lesions prior to treatment were sensitive to penicillin. The two remaining strains had not been previously exposed to penicillin and displayed a natural resistance to penicillin. We are indebted to Dr. Donald G. Anderson of Boston for providing us with three strains.

Determination of in vitro sensitivity to penicillin. The sensitivity of the strains of staphylococci to sodium penicillin was determined in the following manner: each of the strains was grown for two to three generations in Gladstone's synthetic and water-clear medium, the preparation of which has been described elsewhere.[7] Then 0.1 ml of a 10^{-3} dilution of a 24-hour culture was seeded to each of several tubes containing Gladstone's medium. The final volume of each tube was 10 ml. Poured plates indicated that such an inoculum contained approximately from 300,000 to 900,000 organisms. Freshly prepared aqueous solutions of commercial sodium penicillin were added in increasing concentrations to the series of tubes containing the bacterial suspensions. Incubation at 37 degrees C. was then carried out for 48 hours. The amount of penicillin necessary to inhibit growth completely was ascertained by selecting that tube which revealed no turbidity with the lowest concentration of penicillin.

[1] From the Division of Internal Medicine, University of Minnesota Hospitals and Medical School, Minneapolis.

[2] Supported by grants from Sharp and Dohme, Inc.; The Graduate School, University of Minnesota; and from the Committee on Scientific Research, American Medical Association.

[3] W. M. M. Kirby, SCIENCE, 99: 452, 1944.

[4] *Idem, Jour. Clin. Invest.*, 24: 170, 1945.

[5] W. W. Spink and V. Ferris, *Proc. Soc. Exp. Biol. and Med.* (in press).

[6] W. W. Spink, L. D. Wright, J. J. Vivino and H. R. Skeggs, *Jour. Exp. Med.*, 79: 331, 1944.

[7] W. W. Spink and J. J. Vivino, *Jour. Clin. Invest.*, 23: 267, 1944.

Determination of potency of penicillin inhibitor. The potency of penicillin inhibitor produced by each strain was defined as follows: crude inhibitor was obtained from a 24-hour culture grown on agar plates by extracting a concentrated suspension of organisms with acetone and ether according to the method of Harper[8] as described by him for the paracolon bacillus. The extracted bacteria were then dried and tested for sterility. Then 0.1 ml of a 10^{-3} dilution of a 24-hour culture of staphylococcus, which had been previously established as being sensitive to penicillin, was added to each of several tubes with Gladstone's medium and increasing concentrations of sodium penicillin. Either 0.01 or 0.1 mg per ml of dried bacteria, depending upon the potency of the inhibitor, was added to the tubes which were then left at 37 degrees C. for 48 hours. The tube showing bacterial growth with the highest concentration of penicillin indicated the number of units of the antibiotic which were inactivated by the given quantity of dried bacteria.

Quantitative action of penicillin inhibitor. The quantitative action of inhibitor was resolved by the following method: the same amount of dried bacteria was added to each of several sterile and standardized colorimetric tubes containing the foregoing inoculum of a 24-hour culture of a strain of penicillin-sensitive staphylococcus and increasing concentrations of sodium penicillin. Growth curves of the organisms were then made by incubating the contents at 37 degrees C. and measuring the density of the bacterial culture in the Evelyn photoelectric colorimeter with a 540 filter at appropriate time intervals over a period of 60 hours. A similar series of observations were made by using varying amounts of dried bacteria and the same concentration of penicillin in each tube. Repeated observations were carried out with different sources of commercial penicillin, including 8 preparations of sodium penicillin, 2 of calcium penicillin and 2 of ammonium penicillin.

RESULTS

The results of a representative series of observations on the resistance *in vitro* of 7 strains of coagulase-positive staphylococci to the same lot of sodium penicillin are presented in Table 1. The number of units of penicillin necessary to inhibit growth completely varied from 0.6 to 20 units per mil. For comparison, the inhibitory effect of 0.01 or 0.1 mgr per ml of dried bacteria obtained from these strains upon the action of penicillin against a penicillin-sensitive strain of staphylococcus is presented. It is to be noted that 800 units of penicillin per ml were necessary to overcome the inhibitory effect of 0.01 mg per ml of extracted organisms from strain 1. Control observations revealed that only 0.1 unit of penicillin per ml was required to prevent growth of the test strain in the absence of inhibitor. There appears to be a rough correlation between the resistance of a strain to penicillin and the potency of inhibitor produced by that strain.

