EVALUATION OF GERMICIDES BY A MANOMETRIC METHOD

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The inhibition of the metabolic activity of a bacterial suspension produced by a germicide has been used by a number of workers as a criterion of the bactericidal or bacteriostatic effectiveness of the germicide. The earlier work of Dreser (1917), Pilcher and Sollman (1923), and Peterson (1926) was reviewed by Branham (1929), who made a few improvements on the previous methods. Yeast was used in most cases as the test organism and the highest dilution of germicide necessary to prevent fermentation for a certain period of time was determined. Bronfenbrenner, Hershey and Doubly (1939) used a definite concentration of bacteria in the presence of horse serum, buffer, and glucose, and determined the concentration of disinfectant necessary to cause a 50 per cent reduction in the rate of oxygen uptake between the 15th and 20th minute. These investigators concluded that the manometric phenol coefficient was a reliable index of the germicidal value of a disinfectant.

The inhibition of the oxygen uptake caused by various drugs upon a suspension of *Escherichia coli* and the correlation of this inhibition with the number of organisms killed were studied manometrically by Ely (1939). Sykes (1939) used as a test of germicidal activity the measurement of the effect of various compounds on the dehydrogenating capacity of *E. coli* for succinate. Since, however, the succinoxidase system is present in only a relatively small number of bacterial species Sykes' method does not seem to be entirely suitable for the evaluation of bactericidal activity. Moreover, this system is specifically

inhibited by the presence of malonate or pyrophosphate, which, on the basis of this method, would be judged to be good germicides.

Heavy suspensions of bacteria in which little or no growth could occur were used by these workers, who measured the inhibition of the oxidation of glucose, or in the case of Sykes, of succinate, and sought to correlate this inhibition with the bactericidal effect of the germicide. However, since the initial effect of a germicide in low concentration is usually the inhibition of growth, the probability was considered of using this inhibition of growth as the basis of a method for the evaluation of germicides.

It has been shown (Greig and Hoogerheide, 1940) that under certain conditions the increase in oxygen uptake of actively growing cultures of several bacterial species is directly proportional to bacterial content. It was thought, therefore, that this relationship could be used to develop a manometric method for the testing of germicides. This, indeed, proved to be possible.

EXPERIMENTAL

The procedure for the preparation of the bacterial suspension was described by Greig and Hoogerheide (1940). The culture medium consisted of a 1 per cent bacto-peptone, 1 per cent lactate solution in phosphate buffer (M/15 or M/20) of pH 7.0. The temperature at which the experiments were carried out was 30°C. in all cases. At this temperature, the rate of oxygen uptake per turbidity unit remained constant during all phases of growth for all organisms investigated, hence the oxygen uptake observed was directly proportional to bacterial content. The solutions of the germicides, adjusted if necessary to pH 7.0, were added in serial dilution at the beginning of the experiment to the suspension in the main part of the manometer vessels. The oxygen uptake was recorded at 15 minute intervals over a period of 5 or 6 hours and curves were plotted for oxygen uptake against time.

When so much germicide was added that growth was completely inhibited, no oxygen uptake was observed, for the initial inoculum was so small that even though the bacteria were not killed, the oxygen consumption was negligible. Whenever there

was partial inhibition of growth, the curve for total oxygen consumption was still logarithmic, but was lower than that of the blank without germicide.

Oxygen uptake curves for a typical experiment are shown in figure 1. Turbidities were measured at the end of each experiment by means of the photoelectric colorimeter and it was found that the inhibition in the rate of increase of oxygen uptake caused by the germicides corresponded closely with the inhibition of

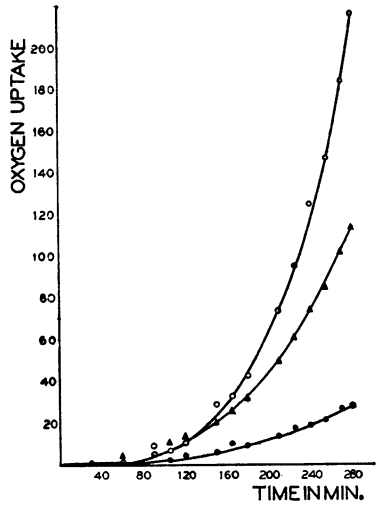

FIG. 1. THE EFFECT OF ADDED PHENOL ON THE OXYGEN UPTAKE OF A CULTURE OF E. COLI

O——O, normal; ▲——▲, phenol in a concentration of 1 to 1600; ●——●, phenol in a concentration of 1 to 800.

growth. In a few cases in which *Proteus vulgaris* was the test organism, counts were made at the end of the experiment (change in cell size was not so marked for this organism as for others) and in these cases, also, inhibition in the normal rate of increase of oxygen uptake corresponded to inhibition of cell multiplication. The results for sulfanilamide are recorded in table 1, those for several other germicides in figure 2.

