least ten single cell cultures of each isolate were examined. The formulae of the three agar solidified sporulation media that were investigated have been described (Merritt & Hurley, 1971). Slopes were made of 5 ml volumes of media in 20 ml screw capped glass bottles. Inoculated slopes were incubated at 25°. Samples were taken from each slope at regular intervals, heat fixed to a glass slide and stained with 0·5% safranin. A count was made of at least 500 structures to find the percentage of cultures in which at least 1% of the structures were asci. The ascospores of *K. fragilis* are released from the ascus as they mature, so the number of ascospores could be determined, but not the number of asci. The results for *K. fragilis* are for the percentage cultures in which at least 1% of the structures are ascospores.

Of the three media studied, the sodium acetate medium allowed optimum sporulation of all four species. The 6% *S. cerevisiae* cultures which were apparently nonsporing, all contained some asci, but not to 1% of the structures. The asci production on this medium was predictable and large variations in asci yields between replicates was unusual. The Gorodkowa medium was of little value for three of the species studied, although some of the cultures contained a few asci. The V8 medium was useful and occasionally gave relatively higher yields of asci than the sodium acetate medium. However, the medium was unreliable because a few cultures would fail to contain any asci when replicates were sporing freely.

For the yeasts of medical importance that I have investigated the sodium acetate medium is very suitable for inducing sporulation. The small amount of nutrient included in the formulation allows some cell division and compensates for some variation in inoculum levels, although this may delay the onset of sporulation by 1–2 days. I can recommend this unbuffered sodium acetate sporulation medium for routine isolates of yeast-like fungi which may be *Candida* species.

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The effect of cetyltrimethylammonium bromide on the cytochrome system of *Escherichia coli*

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The effect of a number of agents on cytochrome difference spectra of cells of *Escherichia coli* NCTC 1093 have been studied. Cells were grown as described by Rye & Wiseman (1966). harvested from the exponential phase of growth by membrane filtration and suspended in glucose-free medium to give a cell concentration of between 15 and 20 mg/ml. Up to 0·2 ml volumes of water or of solutions of substrates or reagents were added to 4 ml aliquots of these suspensions and difference spectra between pairs of them measured using a Unicam SP 700 recording spectrophotometer.

The spectra obtained between blanks of washed aerated cells and similar test suspensions to which had been added sodium succinate showed absorbancy peaks in the visible light region at 533, 560, 593 and 630 nm corresponding to those reported for *E. coli* by Smith (1954). The addition of potassium cyanide to the succinate respiring cells in such pairs of suspensions resulted in the elimination of the 630 nm cytochrome a_2 peak whilst the other peaks remained unaffected. This indicates that the terminal cytochrome had become oxidised, being no longer reducible by the remainder of the electron transport chain when complexed with cyanide.

In the spectra obtained between blanks of washed aerated cells and test suspensions of succinate respiring cells treated with some concentrations of CTAB the 630 nm cytochrome a_2 peak was again eliminated, with the other peaks unaffected. This indicated that CTAB is capable of specifically uncoupling the terminal cytochrome from the remainder of the electron transport chain. Similar elimination of the 630 nm peak also followed treatment of respiring cells with chlorhexidine diacetate. The final concentrations of CTAB and of chlorhexidine having these effects were 300 to 400 and 100 to 200 μg/ml respectively; lower concentrations had no effect on the cytochrome spectra whilst higher concentrations caused

the elimination of all peaks and agglutination of the suspensions. On extrapolation to the appropriate cell densities these concentrations approximate to those that would be expected to partially inhibit the growth of cultures of this organism and it seems possible that inhibition of bacterial growth by low concentrations of these membrane active agents results from the reversible uncoupling of the terminal cytochrome from the electron transport chain without any gross membrane damage or penetration of agent into the cells.

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Preliminary compaction studies using a device to simulate a rotary tabletting machine

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Rotary compressing machines commonly used for the manufacture of pharmaceutical tablets compress powders in a die between two moving punches. However, preliminary compaction studies are often made using hydraulic presses or eccentric compressing machines, in which one moving punch compresses the material against a second stationary punch. Since the frictional conditions at the die wall, and the stress distribution within the compact differ between these two types of compression it is often difficult to relate the results of preliminary studies to the subsequent behaviour of the material on a rotary machine. Nevertheless it is inadvisable to use precision equipment such as a rotary tabletting machine for initial compression studies with unlubricated powders or with formulations having ill-defined compaction properties.

A device has therefore been developed to simulate the compression conditions on a rotary machine. We have used the apparatus in conjunction with a universal testing instrument ("Instron") but the principle could also be applied to a hydraulic press or an eccentric tabletting machine. The lower punch is supported by a load cell located on the fixed platten of the universal testing instrument and the upper punch is attached to the movable crosshead. As the upper punch compresses the powder, the movement of the crosshead is translated to the die which also begins to move downwards but at a slower rate than the upper punch. The relative rate of movement of the die and the upper punch is adjustable, and the instant at which the die begins to move can be controlled.

Using this system with plane-faced punches of 33 mm diameter, the compaction properties of 40–60 mesh fractions (250–420 μm) of crystalline sodium chloride, potassium chloride, potassium citrate and lactose were investigated. Deformation of the material was measured at a range of applied loads up to 49·0 kN. For a range of maximum loads, when samples of each material had been compressed the upper punch movement was stopped. Decay in the load on the compact at constant strain, due to continuing deformation of the material, was studied. The effect of load on ejection force and compact strength was determined.

Differences in the compaction behaviour and the properties of the compacts of the four materials studied indicate differences in the mechanism of consolidation. Evidence of plastic deformation of sodium chloride and potassium chloride is shown by large stress relaxations, high ejection forces and high strength values. This effect is most apparent with potassium chloride. Conversely, lactose and potassium citrate exhibit much less stress relaxation and produce far weaker compacts. Differences between potassium citrate and lactose are explained by more extensive size reduction of lactose by fragmentation during compaction.

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