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

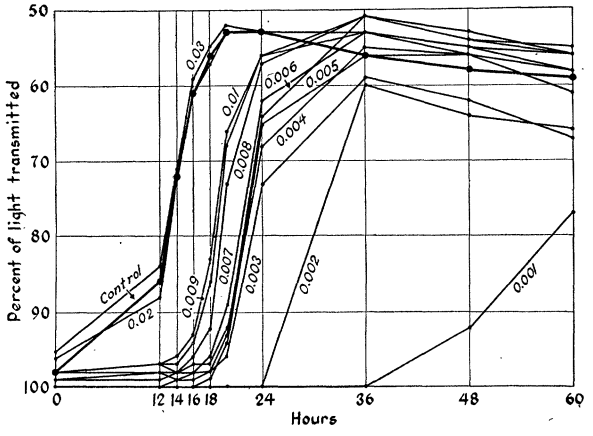

FIG. 2. Effect of concentrations of penicillin inhibitor varying from 0.001 to 0.03 mg per ml upon antistaphylococcic action of 1 unit per ml of sodium penicillin. Turbidity of bacterial growth expressed in per cent. of light transmitted in Evelyn photoelectric colorimeter.

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.

proved sterile by subculture on blood agar streak plates.

In eliminating the serum inhibiting factor from streptomycin assays, Stebbins and Robinson[12] have substituted a strain of *Staphylococcus aureus* for *B. subtilis*. This strain of *S. aureus* was included in part of the following series of tests.

EXPERIMENTAL

Sera from three series of persons, with no recent history of penicillin or sulfonamide treatment, were tested for their inhibitory activity on the streptococcal strain C203 by the procedure outlined by György *et al.*[4] The same sera were employed in determining the sensitivity of the strain of *B. subtilis* used in the method proposed by Randall[8] with the exception that the tube dilution system of György's replaced the serial dilution method. Tube dilutions have the advantage of providing closer determinations. The strain of *S. aureus* of Stebbins[12] was eliminated during the investigation for reasons discussed later. However, the test procedure was identical to that used for *B. subtilis*.

The first series consisted of sera from 100 normal adults, kindly supplied by Dr. A. H. Price, of the Jefferson Medical College, Philadelphia, Pa. The second series of 26 sera were obtained through the kindness of Dr. John Sinnott, Jr., of the Pennsylvania Hospital, Philadelphia, Pa., and represent sera from adults suffering from a wide variety of ailments. The sera in the third series, consisting of sera from 12 children, were obtained through the courtesy of Dr. Paul György, of the Department of Pediatrics, University of Pennsylvania School of Medicine.

The second series was included in this work to demonstrate the difference between sera from normal adults and those suffering from some ailment. The series of children was included in order to verify or disprove a contention that sera from children (under 12 years) were not inhibitory to the streptococcal strain C203 to the same degree as were the sera from adults. The contrast in sensitivity between the streptococcal and subtilis cultures was also shown in sera from children.

It has been demonstrated[4] that five minutes in a water bath at 56° C. did not affect the penicillin content of serum. Thus, when sufficient serum was available, 1 ml samples were so treated and any alterations in the inhibitory effect on the two cultures were noted.

Tube results were read after 16 hours, 24 hours and 40 hours of incubation at 37° C. In the initial fifty normal adult sera, subcultures were made on blood agar plates from the tubes showing no visible growth only at the 40-hour period. With the desire to shorten

TABLE 1

THE INHIBITORY AND BACTERICIDAL EFFECT OF HUMAN SERA ON THE GROWTH OF AN HEMOLYTIC STREPTOCOCCUS (C203) AND *B. subtilis**

Tube inhibition at incubation period of	Volume of serum in 2 ml final volume	Normal adult sera—100 cases		Sera from Ill (otherwise sick, or ailing, adult's)—26 cases		Sera from children—12 cases	
		Strept. C203	*B. subtilis*	Strept. C203	*B. subtilis*	Strept. C203	*B. subtilis*
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
16 hrs.	1.0 ml	12	18	11.5	7.7	33.3	8.3
	.8 "	18	17	3.8	19.2	25.0	8.3
	.6 "	12	30	34.5	30.8	25.0	50.0
	.4 "	5	21	19.2	26.9	0	16.7
	.2 "	2	3	15.4	7.7	0	8.3
	Total cases	49	89	84.4	92.3	83.3	91.6
24 hrs.	1.0 ml	10	24	7.7	26.9	8.3	16.7
	.8 "	8	23	7.7	23.1	0	33.3
	.6 "	1	18	19.2	19.2	0	16.7
	.4 "	1	6	19.2	15.4	0	0
	.2 "	0	0	11.5	3.8	0	0
	Total cases	20	71	65.3	88.4	8.3	66.7
40 hrs.	1.0 ml	7	29	11.5	26.9	8.3	16.7
	.8 "	5	10	11.5	23.1	0	16.7
	.6 "	1	2	19.2	7.7	0	0
	.4 "	1	1	3.8	3.8	0	0
	.2 "	0	0	7.7	0	0	0
	Total cases	14	42	53.7	61.5	8.3	33.4

		24 hrs.†	40 hrs.	24 hrs.	40 hrs.	24 hrs.	40 hrs.	24 hrs.	40 hrs.	24 hrs.	40 hrs.	24 hrs.	40 hrs.
Bactericidal activity as demonstrated by absence of growth on subculture	1.0 ml	0	0	20	18	3.8	3.8	30.7	30.7	8.3	8.3	16.7	8.3
	.8 "	0	1	6	5	15.4	19.3	0	0	0	0	8.3	8.3
	.6 "	0	0	2	1	15.4	11.5	0	0	0	0	8.3	0
	.4 "	0	1	0	0	0	0	3.8	3.8	0	0	0	0
	.2 "	0	0	0	0	7.7	7.7	0	0	0	0	0	0
	Total cases	0	2	28	24	42.3	42.3	34.5	34.5	8.3	8.3	33.3	16.6

* Results are expressed as the per cent. of the total cases showing inhibitory activity at each particular volume of serum. These results, with the exception of the summations, are not cumulative.

