A comparison of colicines K and V extracted from solid medium

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Following the extraction of colicine K and colicine V from digest nutrient agar, the crude colicine was divided into 3 portions. Each portion was subjected to a primary treatment with either 30% chloroform, 90% acetone or 66% absolute alcohol. Aliquots of the active fractions obtained from each of these primary treatments were subsequently exposed to either of the other two extractants. It was noted that the best method for primary purification of both colicines was blending with chloroform but the results of subsequent fractionation differed. Colicine K was insoluble in 66% absolute alcohol whereas colicine V remained soluble in alcohol but was precipitated by 90% acetone.

Mouse toxicity tests revealed that the toxic fraction of the crude colicine V was precipitated by 66% alcohol and that the non-toxic fraction, soluble in alcohol, was associated with the activity of the colicine. All the active fractions of crude colicine K were lethal for mice.

INTRODUCTION

Although the existence of bacteriocins has been recognized for nearly 90 years, since Pasteur and Joubert (1877) observed the inhibition of an anthrax bacillus by a coliform bacillus, attempts to purify and characterize the colicines have been sporadic. Gratia (1925, 1932) described some of the characteristics of colicine V. Goebel and his colleagues (Goebel et al., 1955; Goebel and Barry, 1958; Hutton and Goebel, 1962) described in detail the purification and characteristics of colicines K and V; Barry et al. (1965) have similarly investigated colicine A; and other characteristics have been reported (Fredericq, 1948; Heatley and Florey, 1947; Gardner, 1950; Ikawa et al., 1952; Nüske et al., 1957; Smarda and Obdržálek, 1966). We had been concerned with the purification of colicine V from solid media and our unpublished results had differed somewhat from those of Hutton and Goebel (1962) but agreed with those of Gratia (1932). We decided, therefore, to compare the extraction and purification of
colicine V and colicine K from cultures of recognized colicinogenic strains on solid media under identical conditions.

**MATERIALS AND METHODS**

**Microorganisms**

*Colicinogenic strains*. *Escherichia coli*, strain CL 131 (CA 235 of Fredericq) producing colicine K and *E. coli*, strain CL I (CA 7 of Fredericq) producing colicine V, were obtained from Dr. B. A. D. Stocker. *E. coli*, strain CA 23 producing colicine D was received from Professor P. Fredericq. *E. coli*, strain RC 208, was obtained from the urine of a patient with significant bacteriuria. This organism produced two colicines D and V.

*Indicator strains*. *E. coli*, strain phi (φ of Gratia) which was sensitive to colicines K, V and D was received from Dr. W. F. Goebel.

For typing the colicines by patterns of inhibition, the Abbott & Shannon indicators were used as previously described (McGeachie and McCormick, 1967).

**Media**

For the production of colicine, digest nutrient agar was used consisting of digest nutrient broth prepared according to Cruickshank (1960), with 1.2% New Zealand agar (Oxoid) added. One and a half litres of this digest nutrient agar were divided into approximately 100 ml aliquots and poured into 15 petri dishes, 20 cm diameter.

One per cent peptone water agar (Cruickshank, 1960) was used as the medium for the indicator plates.

**Methods**

1. *Production and crude extraction of colicines*. Each digest nutrient agar plate was inoculated with a loopful of an overnight culture of the colicinogenic organism and incubated at 37 C for 18 hr. The plates were then sterilized by exposure to chloroform vapour for 1 hr at room temperature following which 30 min were allowed for any chloroform vapour which had condensed on the surface of the plate to re-evaporate. The plates were then placed at -20 C overnight and allowed to thaw at room temperature. The extract produced was centrifuged at 16,000 g for 10 min to remove the cells and any particles of agar. Approximately 570 ml of clear supernatant were obtained from each litre of nutrient agar used. This was termed the crude extract of colicine.

2. *Purification of colicine*. Three extraction procedures were performed in parallel using chloroform, alcohol and acetone. These were used in primary purification procedures and subsequently, aliquots of each of the primary active fractions were treated with either of the other two precipitants (Fig. 1).