If an adequate fronto-nasal duct is provided the frontal sinus is in the great majority of cases able to return to a normal state, provided the lining mucosa has not been disturbed. In four cases I have had to remove the anterior wall of a shallow sinus to obliterate outlying recesses. In these the mucosa failed to recover after reconstruction of the fronto-nasal duct; but such cases are rare, and even with them it is essential to provide a free communication between the remains of the sinus and the nasal fossa, so that the first operation is not wasted. In this connexion it is interesting to note that in five cases in which previously unsuccessful attempts at obliteration had been made I have secured a good result by establishing an adequate fronto-nasal duct while at the same time reconstructing the frontal sinus by means of a saccular skin-graft. Not only has suppuration been eliminated thereby, with cure of the previous symptoms, but a good cosmetic result has been attained by restoring the contour of the forehead.

Reviewing the records of over a hundred patients on whom I have operated, the conclusion has been arrived at that a cure can usually be obtained by re-establishing ventilation and drainage of the sinus, the lining being undisturbed and allowed to return to normal. I do not believe that curettage of the cavity is necessary or desirable. I have, in various instances, found pockets of infection in such cases and have had to reoperate, sometimes replacing the denuded lining of the sinus by the insertion of skin, as referred to above. A considerable number of the patients I have dealt with had had one or more previous operations on the frontal sinus, and one young man had undergone, according to his own account, a total of seventeen.

Leaving, therefore, the anterior wall and the lining of the sinus, one has to decide on the route to adopt. I am of the opinion that a short incision, curving round the inner margin of the orbit, well away from the inner canthus, gives the best approach. A clear view is provided for clearance of any remaining infected or obstructing ethmoidal cells; there is no danger of damage to the trochlea or lacrimal sac if the orbital periosteum is carefully detached. The base of the skull is visible and safety is attained in enlarging the opening between the sinus and the fossa. It is unnecessary to remove more than a small portion of the floor of the sinus, but it is essential to take away a considerable part of the nasal process of the maxilla, as advocated by Howarth. Approach by an incision lower down seems to me to be inconvenient and to make permanent restoration of the fronto-nasal duct difficult or impossible. The operation described above allows of the gentle removal of exuberant hyperplastic mucosa when necessary, but curettage is carefully avoided. Examination of the posterior wall of the sinus for possible caries is, in my opinion, unnecessary, for the reason that even if present it is better left alone, in the certainty of ultimate recovery. This applies, naturally, to the usual chronic case and not to those isolated instances where an extradural or a frontal-lobe abscess is suspected. In not one of over a hundred cases have I had reason to regret leaving the posterior wall and its covering mucosa intact.

Finally, one comes to the means of maintaining the integrity of the fronto-nasal duct; this is readily attained by insertion of a skin-graft, applied to the raw orbital periosteum and to the exposed margins of the bone at the entrance of the frontal sinus. The graft is tucked into position after the insertion of a large tube of rubber or plastic material; it is not wrapped round the tube, since no graft is required over the nasal septum or posterior wall of the frontal sinus. Details of the operation are given elsewhere (StClair, Thomson, and Negus, 1937). In ten days the tube is removed, and no subsequent dilatation or passage

of sounds should be necessary, as the new duct maintains its patency and provides adequate ventilation and drainage.

Conclusion

The operation requires care and meticulous attention to detail, but in the hands of my colleagues and myself it has proved most satisfactory in curing the symptoms without disfigurement. It is advisable, however, again to emphasize the signs and symptoms that require an external operation. Unilateral frontal headache, worse in the morning, either repeated daily over a prolonged period or recurring in acute or subacute attacks; clinical and radiological evidence of infection in the frontal sinus; evidence that maxillary sinusitis has been adequately treated and that freedom to the nasal orifice of the frontal sinus in the nasal fossa has been attained: these are the factors that indicate blockage of the fronto-nasal duct, either permanent or recurrent, and these are the reasons for which the external operation should be performed.

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A SIMPLE METHOD OF ESTIMATING PENICILLIN AND OTHER BACTERIOSTATICS

BY

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The methods in current use for the estimation of penicillin have been reviewed by Garrod and Heatley (1944). They include titration in broth, inhibition zones on solid media, turbidimetric methods, and the inhibition of haemolysis by a β -haemolytic streptococcus. Most of these methods require comparatively large volumes of test fluid, or are not easily applied in a routine clinical laboratory where many estimations of body fluids may be needed.

The method presented here utilizes the fermentation of glucose in a serum-water medium, with acid production and subsequent clotting of the serum as an indicator of growth. It combines the advantages of using small amounts of test fluids, easily available materials, a clear-cut end-point, with few manipulations and sufficient accuracy for all clinical purposes.

Technique

The glucose-serum medium (see below) is inoculated with 10 c.mm. of a 24-hour broth culture of the Oxford strain of staphylococcus per ml. of medium, and well mixed. Several 25-c.mm. volumes of the seeded medium are placed in a row on the paraffin-waxed surface of a glass slide. An equal volume of the serum or other fluid to be estimated for penicillin content is mixed with the first 25-c.mm. volume of medium on the slide, and serial dilutions are then made along the row by transferring 25 c.mm. of the mixture to the next volume, and so on. The last volume is left unmixed, as a control. The 25-c.mm. mixtures and the control are then taken up into capillary tubes, the ends sealed in a flame, and the tubes incubated overnight. This gives 1:2, 1:4, 1:8, etc., dilutions of the fluid under test and will correspond as end-points to 0.04, 0.08, 0.16, etc., unit of penicillin per ml. in the original fluid.