† As indicated in the text, the initial 50 normal adult sera were subcultured only at 40 hours, providing a total of 50 cases subcultured at 24 hours and a total of 100 subcultured at 40 hours.

[12] R. B. Stebbins and H. J. Robinson, Merck Institute for Therapeutic Research, Rahway, N. J. Personal communication.

the test duration, subcultures from all remaining sera were made at the 24- and 40-hour periods from tubes showing no visible growth or hemolysis.

RESULTS AND CONCLUSIONS

The results of this investigation, including the data presented in Tables 1 and 2, demonstrate that:

(1) Inhibitory substances to the streptococcal strain C203 and *B. subtilis* exist in human sera.

(2) The effect of these substances may be very easily misinterpreted as penicillin activity, especially in concentrations of the order of 0.02 to 0.05 units/ml of serum.

(3) The inhibitory activity of normal adult sera was much more pronounced against *B. subtilis* than against the streptococcus. In the former the effect was bactericidal as well as bacteriostatic; in the latter the effect was only bacteriostatic, which activity could be nullified by subculture after 24 hours' incubation of the tubes.

(4) The contrast between the streptococcus and *B. subtilis* cultures carried over to sera from children. Of twelve sera only one, and even then only in the 1 ml serum volume, showed inhibitory activity against the streptococcus, whereas bactericidal activity was demonstrated in one third of the sera against *B. subtilis* at 24 hours.

(5) Sera from ailing adults showed pronounced bacteriostatic and bactericidal activity against both microorganisms. This agrees with the observations of Tillet and is probably due to the greater concentration of inhibitory substances whose effect can not be eliminated by subculture.

TABLE 2

THE EFFECT OF HEAT (56° C. WATER BATH FOR 5 MINUTES) ON THE INHIBITORY AND BACTERICIDAL ACTIVITY OF SERA FROM 47 NORMAL ADULTS*

Tube inhibition at incubation period of	Strept. C203		*B. subtilis*	
16 hrs.	27.7 per cent.		55.3 per cent.	
24 "	8.5 " "		48.9 " "	
40 "	4.3 " "		14.9 " "	
Bactericidal activity as demonstrated by absence of growth on subculture at	*24 hrs.*	*40 hrs.*	*24 hrs.*	*40 hrs.*
	0 per cent.	0 per cent.	12.8 per cent.	8.5 per cent.

* 1 ml volumes of sera were contained in 2 ml final volumes with broth as diluent. Results are expressed in per cent. of cases showing inhibitory activity as in Table 1

(6) Heating normal adult sera at 56° C. for five minutes lowered the incidence of bacteriostatic activity against the streptococcus and *B. subtilis* but did not eliminate the bactericidal effect against the latter culture.

(7) In the comparison of penicillin dosage forms the streptococcal method, with subculture, has proven of value when sera from children or normal adults are used in such tests.

(8) Neither streptococcal nor *B. subtilis* method are absolutely reliable in the determination of low concentrations of penicillin in ill adults, since neither method can distinguish between penicillin activity and other bacterial inhibitory substances in sera from such individuals.

(9) The sensitivity of a strain of *Staphylococcus aureus*[12] to inhibitory substances in human sera was determined. Although this organism was not inhibited by 1 ml volumes of serum in 50 cases of normal adult serum, because of its insensitivity to penicillin it is not applicable to the determination of low concentrations of penicillin in serum. This organism required 0.08 unit of penicillin per tube for inhibition as compared to 0.02 unit for the streptococcal strain C203.

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CALCIUM CARBONATE AS AN ANTACID FOR ORAL PENICILLIN

EARLY work by Abraham, Florey and associates[1] and Rammelkamp and Keefer[2] indicated penicillin to be ineffective by the oral route because of destruction by stomach acidity. Later, Free *et al.*,[3] using larger doses and more purified penicillin, showed that some penicillin escapes destruction by the stomach acid and is absorbed, thereby renewing interest in the oral route as a possible mode of administration. More recently, somewhat greater absorption has been reported to take place when the penicillin is administered in combination with agents to protect it from destruction by stomach acidity. Libby[4] suspended penicillin in fixed oils and reported a protective effect, but McDermott[5] was not able to show a significant protection by the use of oils. Reports on the administration with certain gastric antacids appear the most favorable. Sodium bicarbonate has been found unsuited for this purpose because of its alkalinity.[1,2,3] Charney *et al.*[6] and Gyorgy *et al.*[7] reported increased absorption by the simultaneous administration of sodium citrate. Sodium phosphate was also

1 E. P. Abraham, H. W. Florey *et al.*, *Lancet*, 2: 177, 1941.
2 C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, 22: 425, 1943.
3 A. H. Free *et al.*, SCIENCE, 100: 431, 1944.
4 R. L. Libby, SCIENCE, 101: 178, 1945.
5 W. McDermott *et al.*, SCIENCE, 101: 228, 1945.
6 J. Charney *et al.*, SCIENCE, 101: 251, 1945.
7 P. Gyorgy *et al.*, *Jour. Am. Med. Asn.*, 127: 639, 1945.