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AMBIGUOUS INHIBITION OF STAPHYLOCOCCI BY PENICILLIN INACTIVATED WITH PENICILLINASE

BY

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For some time past penicillinase prepared by the growth of certain strains of *B. subtilis* in broth has been used in this laboratory to inactivate penicillin before testing for sterility. Each batch of penicillinase is assayed by a method devised in the Bacteriological Laboratory of Messrs. Boots Pure Drug Co., Ltd. (personal communication).

A 1:20 dilution of the penicillinase preparation in water is added in ascending quantities to a series of broth tubes each containing 4,000 units of penicillin. These are inoculated with a loopful of a broth culture of a sensitive staphylococcus, and the end-point is read after 40 hours' incubation at 37° C. On one occasion 20,000 units of penicillin was erroneously added to each tube in setting up the assay. On this being realized the penicillinase was diluted 1:4 instead of 1:20, the assumption being that this would give the same result, since the proportion of penicillinase to penicillin in each tube would be the same. However, no growth of staphylococcus occurred in any of the tubes. The assay was then repeated at the normal level of dilution and an end-point was obtained as usual near the middle of the range.

This incident suggested that the ability of staphylococci to grow in broth plus inactivated penicillin is limited to a certain range of concentration of penicillin.

Experimental Results

It was first shown that failure to grow was not due to a change in pH of the broth. The following experiment was then carried out. Serial dilutions of penicillin in broth were made in geometrical progression, beginning with 200,000 units, the last tube containing 781 units. Corresponding dilutions of penicillinase in water were made and 1 ml. of each dilution was added to each corresponding tube of penicillin, so that the proportion of penicillinase to penicillin was the same in each tube. One loopful of staphylococcus broth culture was added to each tube and the series was incubated at 37° C. The tubes were examined first after 24 hours' incubation; growth had occurred only in the tube containing 6,250 units of penicillin. Examination after 40 hours showed growth in the tubes containing 12,500, 3,125, and 1,562 units, but no growth at the four highest dilutions or at the lowest one.

The experiment was then repeated using quantities of penicillin from 100,000 down to 390 units, and on this occasion examination of the tubes was made at hourly intervals. Growth occurred with 6,250 units at less than 19 hours (as it did in a control tube of plain broth), with 12,500 units in 22 hours, with 3,125 units in 24 hours, and with 1,562 units in 30 hours. No growth occurred with 100,000, 50,000, 25,000, 781, or 390 units up to 3 days.

The results of three further experiments are given in Table I.

TABLE I

Penicillin (Units)	Expt. 1	Expt. 2	Expt. 3
100,000	0	0	—
50,000	0	0	0
25,000	0	0	0
12,500	0	± 3 days	(4) + 3 days
6,250	(2) + 22 hours	(3) + 26 hours	(1) + 21 hours
3,125	(1) + 21 "	(2) + 24 "	(2) + 23 "
1,562	(3) + 23 "	(1) + 22 "	(3) + 40 "
781	(4) + 30 "	(4) + 27 "	0
390	0	(5) + 40 "	0
195	—	± 3 days	0

Control + 18 hours

0 = No growth. + = Normal growth. ± = Trace of growth. — = Not done.

It will be seen that growth always begins first in one of the tubes near the middle of the series and spreads outwards through the tubes on either side of the first, though the order is not constant. If the solutions in the high-concentration tubes in which no growth has occurred are diluted with broth to fall within the range of the positive tubes, growth immediately occurs, sometimes being visible in as short a time as three hours after dilution, showing that the penicillin has in fact been inactivated and that the staphylococci have not been killed by the high concentration, for only the original inoculum is still present. Similar results were obtained in other experiments, using three different samples of penicillinase prepared in media other than papain digest broth.

The penicillin used in all the foregoing experiments was manufactured some time ago and was relatively impure, containing 700 to 800 units per mg., assayed against staphylococcus.

Inhibition of growth is not due to anything in the penicillinase; this was shown by making serial dilutions of penicillinase in broth of the same strengths as used in the experiments with penicillin and inoculating with the same staphylococcus. Growth occurred in 18 hours in every tube. It seems evident, therefore, that there is some substance present with the penicillin which, while not affected by penicillinase, in high concentration inhibits the growth of staphylococci.

