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Cholestanol and Plant Sterols in the Adrenal Gland of the Rat

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Adrenal sterols of rats on the ordinary laboratory diet were subjected to successive chromatography on silica gel and silver nitrate-impregnated silica gel chromatoplates. Gas liquid chromatographic analysis of resulting unsaturated (Δ^5) sterol fraction revealed the presence of cholesterol and plant sterols, campesterol, stigmasterol, and β-sitosterol. Cholestanol and corresponding ring saturated (5α) plant sterols, campestanol, stigmastenol, and β-sitostanol, were found in the stanol fraction where the latter sterol group constituted about 20 % of total stanols. Cholesterol accounted for approximately 94 % of the total, the rest being mainly due to plant sterols (5 %) and cholestanol (1 %). In addition, small amounts of cholesterol precursors were present.

Much attention has been paid recently to the metabolism of cholestanol in the animal organism, including the adrenal gland. [1–4] For these studies new methods have had to be developed for the determination of cholestanol in the presence of a large excess of cholesterol.[4–6] On account of its Δ^5 double bond, cholesterol can be converted to polar derivatives that are easily separated from the remaining intact cholestanol. Subsequent quantification of stanol has been carried out nonspecifically, with the result that cholestanol is overestimated if other stanols are present in the sample studied.

Recent investigations have shown that, in addition to cholesterol, small amounts of other sterols are present in the adrenal gland of the cow. But identification of these minor components, even with a method involving successive column chromatography, thin layer chromatography (TLC), and gas liquid chromatography (GLC), proved difficult.[7] However, the presence of cholestanol, methostenol, lanosterol, 7α- and 7β-hydroxycholesterol, and some other minor steroids was verified. The occurrence of plant sterols has not been demonstrated in the adrenals or in any other tissue. During our studies on the genesis of adrenal cholesterol, the gland was found to contain a large number of sterols, including stanols.[8] The present report demonstrates that the adrenals of rats on a regular diet contain both unsaturated (Δ^5) and saturated (5α) plant sterols. Yet, by using a combination of the TLC and GLC techniques,[9–10] cholesterol and cholestanol could be quantified separately from the respective Δ^5 and 5α plant sterol series.

MATERIAL AND METHODS

Male rats of the Wistar strain were kept on the ordinary laboratory diet and killed by decapitation. The adrenals were removed quickly, and placed in an approximately 30-fold amount (w/v) of N NaOH in 90 % ethanol and refluxed under a nitrogen atmosphere for 1 h. The sterols were extracted and subjected to successive TLC on silica gel G and silver nitrate-impregnated silica gel G chromatoplates and to subsequent GLC as presented earlier for faecal sterols.[9-10] TLC on silica gel G gave a broad band with the mobility of cholesterol and in front of it two weak bands with the respective mobilities of lanosterol* and methostenol. The "cholesterol fraction" was rechromatographed on silver nitrate-impregnated silica gel G, and three separate zones were found, designated fractions I, II, and III in descending order of mobility. Sterols of these fractions were desorbed and subjected to GLC as their TMS derivatives. Qualitative and quantitative studies could be carried out on the adrenals of one rat or even on a single gland if necessary. In quantitative analyses, losses occurring during the procedure were corrected in accordance with the recovery of added cholesterol-4-^{14}C (New England Nuclear, Inc.) of high specific activity. The label was purified before use on a silver nitrate-impregnated chromatoplate. Rechromatography of the label after mixing with cold cholestanol and cholesterol gave no counts in the cholestanol fraction.

Available sterol references were: cholestanol, lanosterol, Δ^7-cholestenol (obtained from Dr. Ahrens, New York), cholesterol (Merck), methostenol and Δ^8-methostenol (obtained from W. W. Welles, Pittsburgh Pa.). A mixture of plant sterols was isolated from corn oil by using TLC on florisil.[9] To obtain ring-saturated 5α plant sterols, this material was rechromatographed on a silver nitrate-impregnated chromatoplate. Two fractions were obtained, a weak one with the mobility of cholestanol and a strong band with the mobility of cholesterol. On GLC, both fractions gave three major peaks with the respective retention times found earlier for ring-saturated (5α) campestanol, stigmastenol and β-sitostanol, and unsaturated (Δ^5) campesterol, stigmasterol and β-sitosterol.[9-10]

