Separation of cholesterol and desmosterol 
by thin-layer chromatography

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Studies of inhibitors of cholesterol biosynthesis and the recognition of 24-dehydrocholesterol (desmosterol) as a potential intermediate in this biosynthetic pathway have stimulated attempts to devise a suitable technique for the separation of these sterols (1, 2). However, a simple, rapid method for the separation of free cholesterol and free desmosterol has not been described.

Kaufmann and Makus (3) have proposed a method for the separation of fatty acids, which involves the coating of the silica gel layer with undecane, enabling the separation to proceed primarily via partition rather than adsorptive chromatography. The separation of the fatty acids appeared to be related to the number of double bonds present as well as the number of methyl groups. It occurred to us that this technique might be applicable to the separation of desmosterol and cholesterol, which differ only by the presence of a double bond. We have applied this basic technique to the separation of these sterols and have achieved excellent results. The procedure follows:

Glass plates, 20 x 20 cm, are coated with a 275 μ layer of silica gel1 (proportion of gel to water, 1:1.5). The plates are activated for 1 hr at 120°, and stored in a desiccator until used. The coated plate is developed in a 15% solution of undecane in petroleum ether to the

1 Adsorbil 1, purchased from Applied Science Laboratories, Inc., State College, Pennsylvania.
cholesterol (4), and its disappearance following cessation of the drug, was clearly demonstrable with this technique. The method described is rapid, convenient, and reproducible and should find many applications in studies of lipid metabolism.

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REFERENCES


Fig. 1. Separation of cholesterol and desmosterol by thin-layer chromatography. Cholesterol (1,4); desmosterol (2,5); mixture of cholesterol and desmosterol (3,6).