POTASSIUM FLUXES. FIRST INDICATIONS OF MEMBRANE DAMAGE IN MICRO-ORGANISMS.

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SUMMARY

A method is described whereby the leakage of potassium ions can be measured from microbial cells which have been treated with membrane active antimicrobial compounds. The method involves the use of specific ion electrodes and does not require the cells to be removed from suspension. The effect of two membrane-active antimicrobials; cetrimide upon E.coli and amphotericin upon C.albicans is related to the leakage of phosphate and 260 nm material.

Membrane active antimicrobial agents have been shown to release various cytoplasmic constituents from treated cells. (1) Components which have been detected include 260 nm. absorbing material, by spectrophotometry, phosphate and pentoses, by colorimetric methods; and potassium ions by flame photometry. All these assay procedures require the removal of the treated cells from the suspension before an analysis can be made upon the supernatant solutions. We would like to report methods whereby the leakage of potassium from treated cells can be measured in situ using specific ion electrodes.

MATERIALS AND METHODS

Two types of potassium sensitive specific ion electrodes are commercially available; glass electrodes (2) and liquid membrane electrodes. (3) Glass electrodes must be used under conditions of constant pH, preferably pH 7 or above, and in the absence of sodium and ammonium ions. Liquid membrane electrodes may be used at any pH and, being far more selective than glass electrodes, may be used in the presence of sodium or ammonium ions, but have recently been shown to be affected by certain compounds. (4)

We have used both electrodes to measure the potassium effluxes caused by the action of various antimicrobial agents upon bacterial and fungal suspensions.

Cells were harvested, washed twice in distilled water, and resuspended in appropriate buffer solution to give cell densities of 1mg./ml. dry weight. 20ml of the suspension was placed in a thermostatically controlled vessel, magnetically stirred, and the electrode inserted. The potential derived by the electrode was measured using a Vibret 46A mv/pH meter. The antimicrobial agent was added to the suspension and the potassium concentration was measured in situ by the electrode, readings being taken at ten second intervals.

RESULTS AND DISCUSSION

Figure 1 shows the leakage of potassium, phosphate and 260 nm.

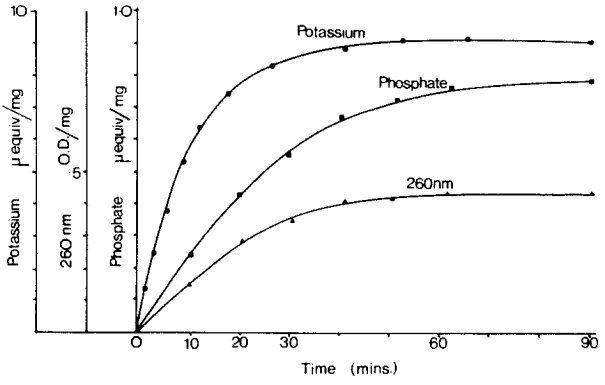

FIG. 1. Time course for leakage of components from E.coli. treated with 0.2mM. cetrimide at 25°C. & pH 7.

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absorbing material caused by the action of 0.2 mM. cetrimide upon a suspension of E.coli. NCIB 8277 (10^9 cells/ml.) in TRIS/HCl buffer pH 7 at 25°C. 260 nm. material and phosphate were measured in the supernatant after removing the cells by centrifugation at 10,000 g. for 5 mins, 260 nm. absorbing material directly, while phosphate was determined by the method of King. (5) The results show that the initial effect of cetrimide upon E.coli under these conditions was to initiate a flux of potassium ions which was complete within

30 minutes. This was followed by a slower release of phosphate and 260 nm. material as reported by Salton (6) and Newton (7).

Figure 2 shows the effect of amphotericin B, a membrane active

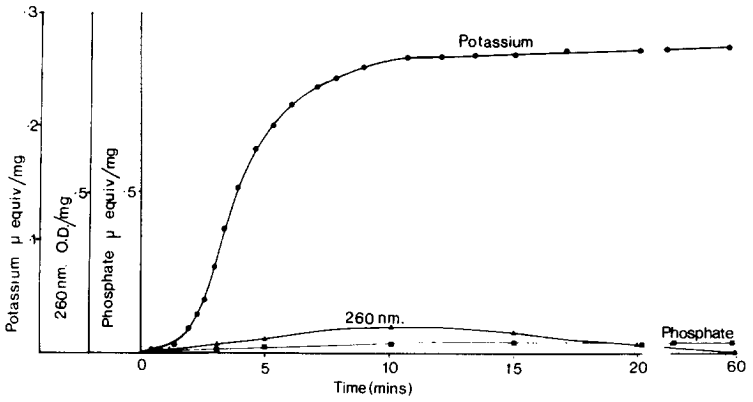

FIG.2. Time course for leakage of components from C.albicans treated with 20 μg amphotericin B at 25°C. & pH 6.

polyene antibiotic, against the yeast Candida albicans NTCC 713, detected by a Philips 560-K valinomycin-based potassium sensitive liquid membrane electrode. 20 μg of amphotericin B causes insignificant leakage of phosphate and 260 nm. absorbing material from a washed suspension of the yeast, (3×10^6 cells/ml.) but potassium leakage is marked.

Specific ion electrodes permit rapid accurate measurement of potassium leakage following treatment of washed microbial suspensions with membrane active antimicrobial agents. The leakage of potassium ions occurs extremely rapidly after treatment. We have detected leakage within 5 seconds using these methods. It is proposed that the efflux of this ion is one of the first indications of the changes induced in the selective permeability of microbial membranes by membrane active antimicrobial agents.

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