RESULTS

Thin layer chromatography of reference sterols (Table 1) showed that cholesterol, cholestanol, Δ^7-cholestenol, and saturated (5α) and unsaturated (Δ^5) plant sterols moved on silica gel G as a single band with a mobility corresponding to that of the "cholesterol fraction" obtained from non-saponifiable material of the adrenal gland. Rechromatography of the "cholesterol fraction" on a silver nitrate-impregnated chromatoplate showed three separate zones, of which fraction I had the mobility of cholestanol and ring-saturated (5α) plant sterols. Fraction II moved with Δ^7-cholestenol and the slowest zone, fraction III, migrated with cholesterol and unsaturated (Δ^5) plant sterols.

Gas liquid chromatographic patterns obtained from fractions I, II, and III are illustrated in Fig. 1 and retention times of reference and adrenal sterols in relation to 5α-cholestane are given in Table 1. According to TLC and GLC criteria, fraction I contained cholestanol and a relatively large amount of ring-saturated plant sterols, campestanol, stigmastenol, and β-sitostanol.

* Common names of sterols used in this report: Cholesterol, cholest-5-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; methostenol, 4α-methyl-5α-cholest-7-en-3β-ol; Δ^8-methostenol, 4α-methyl-5α-cholest-8-en-3β-ol; lanosterol, 4,4,14α-trimethyl-5α-cholest-8,24-dien-3β-ol; Δ^7-cholestenol, 5α-cholest-7-en-3β-ol; Δ^8-cholestenol, 5α-cholest-8-en-3β-ol; campesterol, 24α-methyl-cholest-5-en-3β-ol; stigmasterol, 24β-ethyl-cholest-5,22-dien-3β-ol; β-sitosterol, 24β-ethyl-cholest-5-en-3β-ol; campestanol, 24α-methyl-5α-cholestan-3β-ol; stigmastenol, 24β-ethyl-5α-cholest-22-en-3β-ol; β-stigmastanol, 24β-ethyl-5α-cholestan-3β-ol.

Table 1. Thin layer chromatography and gas liquid chromatography of major adrenal sterols.

TLC fraction from silica gel G	Reference compound	TLC fraction from $AgNO_3$-impregnated silica gel G[b]	Relative retention times[c]	
			Reference	Found for adrenal sterols
	Cholestanol	I	2.33	2.32
	Campestanol	I	3.04	3.04
	Stigmastenol	I	3.27	3.25
	β-Sitostanol	I	3.83	3.82
"Cholesterol	Δ^7-Cholestenol	II	2.59	2.58
Fraction"[a]	Cholesterol	III	2.28	2.28
	Campesterol	III	2.95	2.96
	Stigmasterol	III	3.18	3.19
	β-Sitosterol	III	3.75	3.74

[a] Compounds with the mobility of cholesterol.
[b] The "Cholesterol fraction" was divided by this system into subfractions I, II, and III.
[c] Relative to 5α-cholestane. Instrument, F & M, Model 400; Column, 6 feet long, 1 % DC 560; Temperature, 244°C.

Fraction II gave two peaks in GLC, of which the major one had the retention time of Δ^7-cholestenol. The small peak did not disappear when the fraction was rechromatographed on a silver nitrate-impregnated chromatoplate; hence it was not due to contamination by cholesterol or cholestanol. Its retention

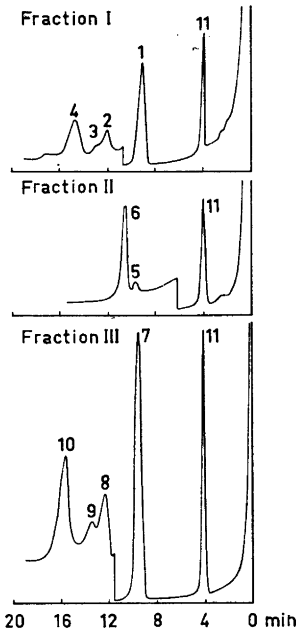

Fig. 1. Gas liquid chromatographic patterns of adrenal sterols. Conditions and fractions as in Table 1. The following tentative identifications were made: 1, cholestanol; 2, campestanol; 3, stigmastenol; 4, β-sitostanol; 5, Δ^8-cholestenol; 6, Δ^7-cholestenol ??; 7, cholesterol; 8, campesterol; 9, stigmasterol; 10, β-sitosterol, and 11, 5α-cholestane added as internal standard.

