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Effect of phenol on the oxygen uptake of *Bacillus subtilis* spores

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The effect of phenol on the oxygen uptake of *Bacillus subtilis* spores in glucose is described. All concentrations of phenol tested inhibited respiration, but a comparison with earlier work showed no correlation between this effect and death of the spores.

INVESTIGATIONS into the effect of phenol on the uptake of oxygen by micro-organisms have been made by Hugo (1956), who found that low phenol concentrations stimulated the rate of uptake of oxygen in *Escherichia coli* when glucose, mannitol or lactose was used as substrate, but not when succinate, lactate, pyruvate or acetate was the substrate, and by Chauhan, Rivers & Walters (1963), who showed that 0·05–0·2% of phenol progressively reduced uptake of oxygen by *Penicillium notatum* spores.

Loosemore & Russell (1963) have indicated that phenol concentrations as high as 2·5 and 5% have little lethal action on *Bacillus subtilis* spores, whereas, depending on the number of spores present, the minimum concentration of phenol required to inhibit growth in nutrient broth was 0·1–0·2%.

The present investigation was made to find out if metabolic processes were continuing during phenol treatment of the spores.

Experimental and results

All chemicals were of analytical reagent quality.

The organism was a laboratory strain of *Bacillus subtilis*. It was grown for 48 hr at 37° on the surface of nutrient agar (Oxoid, pH 7·4) in Roux flasks, washed from the surface with sterile water, and then washed twice with sterile water. The suspension was shaken with sterile glass beads to break up any clumps, heat-shocked at 75° for 20 min, and adjusted to a density of approximately 7×10^8 or 7×10^9 viable spores/ml.

Oxygen uptake was determined using the Warburg apparatus, by the method described by Umbreit, Burris & Stauffer (1959).

Effect of spore concentration on rate of oxygen uptake. It was necessary to determine first the optimum numbers of spores for measuring the rate of oxygen uptake over a period of time.

The results of this experiment are shown in Fig. 1, from which it is apparent that the higher spore inoculum gives an easily measurable response. Such an inoculum was therefore used in later experiments with phenol.

Effect of phenol on oxygen uptake. The effect of various phenol concentrations in duplicate on the respiration of the higher spore inoculum was investigated. Manometer readings (Fig. 2) were made at frequent intervals over several days to obtain some comparison with sporicidal tests (Loosemore & Russell, 1963).

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Discussion

With glucose as substrate, a heavy inoculum of *B. subtilis* spores is needed before oxygen uptake can be detected. Respiration of spores is

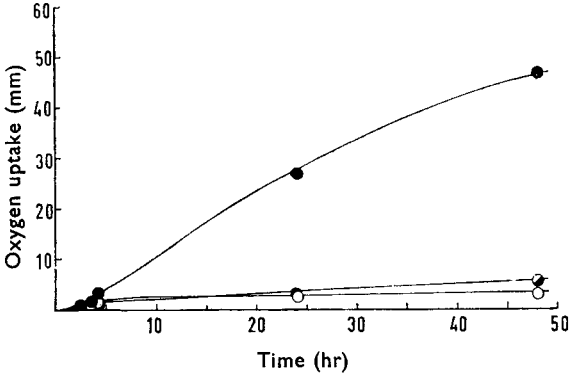

FIG. 1. Oxygen uptake of *B. subtilis* spores. ●—● Approx. 44×10^7 spores/ml, substrate glucose. ◒—◒ Approx. 44×10^6 spores/ml, substrate glucose. ○—○ Approx. 44×10^7 spores/ml, glucose absent.

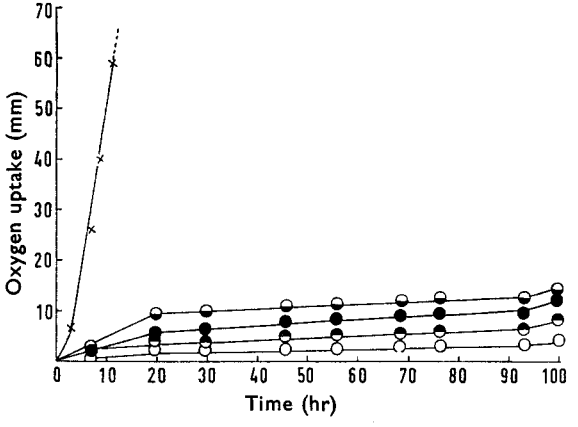

FIG. 2. Effect of phenol on the oxygen uptake of *B. subtilis* spores. ×—× Phenol absent. ●—● Phenol 0·5%. ◒—◒ Phenol 1·0%. ◓—◓ Phenol 2·5%. ○—○ Phenol 5·0%

greatly inhibited in the presence of phenol. The minimum concentration of phenol which inhibits germination and subsequent growth of approximately 10^7 spores of this organism in nutrient broth is about 0·2%. Preliminary experiments indicated that 0·25% phenol inhibited oxygen uptake, and it is therefore, apparent that such concentrations of the antibacterial agent can inhibit germination and subsequent growth, and prevent

respiration. However, there is no correlation of the inhibition of these processes and the sporicidal activity of phenol, which even in concentrations as high as 2·5 and 5% w/v, possesses little lethal effect against spores of this organism (Loosemore & Russell, 1963). It is interesting to note that Chauhan & others (1963) have concluded that measurement of oxygen uptake cannot be used for quantitative evaluation of fungicidal action.

Since glucose is a known germination stimulant, it is likely that the spores germinate in the absence of phenol. Phenol itself is known to inhibit a stage in the germination process (Lund, 1962).

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