Paper chromatography of cholesterol and desmosterol after bromination

SERGIO FABRO

Medical and Clinical Research Department,
Lepetit S.p.A.,
Via Durando, 38,
Milan, Italy

[Received for publication April 11, 1962]

Desmosterol ($\Delta^{5,24}$-cholestadien-3β-ol) differs from cholesterol (Δ^{5}-cholesten-3β-ol) in possessing a double bond in position 24. Because of the great similarity of these two molecules, their chromatographic separation has been difficult. Stokes *et al.* (1) and Avigan *et al.* (2) succeeded in separating their esters by adopting and partially modifying the column chromatography technique of Idler and Baumann (3).

More recently, Holmes and Stack (4) have used gas chromatography for their separation.

As the cholesterol and desmosterol molecules take up different quantities of bromine (2 and 4 atoms per mole respectively), I thought it would be rewarding to exploit this difference and investigate a method of separating these two compounds by paper chromatography after bromination. Descending chromatography on paper impregnated with β-hydroxyethylphenylether, using hexane as solvent, proved to be the most suitable system.

The desmosterol was supplied by W. S. Merrell Company, Cincinnati, Ohio. All the other substances were reagent grade. Sheets of 3 MM Whatman paper 18–30 cm wide by 53–55 cm long were impregnated with a 10% solution of β-hydroxyethylphenylether in acetone. After removal of the acetone by aeration, the sterols in a sulphuric ether solution were placed on the starting line of the impregnated sheet at a distance of 4 cm from one another. The quantity of sterols ranged from 50 to 200 μg.

In the first experiments, bromination was effected by applying a few drops of a 10% solution of bromine in CCl_4 directly to the product at its starting point on the paper, the excess bromine being removed by a draught of cold air. In other experiments, the process was accomplished with the sterols in a sulphuric ether solution by adding bromine drop by drop until the solution assumed a stable orange color. After 15 min., the excess bromine was removed by washing the solution 3 times with sodium thiosulphate, 0.1 M, and then with H_2O. The ether solution of brominated sterols was reduced to a low volume under vacuum and then placed on the paper. The two methods yielded identical results.

Descending chromatography was carried out in an atmosphere saturated with β-hydroxyethylphenylether-hexane at a temperature of 22° ± 3°, using as solvent a solution of hexane saturated with β-hydroxyethylphenylether at the experimental temperature. The time taken by the front of the solvent to effect a run of about 45 cm was generally 2 hr. The brominated compounds were revealed, after the chromatographic run, by placing the sheet, previously heated to 115° for 20 min., under UV light.

The brominated products of cholesterol and desmosterol exhibited an intense yellowish-white fluorescence under the above conditions, whereas the respective unbrominated compounds were not fluorescent. The cholesterol and desmosterol in the system used proved to have a practically identical R_f value (0.72 for cholesterol and 0.68 for desmosterol), whereas their brominated compounds were sharply separated: cholesterol 0.69 ± 0.06 (16 experiments) and desmosterol 0.42 ± 0.07 (13 experiments). The slight variation from experiment to experiment was due, in all probability, to the difficulty of impregnating the sheet with β-hydroxyethylphenylether in a rigorously constant manner, as Neher and Wettstein (5) pointed out some time ago. On chromatographic separation of brominated desmosterol (100–200 μg), two other small fluorescent spots become visible under UV light after heating in addition to the one with R_f 0.42: one

