MICROBIOLOGY

Influence of Bacterial Cell Age on Phenol Action

It has been suggested that the bacterial cell may vary in its response to antiseptics with age (Chick¹, Sherman and Albus², Hinshelwood³, and Jacobs⁴). Until recently, it has been customary to describe bacteria as old or young according to the length of time for which the cultures have been incubated. If an individual cell is regarded as new-born the moment it has separated from its parent/sister and old-aged the moment before such separation occurs, it is clear that in any randomly growing culture there will always be present cells of all ages.

In such cultures the length of incubation time will determine not the age of the majority of cells but to some extent the proportion of cells which are actively dividing.

The principle of synchrony (Scherbaum and Zeuthen⁵ and Hotchkiss⁶) which enables bacterial cultures to be prepared so that all the cells will divide at the same time, and therefore be of the same age, makes possible the assessment of the effect of cell age on resistance to antiseptics.

A synchronous culture of *Escherichia coli* was prepared by a modification of the method described by Yanagita and Kaneko⁷. Two samples were taken from the culture at 5-min intervals for 120 min. One sample served as a control, while the other was treated with 0.5 per cent phenol for 5 min. Viable counts were performed on all samples by a modification of the method of Miles and Misra⁸ and the results are shown in Fig. 1. The antibacterial effect of the phenol was stopped by diluting the sample 2,000 times with distilled water.

Fig. 1. Synchronized culture of *Escherichia coli* showing the effect of phenol treatment on cells of different ages

It will be seen that in the first 50 min of the experiment (lag period) the proportion of surviving cells after phenol treatment declined to 72 per cent, and that the control showed no increase of viable organisms. At the fifty-fifth minute, examination of the control showed that the cells were at the dividing stage and there was a dramatic decline to 14.9 per cent in the surviving cells in the phenol-treated sample. During the next 25 min the cells aged but did not increase in number. During this period the percentage of cells surviving phenol treatment rose to 79.9 per cent at the seventy-fifth minute. At the next cell division the cycle was repeated, the survivors in the phenol-treated culture falling to 20 per cent by the eighty-fifth minute.

This experiment demonstrates conclusively that *Escherichia coli* cells are most sensitive to the action of phenol

at the time of division and that their resistance increases with increasing age.

A fuller account of this work will be published elsewhere.

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¹ Chick, H., *J. Hyg., Camb.*, **8**, 92 (1908).

² Sherman, J. M., and Albus, W. R., *J. Bact.*, **8**, 127 (1923).

³ Hinshelwood, C., *Nature*, **167**, 666 (1951).

⁴ Jacobs, S. E., *J. Pharm. Lond., Supp.*, **12**, 21 T (1960).

⁵ Scherbaum, O., and Zeuthen, E., *Exp. Cell Res.*, **6**, 221 (1954).

⁶ Hotchkiss, R. D., *Proc. U.S. Nat. Acad. Sci., Wash.*, **40**, 49 (1954).

⁷ Yanagita, T., and Kaneko, K., *Plant and Cell Physiol.*, **2**, 443 (1961).

⁸ Miles, A. A., and Misra, S. S., *J. Hyg., Camb.*, **38**, 732 (1938).

AGRICULTURE

Solubilization of Copper and the Mode of Action of Bordeaux Mixture

SINCE the discovery of Bordeaux mixture over 80 years ago the question has often been posed as to how the copper reaches the fungal cell in toxic concentrations when the solubility of dried Bordeaux deposits in water is so low. Of the three possible agencies for solubilizing copper, namely the atmosphere, the spore, and the host plant, the last two have been regarded as the most important and it is established that spore^{1,2} and leaf^{3,4} exudates can dissolve copper from Bordeaux spray deposits. The assumption from this work is that a soluble copper complex is formed, possibly with the amino-, hydroxy-, or dicarboxylic acids in the exudates, and this complex is accumulated by the fungal cell where it may dissociate to give free cupric ion. However, most workers have shown that the copper complexes which could possibly be formed with leaf or spore exudates are much less toxic than the free ion⁵⁻¹⁰. In fact, under conditions of high spore/copper ratios, copper can stimulate germination by leaking growth factors in the spore exudate^{11,12}.

It is well known that fungal conidia can accumulate large quantities of metallic ions against a concentration gradient¹³ and recently quantitative determinations have been made of the uptake of copper by spores from aqueous solution¹⁴. No determinations have been made, however, of the accumulation of copper by spores in a three component system, spores/leaf/Bordeaux deposit, and it is implicit in the solubilization hypothesis that spore and leaf exudates should increase copper uptake. We have therefore measured the solubilization of Bordeaux deposits on leaf surfaces bathed in dense suspensions of *Neurospora crassa*, macroconidial wild-type *Em* 5297a, conidia, using the techniques previously described³. Dried spray deposits of 10:12.5:100 Bordeaux mixture were immersed in 30 ml. of glass-distilled water or *N. crassa* suspension at 100 million spores/ml., and gently agitated for 24 h at 25°C. The liquid was centrifuged and the copper content of the supernatant and of the spores (washed once) was determined after acid digestion.

Table 1. COPPER DISSOLVED FROM BORDEAUX DEPOSITS AND ACCUMULATED BY *Neurospora crassa* SPORES

Surface	Water ($\mu\text{g Cu/ml.}$)	Spore suspension Supernatant ($\mu\text{g Cu/ml.}$)	Spores ($\mu\text{g Cu/million}$)
Glass	0.4	18.3	8.5
Broad bean	1.3	0.0	5.3
Laurel	0.9	10.9	4.8
Potato	0.7	13.2	8.7

The results in Table 1 show the usual increase in soluble copper when a glass surface is replaced by a leaf, although to a lesser extent than previously reported³. In the presence of the spore suspension there was a large increase in