It will be found an advantage to use a Pasteur pipette graduated in four or five 25-c.mm. divisions. This is the only special piece of apparatus required, and may be used for all manipulations.

The capillary tubes should be about 1 mm. bore, to give a fluid column 2 to 3 cm. long. Too long a column reduces the rate of growth in its middle section.

Test Organism.—Any organism which is penicillin-sensitive, which will grow readily in fluid serum media and ferment

glucose, is suitable. In routine work the Oxford strain of staphylococcus serves very well, and has the advantage that the reading is made direct in units per ml. Where it is required to estimate accurately penicillin concentrations of less than 0.04 unit per ml., a more sensitive strain of staphylococcus or streptococcus must be used. The 10-c.mm. inoculum per ml. of medium, recommended above, has been found to produce the clearest results, but the same end-point is given by a very wide range of inocula.

Medium.—This has the following composition:

Sterile horse serum, 1 part; distilled water, 3 parts; glucose to give 1% solution; Andrade's indicator to give 1% solution. Tube into sterile containers and sterilize in steamer for 30 minutes.

It is, in fact, the standard His's serum-water medium used in testing fermentation reactions, and is supplied in the L.C.C. Pathological Service in 2- to 3-ml. volumes in screw-capped bijou bottles, which are both convenient and economical and widely available to many laboratories. The medium should be quite clear after sterilization. Some batches become turbid and should be rejected. This turbidity is apparently caused by heating a too acid medium, and is avoided by adjusting the serum to pH 8 before use (Mackie and McCartney, 1945). Here again the end-point is the same over a wide range of serum concentrations. 1:2 and 1:4 dilutions of the medium given above produce similar results. It is difficult in any serial dilution technique to secure exactly the same medium and inoculum in each dilution under test without elaborating a method too complicated for routine use. The use of a 25% serum medium as diluent has the advantage of approximating to these conditions in estimations of blood-serum levels.

End-point of Titration.—Where full growth of the staphylococcus has occurred the column of fluid in the capillary tube shows opaque clotted serum, coloured uniformly pink by the indicator; where no growth has occurred the fluid retains its original transparency. The end-point is sensitive enough to justify the use of concentrations intermediate to those obtained in a single serial dilution, by utilizing two or three series starting with, say, 1:2, 1:3, and 1:5 dilutions of the fluid under test. The Table shows the results of titrating saline or serum containing 1 unit of penicillin per ml. against the Oxford staphylococcus.

Table Showing Results of Titrating Saline or Serum Containing 1 unit Penicillin per ml. Against Oxford Staphylococcus H.

Dil.	Result	Dil.	Result	Dil.	Result	Dil.	Result
1:2	—	1:3	—	1:5	—	1:7	—
1:4	—	1:6	—	1:10	—	1:14	—
1:8	—	1:12	—	1:20	—	1:28	—
1:16	—	1:24	—	1:40	—	1:56	±
1:32	—	1:48	±	1:80	+	1:112	+
1:64	+	1:96	+	1:160	+	1:224	+
1:128	+	1:192	+	1:320	+	1:448	+

— = no acid, no clot. ± = acid only. + = acid and clot.

In routine work a single series is generally used, and here the end-point is quite sharp. One capillary-tube dilution contains clear fluid and the next completely clotted pink serum. The table shows that the dilution half-way between these tubes contains a single inhibition dose—that is, 0.02 unit of penicillin per ml. Sometimes a single tube shows acid production only without clot formation, and this is then taken as the end-point. The table indicates the range of error in this assumption. It compares well with other serial dilution methods; the illustration shows such a titration. Occasionally one tube is found to be clear while the next is acid, with the beginning of clot formation, showing as a turbidity. In this case the end-point is nearer by one-third to the second of these tubes than to the first.

Comment

The above method has been used for the estimation of unknown penicillin solutions, blood serum, cerebrospinal fluid, and urines with consistent results. Although it is described as a micro-estimation, using the dilution technique of Fleming (1944), similar results are obtained with larger quantities of materials in test-tubes. In the case of

blood-serum titrations, haemolysis does not seriously interfere with the readings. Where test fluids are somewhat contaminated—for example, urines—care must be taken to read the end-point correctly. A penicillin-insensitive contaminant may give an isolated colony in the fluid column

Titration of a blood serum. Right-hand tube showing acid and clot is a control. From right to left, serum dilutions are 1:2, 1:4, 1:8, etc. The fourth tube from the left shows acid without clot, indicating a blood-serum level of 1.28 units of penicillin per ml.

surrounded by a small zone of acid and perhaps clot formation. Very little convection occurs in capillary tubes, and the effect is not distributed throughout the column.

The method may be adapted for comparing the sensitivities to penicillin of organisms which satisfy the cultural requirements given above—for example, streptococci, staphylococci, Klebs-Loeffler bacilli. The fermentable carbohydrate may of course be changed to suit the organism.

While the above description has mentioned penicillin alone, this method may be used in work with other antibacterial substances—for example, tellurite, sulphonamides, etc.

Summary

A method for the estimation of penicillin in body fluids, suitable for the clinical laboratory, is described in which the fermentation of a carbohydrate in a serum-water medium is used as the indicator of growth.

This work was started at the Emergency Public Health Laboratory, St. Mary's Hospital, W.2, and I wish to express my thanks to Sir Alexander Fleming for the interest he has taken in it, and to Dr. A. B. Rosher for his helpful criticism.

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