Confirmation of this hypothesis was obtained by repeating the experiment using purified penicillin, containing 1,600 units per mg., assayed against staphylococcus. An amount of this material containing 100,000 units was diluted serially and each tube was treated with the corresponding quantity of the penicillinase used in previous experiments. The tubes were inoculated from a broth culture of the same staphylococcus. Growth occurred simultaneously in 18 hours with the six highest concentrations, from 100,000 units down to and including 6,250 units. It occurred with 3,125 units in 21 hours, with 1,562 and 781 units in 40 hours, and with 390 units in 3 days. No growth occurred with 195 units up to 5 days. Inhibition at the low end of the scale thus occurs even with pure penicillin.

Three further experiments were done with penicillin from batches having potencies of 1,000, 1,210, and 1,450 units per mg. respectively. The results were as shown in Table II.

TABLE II

Penicillin (Units)	1,000 u./mg.	1,210 u./mg.	1,450 u./mg.
100,000	(7) ± 3 days	(5) — 3 days	(5) + 42 hours
50,000	(3) + 20 hours	(1) { + 18 hours	(1) + 20 "
25,000	(2) + 18 "	{ + 18 "	(3) + 24 "
12,500	(1) + 16 "	(2) + 20 hours	(2) + 22 "
6,250	(4) + 22 "	(3) + 24 "	(4) + 40 "
3,125	(5) + 40 "	(4) + 40 "	(6) + 3 days
1,562	(6) + 3 days	0	0
781	0	0	0
390	0	0	0
195	0	0	0

Control + 16 hours

It will be seen that, as the potency of the penicillin in units per mg. increases, the first tube to show growth is one step higher in the series. As has been shown, when a potency of 1,600 units per mg. is reached the phenomenon no longer occurs, growth occurring simultaneously with all the higher concentrations.

Further evidence that inhibition with high concentrations of penicillin is due to impurities was obtained by inactivating in the autoclave a solution of penicillin containing 100,000 units in 5 ml., or 20,000 units per ml., and inoculating with one loopful of staphylococcus broth culture. No growth occurred in 7 days, though it occurred in lower concentrations.

Speculation about the nature of any unknown inhibitory substances and their possible therapeutic action is beyond the scope of this paper.

Failure of growth at the very low concentrations of penicillin must be a phenomenon quite distinct from that observed at the other end of the scale and is much more difficult to interpret. In an attempt to elucidate it the following experiment was done:

To four tubes of broth each containing 195 units of penicillin were added the corresponding concentration of penicillinase and one loopful of staphylococcus broth culture. They were incubated for three days; no growth occurred. They were then treated as follows:

Tube 1: excess penicillinase added. Tube 2: one loopful of staphylococcus broth culture added. Tube 3: excess penicillinase and one loopful of staphylococcus broth culture added. Tube 4: no additions. All were incubated at 37° C. with a broth control.

The results were:—Tube 1: +, 24 hours; tube 2: +, 4 hours; tube 3: +, 4 hours; tube 4: 0, 7 days. Control: +, 24 hours.

Tube 1 shows that the original inoculum was still living, but that the original amount of penicillinase added was not sufficient to inactivate the penicillin. Tube 2 indicated that the penicillin had been sufficiently reduced in activity to allow a fresh inoculum to grow; tube 3 is thus redundant, in view of the results with tubes 1 and 2. Tube 4 confirms that the penicillin had not been inactivated by the original addition of penicillinase. The extremely rapid growth in tubes 2 and 3—full growth with maximum turbidity in 4 hours—is difficult to explain. It seems to be analogous to the very rapid growth, noted above, with high concentrations diluted to fall within the critical range of concentration. Both these observations seem to indicate that treatment of penicillin with penicillinase at sub-maximal levels for several days increases the susceptibility of the medium to growth of staphylococcus when the conditions are altered sufficiently to allow growth to take place. That the alteration is in the medium and not in the inoculum seems clear from tubes 2 and 3, where the inoculum was fresh.

Discussion

The observations here reported are, apart from any academic interest, of practical importance for sterility-testing of penicillin.