time corresponded well with that of Δ^8-cholestenol calculated from the respective steroid numbers[10] of methostenol, Δ^8-methostenol and Δ^7-cholestenol. GLC of fraction III revealed the presence of four peaks that were identified tentatively on the basis of retention times and of behaviour on TLC as cholesterol and the unsaturated plant sterols campesterol, stigmasterol and β-sitosterol.

GLC of the adrenal sterols moving ahead of the "cholesterol fraction" on silica gel G gave several peaks including those with the retention times of lanosterol, dihydrolanosterol, methostenol, and Δ^8-methostenol.

Table 2. Composition of adrenal sterols in rats on the ordinary laboratory diet.

	Cholesterol	Δ^5-plant sterols[a]	Cholestanol	5α plant sterols[b]	Δ^5-Cholestenol	Total
μg/rat[c]	348 ± 36	16.9 ± 1.6	4.23 ± 0.66	1.12 ± 0.24	0.82 ± 0.18	371.07
% of total[d]	93.8	4.6	1.1	0.3	0.2	100.0

[a] Sum of campesterol, stigmasterol and β-sitosterol.
[b] Sum of campestanol, stigmastenol and β-sitostanol.
[c] Average ± SE of both adrenals of 8 rats.
[d] Other minor sterols are not included.

Quantitative data of adrenal sterols are presented in Table 2. Cholesterol comprised approximately 94 % of the total, the rest being mainly due to plant sterols and cholestanol. The amount of Δ^7-cholestenol and other cholesterol precursors accounted for about 1 % of the total. Further, it is clear from Table 2 that if the cholestanol of fraction I is determined non-specifically it is overestimated by some 20 %, owing to the presence of 5α-plant sterols.

DISCUSSION

Plant sterols are assumed to be poorly absorbable compounds [11] and consequently their occurrence in the animal organism has not been established earlier. Moreover, their detection in the presence of a large excess of cholesterol is difficult by ordinary methods. The present paper shows that in the adrenals of rats on a regular diet plant sterols constitute about 5 % of total sterols. Some additional studies with rat and human serum, muscle and liver similarly showed the presence of small amounts of these sterols. This clearly indicates that plant sterols are absorbed to a small extent and are obviously distributed to all organs analogously with exchangeable cholesterol.

Determination of tissue cholestanol has been carried out by converting unsaturated sterols to 3β-, 5α-, 6β-triol derivatives [5] or to α- and β-epoxides.[6] In these procedures saturated sterols remain intact and could be separated chromatographically as less polar compounds from polar sterol derivatives. The final measurement of cholestanol has been unspecific, either colorimetric or isotopic. In the present procedure saturated sterols are separated and

isolated from unsaturated ones by means of TLC followed by analysis on GLC. The latter appears to be the only accurate technique for quantitating cholesterol or cholestanol in the presence of the respective plant sterol series.[9,10] Consequently, owing to the presence of plant sterol stanols, earlier nonspecific methods for quantification of cholestanol may have overestimated this stanol by some 20 % in adrenals of rats on the ordinary laboratory diet. The question of whether plant sterol stanols are synthesized within the body from the corresponding unsaturated parent compounds in a manner analogous to the synthesis of cholestanol from cholesterol or whether they originate from dietary stanols remains unsolved.

The significance of cholestanol and plant sterols in the adrenals is not known, although cholestanol may influence the synthesis of steroid hormones.[12] That cholestanol and plant sterols may not be readily utilized for this purpose was suggested by our preliminary observations [8] that in rats subjected to stress at a low temperature adrenal cholesterol decreased markedly, and Δ^7-cholestenol decreased slightly, whereas the cholestanol and plant sterol levels remained unchanged.

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