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Note

Improved resolution of cholestanol and cholesterol by gas–liquid chromatography

Application to pigeon testicular sterols

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Cholestanol (5α-cholestan-3β-ol) has been found to accompany cholesterol in all animal tissues[1]. The presence of cholestanol in the atherosclerotic aorta of humans[2] and pigeons[3], its atherogenicity when fed to animals[4], and its occurrence in a significant concentration in the plasma and tissue of patients with cerebrotendinous xanthomatosis[5] have stimulated an interest in this compound. Cholestanol is usually separated from cholesterol by performic acid oxidation[6] or by argentation chromatography[7]. During our studies of the testis of the White Carneau pigeon, which contains the highest cholestanol concentration hitherto reported in tissues[8], it became desirable to develop a direct gas chromatographic system for the efficient separation of cholestanol from cholesterol, particularly when the former is in low concentration. This communication describes such a system and its application to the study of the age-related changes in the concentration of cholestanol in pigeon testis.

EXPERIMENTAL

Standard cholesterol and cholestanol were obtained from Applied Science Labs. (State College, Pa., U.S.A.). The purity of the compounds was determined by thin-layer (TLC) and gas–liquid chromatography (GLC). Sterols were converted into their trifluoroacetates as described previously[9]; briefly, the sterols were heated with trifluoroacetic anhydride at 60° for 40 min, the excess solvent was evaporated, and the compounds were dissolved in chloroform. Sterols were converted into their trimethylsilyl ethers as described previously[3].

Testicular lipids of White Carneau pigeons (*Columba livia*) were extracted with chloroform–methanol (2:1), as described by Folch *et al.*[10]. The sterols and steryl esters were separated by TLC on silica gel G using the solvent system heptane–diisopropyl ether–acetic acid (65:40:4). Sterols from each fraction were recovered as

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described previously[3]. They were then chromatographed as trifluoroacetates with 5α-cholestane as an internal standard. Details of the chromatographic systems used are given in Table I.

RESULTS AND DISCUSSION

Fig. 1 shows the GLC separation of the esterified sterol fraction from pigeon testis on a 10% SP-2401 column. This resolution of a cholestanol–cholesterol mixture is better than that achievable by any other system previously described or by the other chromatographic systems in Table I. It is important to note that, when cholestanol is present to the extent of 1%, it can be easily resolved from cholesterol.

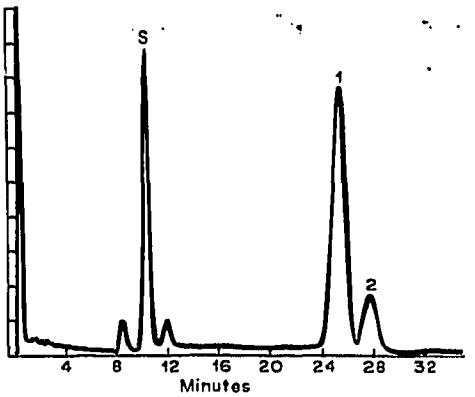

Fig. 1. Gas–liquid chromatogram of esterified sterols from pigeon testis. The sterols were chromatographed as trifluoroacetate derivatives on a 10% SP-2401 column. S = Cholestane (internal standard); 1 = cholesterol; 2 = cholestanol. Operating conditions: oven temperature, 220°; flash heater temperature, 220°; detector temperature, 300°; carrier gas, helium, 50 ml/min.

TABLE I

CHOLESTANOL–CHOLESTEROL SEPARATION ON VARIOUS LIQUID PHASES

Column packing and operating details: W-98 on Diatoport (80–120 mesh), QF-1 on Gas-Chrom Q (80–100 mesh), SP-2401 on Supelport (100–120 mesh); W-98 and SP-2401 columns were fitted to Packard 409 gas chromatograph; QF-1 columns were fitted to an F & M Model 402 gas chromatograph. Operating conditions for Packard: oven temperature, 220°; flash heater temperature, 220°; detector temperature, 300°. Operating conditions for F & M: oven temperature, 225°; flash heater temperature, 240°; detector temperature, 280°. The carrier gas was helium, 50 ml/min, for all columns. Abbreviations: TMS = Trimethylsilyl ether; OH = non-derivatized sterol; TFA = trifluoroacetate.

Liquid phase	*Derivative*	*Separation factor**
3% W-98	TMS	1.00
3% QF-1	OH	1.02
3% QF-1	TFA	1.04
3% SP-2401	TFA	1.06
10% SP-2401	OH	1.05
10% SP-2401	TFA	1.14

* The separation factor was calculated by dividing the retention time of cholestanol by that of cholesterol.

This separation system was used to study the changes with age in the cholestanol concentration in pigeon testis (Table II). Total sterol concentration increased from age 6 months to age 2 years and then decreased. This increase is probably due to the increase in testis growth[11]. Steryl esters showed a decrease around age 1 year, followed by a gradual increase. Whether the decrease in cholesteryl esters is related to the increased production of steroid hormones during this stage is not known. The relative amount of cholestanol, both the free and ester fractions, did not change significantly with age. This suggests that cholestanol has little, if any, role in the control of steroid hormone biogenesis.

TABLE II

TOTAL STEROL AND CHOLESTANOL CONCENTRATIONS* IN TESTIS OF PIGEONS AT VARIOUS AGES

Age (year)	*Total sterols (mg/g)*	*% esters*	*Cholestanol (% of total sterols)*		
			Free	*Esterified*	*Total*
0.5	1.77	25.2	24.2	12.5	21.3
0.75	1.94	20.6	22.8	19.1	22.0
1	1.94	16.8	22.8	16.3	21.7
2	2.07	35.7	24.3	14.9	20.9
7	1.61	41.5	25.2	22.7	24.2

* Each number represents the mean of values obtained from four to ten separate birds at the specific age.

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