This laboratory is frequently called upon to test for sterility dried penicillin in phials containing up to 1,000,000 units. In the light of the observation here reported it is clear that to make certain of getting growth of sensitive contaminants the final dilution in the test medium should be related to the potency of the sample in units per mg. With potencies of less than 1,000 units per mg. it should be of the order of 1:100. However, with penicillin of recent manufacture, potencies of at least 1,400 units per mg. are usual, and it should not be necessary with such samples to employ volumes of test broth larger than those normally used for sterility testing. Where very low concentrations of penicillin are to be tested for sterility care must be taken to add gross excess of penicillinase, of the order of 10 times the equivalent quantity, whatever the potency of the sample. This does not normally arise in practice.

Apart from the above practical considerations, the phenomenon described is interesting from several points of view. Inhibition by high concentrations suggests that impurities in penicillin exert on staphylococci an inhibitory effect that is not a true penicillin effect, for it does not occur with "pure" penicillin. Inhibition at the low end of the scale is more difficult to understand. It would seem that penicillinase does not act proportionately to its concentration—that the relationship of penicillinase activity to concentration of penicillin is in fact

not linear. Consequently the value obtained by the method of assay described holds good only for a limited range of penicillin unitage—round about 4,000 units. The quantitative relationship of penicillinase and penicillin may have a bearing on the generally assumed enzymic action of penicillinase.

The extremely rapid growth of staphylococci (full growth from a single loopful in 3 to 4 hours at 37° C.) after reducing to a normal dilution a concentrated penicillin solution in which growth has so far failed to occur is a most striking phenomenon and difficult to explain. So also is the similar rapid growth in very dilute penicillin in broth after excess penicillinase has been added to a tube in which no growth has so far occurred. Such rapid growth has never previously been observed in this laboratory: it would seem to merit further study.

Summary

Staphylococci are inhibited by penicillin above a certain concentration, notwithstanding that the penicillin is shown to have been inactivated by penicillinase.

This does not occur with "pure" penicillin, suggesting that the inhibition is due to impurities.

Similar inhibition occurs in penicillin dilutions in broth below a certain concentration. In this instance the inhibition is due to the penicillinase having failed to inactivate.

In both instances, if the penicillin in broth is rendered capable of supporting growth by diluting the high concentrations or by adding excess penicillinase to low concentrations, growth of staphylococci occurs with extraordinary rapidity.

The implications of the observations are discussed from the point of view of sterility-testing of penicillin.

MESENTERIC OCCLUSION

A REVIEW OF RECENT METHODS OF TREATMENT, WITH NOTES ON SIX INSTANCES

BY

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Mesenteric occlusion is one of the rarer abdominal disasters and one that is very difficult to diagnose pre-operatively, very difficult to treat, and recovery from which is most exceptional. Having had experience of six cases in the last four years, with three recoveries, my interest was aroused and I was stimulated to consult the literature.

The occlusion may be arterial or venous, and may be thrombotic or embolic. Venous thrombosis is usually a complication of a septic process within the peritoneum. Arterial emboli may arise from cardiac vegetations, whereas arterial thrombi are likely in blood disorders—e.g., purpura. The arterial architecture is very important. The small intestine is supplied entirely from branches of the superior mesenteric artery, dividing into a series of arcades. There is no marginal artery such as is found in the large intestine. When an occlusion occurs in the area of the small intestine it has been suggested by Litten (1875) that a violent spastic contraction occurs from the ligament of Treitz to the middle of the transverse colon. The last case described below is difficult to explain on an arterio-anatomical basis. The symptoms may be acute or sub-acute: constant pain is a regular feature, with only moderate or ill-defined tenderness. Vomiting is more likely if the upper reaches of the alimentary tract are involved. Constipation or diarrhoea may occur.

Diagnosis may be facilitated in subacute cases by a plain skiagram, where gas is seen as far as the splenic flexure and a barium enema reveals no obstruction. Prognosis is always grave, but patients have lived many years after operation.

Treatment must be decided after the state of affairs has been assessed at laparotomy. The appearances found vary considerably: the bowel affected may be black, and profuse haemorrhagic exudate may be present; in others a deeper cyanotic colour is seen, with ecchymoses and less heavily blood-stained exudate. If the affected length of bowel is not too great and the patient's condition is good enough, a resection can be undertaken and anastomosis performed. Ficarra (1944) describes fifteen cases, three of which were cured by resection: Atkins (1937) reports recovery by resection, exteriorization, and subse-