Sulfanilamide (table 1) acts like other germicides tested in first inhibiting cell multiplication without affecting metabolism, i.e., its action is bacteriostatic in low concentrations, but it is not

nearly so potent as standard germicides under the same conditions. It also seems to be equally effective on the organisms tested, namely, *Proteus vulgaris* and *Staphylococcus aureus*.

TABLE 1

The bacteriostatic effect of sulfanilamide on Proteus vulgaris and Staphylococcus aureus at 30°C.

ORGANISM	CONCENTRATION SULFANILAMIDE	INHIBITION OF GROWTH	INHIBITION IN OXYGEN UPTAKE
	per cent	*per cent*	*per cent*
Proteus vulgaris	0.4	79	65
	0.2	53	53
	0.1	40	38
	0.05	36	28
Staphylococcus aureus	0.4	73	68
	0.2	56	56
	0.1	49	49

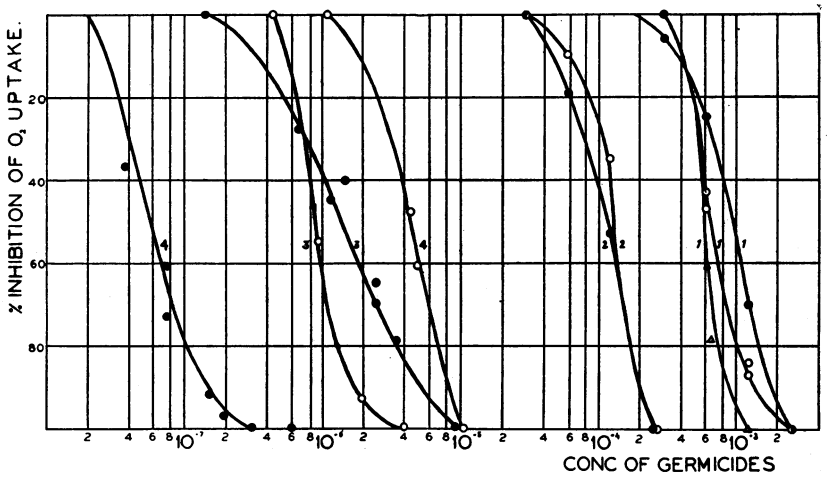

FIG. 2. THE PERCENTAGE INHIBITION OF OXYGEN UPTAKE OF CULTURES OF SEVERAL ORGANISMS PRODUCED BY DIFFERENT CONCENTRATIONS OF ADDED GERMICIDES

●——●, *S. aureus;* ○——○, *E. coli;* △——△, *P. vulgaris.*
1, phenol; *2*, sodium orthophenyl phenate; *3*, mercuric chloride; *4*, metaphen.

That the action of the germicides is usually non-specific is shown in figure 2, the curves for the organisms tested falling quite close together for each antiseptic. Metaphen, however, is an

exception, since to produce 90 per cent inhibition required fifty times as much metaphen for *E. coli* as for *Staphylococcus aureus*. This result compares favorably with those of Birkhaug (1935) who found by his method that to inhibit growth in peptone water of *E. coli* required forty-five times as much metaphen as in the case of *Staphylococcus*.

DISCUSSION

Previous investigators using manometric methods have attempted to correlate the inhibition of the metabolic rate of a heavy suspension of bacteria in the presence of a germicide with the lethal effect which the germicide exerts. By the method here described attempts were made to correlate inhibition of growth with inhibition in the normal rate of increase of oxygen uptake during growth in the presence of a germicide. It differs from other methods in that it measures bacteriostatic rather than bactericidal effects. The germicide in sub-lethal concentrations inhibits cell multiplication but not metabolic rate, since oxygen uptake per turbidity unit remains constant for all concentrations of germicide used, up to almost 100 per cent inhibition. Bactericidal concentrations, however, affect not only growth but also cellular metabolism as was shown by Bronfenbrenner, Hershey and Doubly (1939) and Ely (1939). Ely has shown that so long as a suspension of bacteria absorbs even a trace of oxygen, it contains viable organisms and only when oxygen uptake becomes zero are all the organisms killed. The results obtained for the determination of germicidal potency with the technique described show in general that the compounds tested can be arranged in the same order of effectiveness as they would be by classical methods.

SUMMARY

1. The fact that the rate of oxygen uptake of multiplying bacteria is directly proportional to bacterial content has been used as a basis for the development of a manometric method for the evaluation of germicides.

2. Inhibition in the normal rate of increase of oxygen uptake upon addition of a germicide was found to be due to a corresponding inhibition in growth.

3. Germicides in bacteriostatic concentrations have no effect on metabolic rates of bacteria, but inhibit cell multiplication.

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