Short Communication

Effect of Filipin on the Permeability of Red Beet and Potato Tuber Discs

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The discovery of an acylated steryl glucoside in plant tissue in 1964 (13) raised the number of known forms of sterols in plants to four. These are the free sterols, sterol esters, steryl glycosides, and acylated steryl glucosides. Kiribuchi et al. (12) analyzed the sterol content of soybean and found that sterol ester was present in very small amount and that the sterol was mostly in the form of steryl glucoside and acylated steryl glycoside. The most reliable quantitative analyses of the different forms of sterols in plant tissue are those of Galliard (4, 5). He has found that 9.1% of the total lipid of potato tubers is sterol in the four forms mentioned above: 0.6% free sterol, 0.1% sterol esters, 2.0% steryl glucoside, and 6.4% acylated steryl glucoside (4). In the case of postclimacteric apples 29.2% of the total lipid was sterol derivatives: 20.1% free sterol, 2.1% sterol esters, 5.4% steryl glucoside, and 1.6% acylated steryl glucoside (5). Several recent papers have examined the biosynthesis of steryl glucoside and acylated steryl glucoside (3, 9, 10, 14); however, there has been no indication of the physiological function of these sterols and derivatives. Grunwald (8) has made an approach to this problem by studying the effect of added sterols on the efflux of betacyanin from red beet discs.

The polyene antibiotic filipin, isolated from Streptomyces filipinensis, has been shown to antagonize fungal spore germination and growth (6, 7). A tentative structure has been proposed for filipin showing it to be a 35 C pentaene (1); however, it now appears that filipin is a complex of at least four components (15). The effect on growth can be prevented and reversed by addition of cholesterol to the medium (6, 7). Work with model membrane systems of lipid monolayers and bilayers has shown that the artificial membranes are affected by filipin only when cholesterol has been previously incorporated into the membrane (2, 11). Mycoplasma laidlawii is susceptible to filipin only if the growth conditions have permitted the organism to incorporate cholesterol into the membrane (16). The inhibition of yeast growth by filipin can be antagonized by sitosterol, ergosterol, and stigmasterol as well as cholesterol (6, 7). These observations indicate that filipin may be used as a diagnostic test for sterol-containing membranes. It therefore occurred to us to test the effect of filipin on higher plant tissues.

Cores of plant material were cut with a cork borer 7.5 mm in diameter. Discs approximately 1 mm thick were cut free-hand from this core. The discs were washed in tap water before use. Aging of the discs for 24 hr in tap water did not change the response to filipin. The reaction mixtures consisted of 8.0 ml of water; 20 ml of 0.1 M phosphate buffer, pH 7.2; 0.1 ml of methanol with varying amounts of filipin; and 10 discs of plant tissue. Filipin was kindly supplied by Dr. G. B. Whitfield, Jr., the Upjohn Company, Kalamazoo, Michigan. Filipin was dissolved in methanol at concentrations of 5 mg/ml, the solution being prepared immediately before the experiment. Varying volumes of the filipin solution were added to the reaction mixture and pure methanol was then added to make the final amount of methanol 0.1 ml in all vessels. This amount of methanol was found to have no effect on the efflux of materials from the tissue discs. The reaction mixtures were shaken in a water bath at 26 C.

![Graph](https://example.com/graph.png)

**FIG. 1.** Efflux of materials absorbing at 260 nm from discs of potato tuber. Ten discs of tuber tissue were incubated in 10 ml of solution 0.02 M with respect to phosphate buffer, pH 7.2. Concentrations of filipin in the reaction mixture are indicated. Filipin in the reaction mixture was measured by its absorbance at 355 nm.

At intervals 3.0-ml samples of the reaction mixture were withdrawn with a pipette and transferred to a spectrophotometer cuvette. The absorbance spectrum was measured with a Bausch and Lomb 505 recording spectrophotometer. In the case of potato the spectral range covered 220 to 400 nm, and in the case of red beet discs, 220 to 600 nm. After the measurement of the absorbance spectrum, the sample was returned to the reaction vessel, and the incubation was continued.

Figure 1 shows the efflux of materials from potato discs (*Solanum tuberosum*) as stimulated by filipin. Material which absorbs at 260 nm is released from the discs which are not treated.
with filipin, but the release is greatly stimulated by filipin. The antibiotic itself has absorption maxima at 355, 337, 319, and 304 nm. This filipin absorbance at 260 nm is approximately 25% of the absorbance at 304 nm, and this accounts for the difference in zero time readings. The absorbance due to filipin decreased during the course of the experiments, and this was most readily measured at 355 nm since absorbance at this wave length was least influenced by materials leaking from the discs. Figure 1 also shows the reduction in the 355 nm absorbance during the course of the experiment. This reduction is probably due to the penetration of the filipin into the tissue slices, but the rapid initial rate and slower subsequent rate suggest that at least two processes are involved. It is not possible at the moment to say whether these processes involve adsorption, absorption, or degradation of filipin.

Figure 2 shows the efflux of materials from red beet discs (Beta vulgaris) as stimulated by filipin. The efflux of material absorbing at 260 mu and the reduction in absorbance at 350 nm are shown as in Figure 1. In addition, the efflux of betacyanin as measured at 530 nm is also plotted. It is to be noted in these cases that there is a lag period before the filipin-elicted efflux, and that the lag periods are the same at 260 nm and at 530 nm. It is known that the betacyanin is localized in the vacuoles of the red beet cells, but no estimate can be made at the present time of the localization of the material which absorbs at 260 nm. In any case, these materials are not distinguishable kinetically during efflux under the influence of filipin. Presumably the effect of filipin in causing the efflux of betacyanin is on both the plasmalemma and the tonoplast, permitting the betacyanin to pass through the cytoplasm to the external solution. Electron microscope pictures, kindly taken by Dr. W. W. Thomson, showed no changes in ultrastructure of red beet discs which had been treated with filipin.

The reaction mixture has been repeatedly extracted with diethyl ether in order to examine the absorbance spectrum of the watersoluble materials. The material which absorbs at 260 nm has the spectrum of nucleotides. Hydrolysis of the reaction mixture, followed by ion exchange chromatography, showed that proteins also are present in the material leaking from the plant tissue discs.

Digitonin has been tested for its effect on the tissue discs, and it has an effect at concentrations slightly higher than those used for filipin. Triton X-100 does cause leakage, but the threshold concentration for leakage is 500 µg/ml, 2 orders of magnitude greater than the concentration of filipin causing a leakage from potato discs. We do not suggest that the action of filipin is the same as that of digitonin or Triton X-100.

Preliminary tests have shown that discs of apple fruit and carrot are not affected by concentrations of filipin up to 100 µg/ml. It would be of interest to relate susceptibility of different plant tissues to filipin-induced leakage to the sterol content of their membranes and particularly the form of sterol in the various membranous structures of the cell. The range of effects of filipin on different plant tissues suggests that this should be possible.

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