normal-appearing nuclei. No effort was made at this time to study the growth requirements of the cells.

This method for preparing cell suspensions from insect tissue is reproducible and makes possible quantitative studies involving large numbers of cells. Conceivably it could provide monolayer cultures for viral research. We are hopeful that the liberated cells may dedifferentiate rapidly and be capable of growth in a less complex medium than is required, apparently, for explanted insect tissues.

MAURO E. MARTIGNONI
ELSA M. ZITCER
R. P. WAGNER*
Laboratory of Insect Pathology, Department of Biological Control; Virus Laboratory; and *Department of Biochemistry, University of California, Berkeley*

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Protection of Fungi against Polyene Antibiotics by Sterols

While we were studying the structural chemistry of the polyene antifungal agent, filipin, the question arose of the possible mechanisms of its antifungal action (*1*). The presence of a conjugated polyene structure in filipin suggested that perhaps filipin interfered with the synthesis or the function of carotenoids in the fungi. In order to investigate this hypothesis, a mixture of carotenoids was obtained from carrots. The hexane extract, prepared according to the method of Loomis and Shull (*2*), was tested by the assay disc method on *Penicillium oxalicum* (*3*). The inhibitory activity of filipin was, indeed, completely prevented, and the organism grew normally in the presence of filipin plus the crude hexane extract.

Saponification of the hexane extract followed by chromatography on MgO : Hyflo Super Cel gave an active fraction which followed α- and β-carotene from the column. From this fraction a white material was obtained which was easily crytalized from methanol-water. This material was obviously not the usual type of carotenoid. Moreover, when crystalline preparations of α-carotene, β-carotene, or vitamin A were tested, they showed no activity at levels of 10 times that of filipin. The isolated material melted at 134.5° to 137°C and gave analytical data consistent with a $C_{20}H_{30}O$ structure. C-Methyl analysis completely ruled out the possibility that this material was a perhydrocarotenoid, for the material contained only 1.5 C-methyl groups per 20 carbons. An alternative possibility that the protecting agent might be a sterol was confirmed by positive Lieberman-Burchard and Salkowski tests. The analytical data are also in good agreement for sterols of the C_{29} series—that is, sitosterol and stigmasterol (*4*)—which have been isolated from carrots, if they are calculated for ½ H_2O of crystallization (*5*). $C_{29}H_{50}O \cdot ½ H_2O$: Calculated: C, 82.18 percent; H, 12.11 percent. Found: C, 82.01 percent; H, 12.46 percent.

The observation that the active agent is a sterol led to the testing of a number of such compounds for similar activity. Thus far, three fungi, *Penicillium oxalicum, Aspergillus niger,* and *Hansenula subpelliculosa,* have been used to study this phenomenon. The prevention of filipin inhibition of the *Penicillium* was observed in both the assay plate test and in liquid media. On agar plates, both carrot sterols and soybean sterols allowed growth of *P. oxalicum* to occur in the presence of filipin at a weight ratio of sterol to filipin of 0.5 : 1.0; *Hansenula* grew normally in shaken liquid culture at a ratio of 0.25 : 1.0. The effect of sterols on nine polyene antibiotics showed various degrees of protection, but no definite relationship between the number of unsaturated groups in the antibiotics and their vitiation could be established (*6*). Filipin and fungichromin inhibitions were most readily prevented, followed by amphotericin B, trichomycin, and rimocidin, while candicidin A, candicidin B, ascosin, and nystatin were only slightly affected. Unfortunately, only the purities of filipin and fungichromin were known so that the relative order of protection can only be tentatively suggested.

Of all the sterols examined thus far, cholesterol, ergosterol, sitosterol, stigmasterol, and to a slight degree, lanosterol, have been active in offsetting filipin inhibition of *H. subpelliculosa.* Ergosterone gives effective reversal of filipin activity; cholesterone is ineffective. None of the short-chain steroids that were tested had similar effects even at a ratio of steroid to filipin of 4 to 1. Apparent reversal occurs after long incubation periods, but preliminary data indicate the possible inactivation of filipin under these conditions.

The prevention of the antifungal activity of filipin by sterols has some interesting implications. Evidently, sterols play a much more important role in the growth processes of fungi than has hitherto been suspected. While a few microorganisms have been shown to require sterols for growth (*7–9*), our current studies indicate that such substances are probably essential metabolites for many fungi. This has not been recognized until now because these microbes are for the most part autotrophic for their sterol requirement. Except for *Labrynthula vitallina* var. *pacifica* (*9*) and *Saccharomyces cerevisiae* S C-1 when grown anaerobically (*7*), the need for this compound has not been demonstrated among the fungi.

The mechanism by which filipin and other polyenes inhibit fungi is intriguing. They might either prevent the synthesis of sterols which are necessary for growth or competitively replace the sterol as a cofactor of a reaction vital to the metabolism of the organism. If the synthesis of cholesterol is prevented, then the inhibition probably occurs at a stage beyond lanosterol formation (*10*). Squalene does not reverse the action of filipin on *Hansenula subpelliculosa* even at a squalene to filipin ratio of 20 : 1. Lanosterol reverses the antibiotic only very slightly at a lanosterol to filipin ratio of 4 : 1. This is a sharp contrast to cholesterol, which is active at a ratio of under 4 : 1 and probably at ¼ : 1.

DAVID GOTTLIEB, HERBERT E. CARTER, JAMES H. SLONEKER, ALFRED AMMANN
Departments of Plant Pathology and *Chemistry, University of Illinois, Urbana*

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Pyrrolidine Metabolism: Soluble γ-Aminobutyric Transaminase and Semialdehyde Dehydrogenase

A strain of *Pseudomonas* isolated by the enrichment culture technique with pyrrolidine as the sole carbon source has been found to catalyze the following reactions designated as γ-aminobutyric-