lower concentrations than needed for *Rhipiphorothrips* evidently has not influenced this species from feeding on this plant. Strangely enough in such instances as some garden crotons, rose leaves, etc., where both the species occur side by side along with their nymphal stages, there appears to be an optimum concentration of the free amino acids needed for both species.

Comparing the results relating to the free amino acids in the two allied species of plants—*Jatropha glandulifera* and *J. curcas* regarding host preference by *Rhipiphorothrips* for the latter species, it appears probable that heavy concentrations of alanine, tyrosine, serine, aspartic acid, glycine, glutamic acid appear to be one of the causes for non-infestation of *J. glandulifera* apart from the repellent action of the pyridine derivatives in this plant. The same is true of the brown stemmed and green stemmed *Ricinus*, the former being the preferred host of *Retithrips*. Heavy concentrations of glutamic acid, threonine, alanine, tyrosine, valine, methionine, leucine, lysine, histidine in the green castor have naturally prevented *Retithrips* attack. While it is known that heavy concentration of free amino acids prevents the acceptance of a host plant by thrips, it has also been inferred from the present studies that lower concentrations of varying essential free amino acids may also influence the degree of acceptance of the plant by thrips and should all of them occur in the proportions needed as for instance in *Vitis vinifera*, heavy aggregation of thrips infestation cause total tissue damage and crumpling of leaves. Such plants like *J. glandulifera* and green *Ricinus* are not preferred at all, or are the non-preferred hosts for food and oviposition because of the adverse effect of chemical factors having an inhibiting action and it has been observed that the first and second instar larvae of *Retithrips* failed to survive and develop even when raised on very young leaves of green castor, but readily did so when reared on the brown variety.

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**EFFECT ON ETHYLENEDIAMINE-TETRA-ACETIC ACID ON THE ANTI-BIOTIC ACTION ON AUREOFUNGIN**

Polyene antibiotics are known to cause a change in the permeability of the fungal cell membrane with depletion of essential cell constituents and this is shown to be induced by binding of the antibiotic to the cell membrane via a steroid-antibiotic complex, the presence of a steroid in cell membrane being a necessary prerequisite for polyene sensitivity. Ethylenediaminetetra-acetic acid (EDTA) also is known to have action on cell permeability, being an effective chelating agent. EDTA has been proved to enhance the activity of many antibacterial antibiotics such as benzylpenicillin, ampicillin, polymyxin, neomycin, vancomycin, chlorhexidine, chloramphenicol and tetracycline and in studies involving actinomycin-D, Leive showed non-specific increase in cellular permeability in bacteria. There is little work on the interaction of antibiotics and EDTA in the case of filamentous fungi. Since both EDTA and polyene antibiotics have action on membranes, it is interesting to know the effect of a combination of the two substances on fungi. Since sterols are necessary for the binding of polyene antibiotics to the cell membrane and for the consequent action of these antibiotics, it is also of interest to study the effect of these antibiotics on fungi which lack sterols. In general, Oomycetous fungi are less sensitive to polyene antibiotics presumably due to a lack of sterols in the cell membrane. Some of them need an extraneous source of sterols for growth and sexuality. *Phytophthora arecae* which did not produce sex organs and even sporangia in a culture medium without sterols has been chosen to study the effect of aureofungin along with *Drechslera pedicellata* which is known to be highly polyene sensitive.

The antibiotic action of aureofungin was tested against the fungi on potato-dextrose agar and the interaction of EDTA and aureofungin on *D. pedicellata* alone was studied using modified DPYA medium (dextrose 1%; peptone 0.5%; yeast extract 0.2%; MgSO₄·7 H₂O 0.05%; KH₂PO₄ 0.1%; agar 1.7%). The effect of the antibiotic on the radial growth of the fungi is given in Fig. 1. *D. pedicellata* was more sensitive to the antibiotic than *P. arecae*. The treated colonies of *D. pedicellata* showed mycelial swellings similar to those induced by other polyene antibiotics, while *P. arecae* showed no mycelial abnormalities. The antibiotic-induced mycelial abnormalities were found only in hyphae embedded in the medium and not
in the aerial hyphae. This may indicate that the antibiotic is not translocated in the active form to the aerial hyphae, only those hyphae in physical contact with the antibiotic in the medium being directly affected. The growth inhibition without morphological changes in P. arecae, which lacks sterols for complex formation, indicates that aureofungin has some direct effects on fungal metabolism other than the metabolic changes concomitant of membrane damage.

Fig. 1. Dosage response curve for aureofungin on Drechslera pedicellata (○) and Phytophthora arecae (△) at 120 hrs.

Observations on D. pedicellata indicated that EDTA alone did not induce notable changes in radial growth and morphology (Fig. 2). However, when a combination of aureofungin at ED₉₀ dosage (0.1 μg/ml) and EDTA (10⁻³ M) was supplied in the medium, the dimensions of the mycelial swellings were significantly less than those of colonies treated with only aureofungin. The average diameter of the mycelial swellings in EDTA-aureofungin combination was only 12.2 μm, whereas in aureofungin without EDTA it was 13.3 μm (average of 250 measurements each). By applying t-test this difference between the two treatments is shown to be significant at 1% level (t = 7.3). There was, however, very little difference in colony diameter (Fig. 2).

EDTA is known to cause extensive damage to the cell wall of Pseudomonas aeruginosa⁸-15 and has been shown to induce the release of lipopolysaccharides¹ and also other intracellular substances like phospholipids, demethyl vitamin K₂ and cytochromes b and c from Haemophilus parainfluenzae¹²-¹⁴. An EDTA-induced change in membrane permeability in Saccharomyces cerevisiae has been observed which was considered to be the result of the removal of Mg²⁺ from the membrane by the chelating agent⁵-⁶. EDTA is also known to chelate effectively other divalent cations like Ca²⁺ and Zn²⁺ and alter the permeability of the cell membrane⁶. Therefore, it is possible to assume that the smaller dimension of the mycelial swellings in colonies treated with both aureofungin and EDTA as compared to the ones treated with only aureofungin is due to the leakage of the intracellular substances from the osmotically stressed protoplasts because of changed membrane permeability induced by EDTA along with aureofungin. Thus, EDTA has an additive effect on the antibiotic activity of aureofungin on fungi but to a lesser extent when compared to its effects in enhancing the sensitivity of bacteria to antibacterial antibiotics⁸.

Fig. 2. Histogram showing the effect of aureofungin and EDTA on Drechslera pedicellata (at 96 hrs).
A. Control; B. EDTA; C. Aureofungin; D. Aureofungin + EDTA.

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6. —. Ibid., 1968, 41, 433.
12. —. Ibid., 1970, 102, 508.