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A New Technique for Testing Antibiotic Sensitivity

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The method described below is simple and could easily be used in a routine clinical laboratory. It would also be of value for the investigator who wants to know (a) the quantitative difference between the effects of various concentrations of the same antibiotic; (b) the qualitative difference between the same amounts of different antibiotics; or (c) the effects of antibiotics on the growth phase and metabolism of certain organisms.

Method

Apparatus.—The apparatus used for redox potential measurement is the Jones pH meter. Readings are taken on the cultures incubated in a thermostat at a desired temperature at intervals set by the experimenter. Either several cultures are used and then at each interval a new culture is tested, or one culture is used throughout and then a sample is taken out under sterile conditions at each interval. In these trials both methods gave similar results and in general the one-tube technique was adopted in the experiments.

Media.—The media used in these experiments were as follows:

(1) McConkey's broth, double strength (Fig. 4).
(2) Lactose broth (Figs. 1 and 3).
(3) Glucose broth (Fig. 2).
(4) McConkey's agar.

Organisms.—The organisms tested were Bact. coli and Proteus vulgaris in 14- and 36-hour cultures respectively.

Antibiotics.—The antibiotics tested were aureomycin, achromycin, and penicillin, the first two in the form of powder and pellet, the last in an aqueous solution. (Aureomycin as hydrochloride, crystalline, and spersoids was obtained from the Lederle Laboratory Division; achromycin in a crystalline powder as HCl and pellet from the same firm; and penicillin as "procaine penicillin G" from the Commonwealth Serum Laboratories, Parkville, Victoria, Australia.)

A series of photographs was taken in some of the experiments together with the potential readings in order to compare the technique with the old method of simple observation.

Results

Fig. 1 gives the picture when Bact. coli is grown in the presence of increasing doses of one antibiotic, e.g., penicillin. The doses range from 0.1 µg. to 10 mg. in the following order: 0.1 µg., 10 µg., 100 µg., 1 mg., and 10 mg. in 10 ml. medium.

Fig. 3 presents the effect of a single dose (100 µg.) of each of three different antibiotics (penicillin, achromycin, aureomycin) on the same organism (Bact. coli).
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If there is an agent present which is likely to influence growth and metabolism in the medium in either direction (enhancing or restricting and blocking it), the chemical decomposition in the medium will alter together with the redox potential accordingly. The application of these general principles provided the basis for this technique.

The results in Fig. 1 show the differences in reaction of the organisms, although the dosage was increased only 10 times in all except one case in which it was increased 100 times.

From the experience gained from these results the technique can safely be recommended as a basis for a new method of assessing antibiotic dosage. If the other requirements, i.e., strain, age of test organism, culture medium, temperature, duration of incubation, remain the same, the amount of antibiotic can be determined with great accuracy by this method.

The method can also be used for antibiotic sensitivity tests.

On the basis of experience obtained from the results shown in Fig. 3 the sensitivity of the test will allow comparisons between the effects of the same dose of various antibiotics as applied to the same organisms under similar conditions.

These two factors, speed and accuracy, combined with simplicity and ease, make this method suitable for any type of routine work where the bacteriostatic effect of an agent has to be tested. But the great value of the test lies in the fact that it gives graphic representation of what is happening in the life of the organism. By studying these curves an exact picture of the condition or the metabolic state of the organism at any given moment can be seen, and to obtain this information it is only necessary to take readings at closer intervals or to connect the millivolt meter to an automatic recording machine.

Other photo-electric and electronic methods have been worked out, but they all fail in comparison with this method, because they gave a static rather than a dynamic picture of the metabolic processes involved.

Fig. 2.—Results of growing *Proteus* in glucose broth + aureomycin.

Fig. 2, like Fig. 1, shows the effect of increasing concentrations of one antibiotic, aureomycin, 1:10 and 1:100, on the growth of *Proteus*.

Although the method is extremely sensitive, it is not influenced by such variations as height of the liquid (within limits) or metallic test-tube capsules instead of cotton-wool plugs, etc. (Fig. 4).

Discussion

Recording redox potentials in bacterial cultures is not a direct measurement, i.e., measuring the redox potentials of the bacterial organism itself, but indirect, since the potential changes of the culture medium are measured (Hewitt, 1950). When organisms are present a certain energy-producing ingredient of the medium is decomposed. The quicker the organisms grow, the more energy they need, and the greater the redox potential will be. Hence, by the close correlation between the changes in the medium and the growth of the organisms, the life of the organisms can be followed and a fairly true picture obtained by this measurement (Zador, 1958).
Summary

There is a close relationship between the redox potential and the growth of living cells. This fact is used for developing a new method for testing the antibiotic resistance of microorganisms by measuring redox potentials. The method is described fully, and its field of application, together with advantages and shortcomings, is discussed and the results are examined in detail.

REFERENCES