[8] G. J. Harper, *Lancet*, 2: 569, 1943.

TABLE 1

COMPARATIVE IN VITRO RESISTANCE OF STRAINS OF STAPHYLOCOCCI TO PENICILLIN AND POTENCY OF PENICILLIN INHIBITOR PRODUCED BY THESE STRAINS

Strain No.	No. of units of penicillin per ml required to inhibit growth completely	No. of units of penicillin per ml required to inhibit growth completely of sensitive strain of staphylococcus in presence of 0.1 mg per ml of inhibitor
1	20	800*
2	2	60*
3	1	40
4	1	40
5	0.6	30
6	0.6	20
7	0.6	20

* 0.01 mg per ml of inhibitor.

Another representative group of observations is illustrated in Fig. 1. The effect is shown of 0.005 mg per ml of dried bacteria from strain 2 upon the growth curves of a penicillin-sensitive strain of staphylococcus in the presence of varying concentrations of sodium penicillin. The growth of the strain of staphylococcus employed in the tests was inhibited by 0.05 to 0.1 units per ml of penicillin. Although the control tube without penicillin revealed a dense bacterial growth within 24 hours, the inhibitory

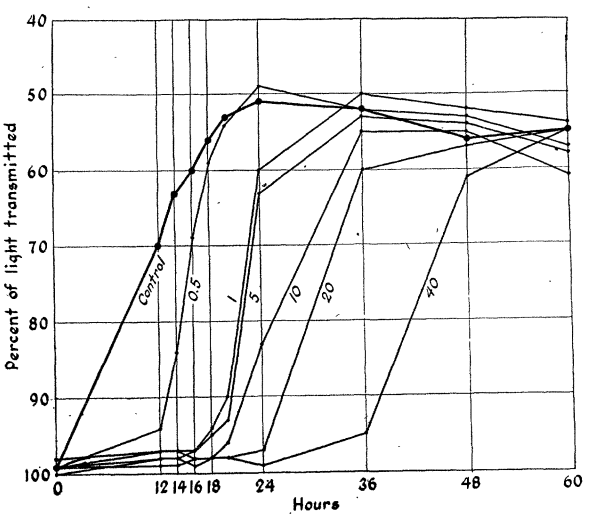

FIG. 1. Effect of 0.005 mgs per ml of penicillin inhibitor upon antistaphylococcic action of sodium penicillin in concentrations varying from 0.5 to 40 units per ml. Turbidity of bacterial growth expressed in per cent. of light transmitted in Evelyn photoelectric colorimeter.

effect of penicillin in the other tubes was still being manifested at this time. With the elapse of more time, the inhibitory effect of penicillin was overcome, and the density of growth in all the tubes eventually approximated that of the control tubes.

Using the same penicillin-sensitive strain of staphylococcus, the effect of different amounts of dried bacteria from strain 2 upon the antistaphylococcic action of 1 unit of penicillin per ml was determined. Fig. 2

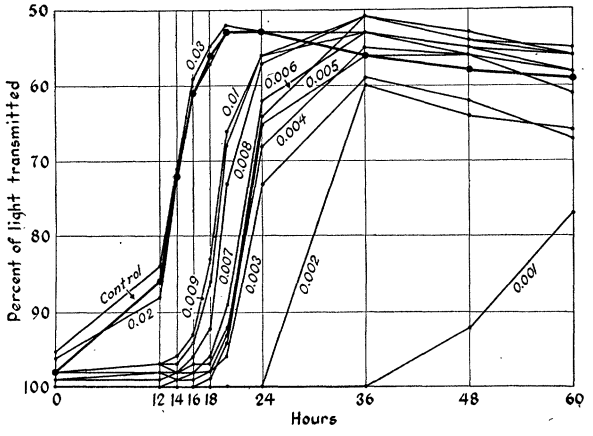

FIG. 2. Effect of concentrations of penicillin inhibitor varying from 0.001 to 0.03 mg per ml upon antistaphylococcic action of 1 unit per ml of sodium penicillin. Turbidity of bacterial growth expressed in per cent. of light transmitted in Evelyn photoelectric colorimeter.

presents the results of one set of growth curves. As the concentration of the penicillin inhibitor increased, a point was reached where the growth of the test strain in the presence of 1 unit of penicillin approximated that of the control tube without penicillin.