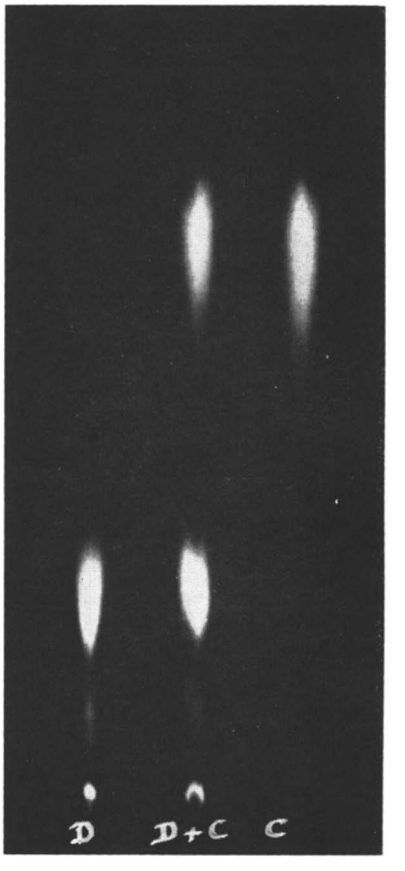

FIG. 1. Chromatographic separation of desmosterol from cholesterol after bromination. D = desmosterol (200 μg); C = (200 μg).

with an R_f of 0.05; and another, very faint and blurred, with an R_f of 0.15. Brominated cholesterol under identical conditions always exhibited a single spot with R_f 0.69 (Fig. 1).

These compounds, which become visible after chromatography of brominated desmosterol, appeared also after chromatography of the unsaponifiable fraction of plasma or of organs (liver, kidney, lung) of animals treated with triparanol (Fig. 2). Here again, two other small spots, definitely comparable in R_f and in

fluorescence to those obtained in the chromatography of desmosterol, are visible under UV light together with the spots of cholesterol and desmosterol. It is not yet possible to say whether they correspond to secondary compounds obtained in the bromination of desmosterol

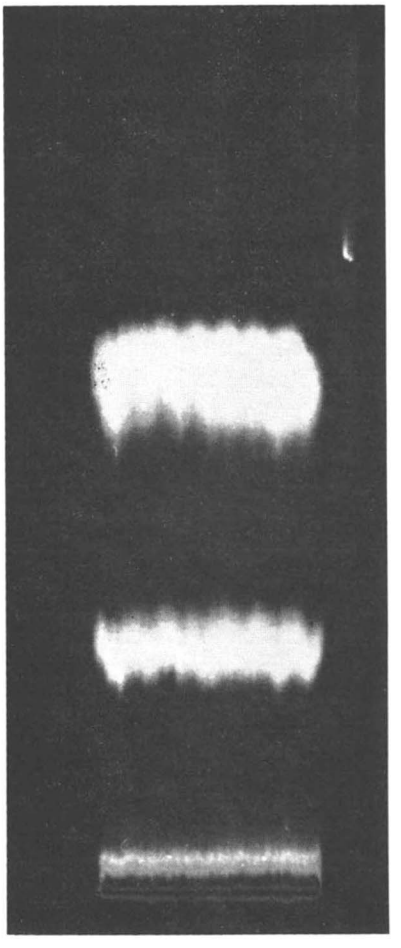

FIG. 2. Chromatography of the brominated unsaponifiable fraction of pooled organs (liver, kidney, lung) of rats treated with triparanol (12.5 mg daily for 30 days). The brominated cholesterol and desmosterol are recognizable. Further, there are two fluorescent zones with R_f values of 0.05 and 0.15 just as occurs with the chromatography of desmosterol. The chromatographic conditions are as described in the text. The photograph was taken under ultraviolet light.

or whether they accompany the desmosterol. If the latter is the case, and the possibility cannot be excluded since the grade of purity of the desmosterol used as standard was not known, then they must correspond to compounds, probably sterols, that form in animals treated with triparanol.

REFERENCES

1. Stokes, W. M., W. A. Fish, and F. C. Hickey. *J. Biol. Chem.* **220**: 415, 1956.
2. Avigan, J., D. Steinberg, H. E. Vroman, M. J. Thompson, and E. Mosettig. *J. Biol. Chem.* **235**: 3123, 1960.
3. Idler, D. R., and C. A. Baumann. *J. Biol. Chem.* **195**: 623, 1952.
4. Holmes, W. L., and E. Stack. *Biochim. et Biophys. Acta* **56**: 163, 1962.
5. Neher, R., and A. Wettstein. *Helv. Chim. Acta* **35**: 276, 1952.