

## NOTES

PERMEABILITY ALTERATIONS  
IN BEET ROOT *BETA VULGARIS*  
VAR. *RUBRA* CAUSED BY  
AMPHOTERICIN B

PAVEL NEMEC and VLADIMÍR BETINA

Department of Microbiology and  
Biochemistry, Slovak Polytechnical  
University, Bratislava, Slovakia, ČSSR

(Received for publication September 10, 1968)

In studies of biological effects of the antibiotic cyanein<sup>1)</sup> inhibition of the growth of *Allium cepa* roots and of mitosis in root tips of *Vicia faba* were observed<sup>2,3)</sup>. This communication is concerned with effects of cyanein, amphotericin B and fusaric acid on permeability of the beet root.

#### Materials and Methods

Cyanein, amphotericin B and fusaric acid were dissolved in dimethylsulfoxide (DMSO) and the solutions diluted with water to obtain final concentrations in the range 25 ~200  $\mu\text{g/ml}$ . The final concentration of DMSO in all dilutions and in the controls was 1% (v/v).

From a beet root 3 mm slices of 1-cm diameter were cut out and washed with water. Individual slices were put into duplicate sets of tubes each containing 6 ml of a diluted antibiotic solution and incubated in darkness at room temperature. Extinctions at 500 m $\mu$  were estimated at intervals. K<sup>+</sup> ion concentrations were estimated by flame photometry.

Anthocyanins were extracted from 3 untreated slices by incubating with 18 ml of methanol for 24 hours under the same conditions. The undiluted extract was considered to contain all of the anthocyanins originally present in the 3 slices. Serial dilutions of the extract with methanol were prepared and their extinctions at 500 m $\mu$  were estimated. A straight line was obtained by plotting extinction against dilution of anthocyanins. Percentage leakage of antho-

cyanins both in the presence and absence of antibiotics was estimated from the extinction values measured. Possible differences between extinctions of methanolic and aqueous solutions of anthocyanins were neglected.

#### Results and Discussion

Amphotericin B at a concentration of 100  $\mu\text{g/ml}$  caused complete leakage of anthocyanins within 24 hours. Fusaric acid was less active and cyanein at up to 200  $\mu\text{g/ml}$  had no effect (Fig. 1).

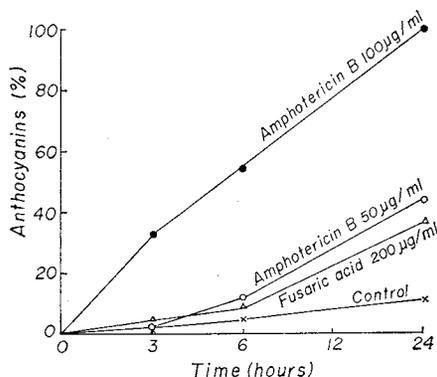
After 24 hours of incubation samples were also taken for the estimation of K<sup>+</sup> ions leaked from the slices. The order of the activities of the antibiotics was the same (Table 1).

Cyanein did not cause leakage either of anthocyanins molecules or of K<sup>+</sup> ions. Up to now only one example of the effects of

Table 1. Leakage of K<sup>+</sup> ions from beet root slices after incubation for 24 hours in presence of antibiotics

	$\mu\text{g/ml}$	$\mu\text{E K}^+/\text{slice}$
None		5.45
Amphotericin B	25	6.18
	50	7.25
	100	15.00
Fusaric acid	100	5.95
	200	9.12
Cyanein	100	5.25
	200	6.00

Fig. 1. Leakage of anthocyanins from beet root slices in presence and in absence of antibiotics



polyene antibiotics on higher plants has been described. Nystatin and N-acetylcandidin inhibited growth of the duck weed *Lemna perpusilla* which was grown photosynthetically in a pure culture<sup>4</sup>). Our observation of the effects of amphotericin B on the permeability of beet root is of interest in view of the hypothesis that the action of polyene antibiotics depends upon their interaction with sterols present in cell membranes of sensitive organism<sup>4</sup>).

We thank Dr. M. MIKO for estimations of K<sup>+</sup> ions by flame photometry.

#### References

1) BETINA, V.; P. NEMEC, J. DOBIAS & Z.

BARÁTH: Cyanein, a new antibiotic from *Penicillium cyaneum*. Folia Microbiol. 7: 353~357, 1962

- 2) BETINA, V.; P. NEMEC & Z. BARÁTH: Growth inhibition of *Allium cepa* roots by the antibiotic cyanein. Naturwiss. 50: 696, 1963
- 3) BETINA, V. & A. MURIN: Inhibition of mitotic activity in root tips of *Vicia faba* by the antibiotic cyanein. Cytologia 29: 370~374, 1964
- 4) LAMPEN, J. O.: Interference by polyenic antifungal antibiotics, especially nystatin and filipin, with specific membrane functions. III. Symposia of the Society for General Microbiology, Number XVI, Studies of Antimicrobial Drugs, Cambridge, Cambridge University Press, 1966