It also has been shown that dried bacteria from both penicillin-sensitive and penicillin-resistant strains of staphylococci act as a growth stimulus for coagulase-positive staphylococci when grown in Gladstone's medium.

COMMENT

The foregoing data indicate that one of the mechanisms whereby staphylococci develop resistance to penicillin is the production of a potent inhibitor of penicillin by the organisms. That this is not the only mechanism involved in penicillin-resistance is shown by the fact that staphylococci which have become resistant to penicillin *in vitro* do not yield a demonstrable inhibitor.[5] However, changes in bacterial metabolism are very likely involved in both instances. A feature of the present observations is that an elapse of time is essential for inhibitor from staphylococci to overcome the antibacterial action of penicillin. This time factor is related to both the concentrations of penicillin and to the amounts of inhibitor which are present. With increasing amounts of inhibitor, the antistaphylococcic effect of penicillin is more promptly overcome. Whereas, decreasing quantities of inhibitor require increasing periods of time to inhibit the penicillin. The application of these biologic phenomena to the clinical use of penicillin bear further investigation. Furthermore, the isolation and identification of penicillin inhibitor from staphylococci are desirable from the viewpoint of defining the precise mode of action of penicillin.

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INHIBITING FACTORS IN THE DETERMINATION OF PENICILLIN IN HUMAN SERA

OF the methods proposed for the measurement of penicillin in serum the majority are based on the activity of penicillin on a selected strain of a hemolytic streptococcus.[1-7] A recent publication[8] demonstrated the possible advantages in the use of *B. subtilis* in place of the streptococcal culture (C203) used by most investigators.

Before accepting either method as being superior, and in view of indications of considerable variations in results obtained by the two methods in recent studies on penicillin oral medication in adults,[9] it was decided to determine the extent of the sensitivity of each organism to the inhibitory power of human sera.

The literature on the inhibitory power of blood and serum is considerable. In this respect the work of Tillet[10, 11] is very inclusive. Tillet found that the streptococcidal property of blood from patients suffering from a variety of diseases was pronounced. This property was absent or greatly diminished in the same patients after recovery. Comparative tests with sera from healthy adults showed this activity to be completely absent. Strain susceptibility of hemolytic streptococci varied considerably; one was destroyed by all sera while another by only a few. A third strain was intermediate in sensitivity.

In a study on oral dosage with penicillin,[4] all the determinations of the penicillin concentration in serum were made on sera from children three weeks to twelve years of age. In that study, in agreement with Rammelkamp, the tubes showing no hemolysis, when tested,

[1] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.
[2] C. Wilson, *Nature*, 152: 475, 1943.
[3] G. Rake and H. Jones, *Proc. Soc. Exp. Biol. and Med.*, 54: 189, 1943.
[4] P. György, *et al.*, *Jour. Am. Med. Asn.*, 127: 639, 1945.
[5] W. M. M. Kirby and L. A. Rantz, *Jour. Bact.*, 48: 603, 1944.
[6] W. McDermott *et al.*, SCIENCE, 101: 228, 1945.
[7] P. Rosenblatt *et al.*, *Jour. Bact.*, 48: 599, 1944.
[8] W. A. Randall *et al.*, SCIENCE, 101: 365, 1945.
[9] P. György *et al.*, to be published.
[10] W. S. Tillet, *Jour. Exp. Med.*, 65: 163, 1937.
[11] W. S. Tillet and C. C. Stock, *Proc. Soc. Exp. Biol. and Med.*, 37: 82, 1937–1938.