Fractionation of Serum and Skin Sterol Esters and Skin Waxes with Chromatography on Silica Gel impregnated with Silver Nitrate *

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Separation of lipid material to fractions differing on carbon-carbon unsaturation and molecular geometry has recently been obtained with both column and thin layer chromatography using silver nitrate impregnated silica gel as adsorbent [1-2]. We have applied the new method for further characterization of the skin surface waxes and sterol esters and for the comparison of the sterol esters of serum and skin surface.

The isolation of the skin surface wax and sterol ester fractions was described in our previous report [3]. The serum sterol esters were isolated from freshly lyophilized human serum by extraction with chloroform-methanol 2:1 (v/v), purification of the extract [4] and subsequent chromatography in silicic acid column [5]. After eluting the column with 2 % benzene in hexane, an eluate obtained with 20 % benzene in hexane was collected. The latter fraction was uniform with respect to sterol esters

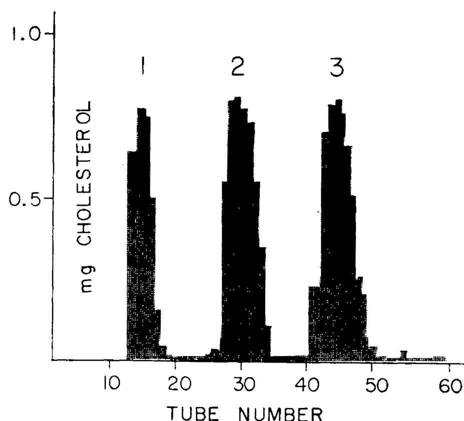

Fig. 1. Fractionation of serum sterol esters in $AgNO_3$-silica gel column. *Eluant:* Benzene-hexane from 1:9 to 7:3. Cholesterol was determined by the Tshugaeff reaction.

1. *Preparative fractionation of serum sterol esters in $AgNO_3$-silicic acid column.* Twenty grams of washed and size graded when chromatographed on thin layer plates of Silica Gel G (Merck) using 10 % benzene in hexane as solvent.
(100 – 150 mesh) silicic acid (Mallinckrodt) were slurried in 100 ml of 20 % (w/v) aqueous silver nitrate solution. The excess of the solution was removed with suction on glass filter. The silicic acid was then dried *in vacuo* at 70°C for 3 h and slurried in hexane. Chromatography columns, 10×120 mm in size, were prepared in conventional manner. The column was protected from light with black tape.

About 40 mg of the serum sterol esters, dissolved in 1 ml of hexane, were pipetted on top of the column. After several washes of the column walls with hexane, a continuous concentration gradient from 10 to 70 % benzene in hexane was applied according to Hirsch and Ahrens.[6] The fraction collector tubes, 70 drops in each, were analyzed with the Tshugaeff reaction for sterol [7]. A clear separation into three main fractions was obtained, as seen in Fig. 1.

The three sterol ester fractions obtained and a sample of the unfractionated serum sterol esters were transesterified in 4 % sulphuric acid in methanol at 70°C. The fatty acid methyl esters were isolated in the usual manner [5] and examined in a gas chromatograph at 180°C using a 4 ft $\times$ 4 mm (i.d.) glass column packed with 10 % ethylene glycol adipate polyester on 100 – 120 mesh siliconized Gas-Chrom P (Applied Science Laboratories, Pa.). An argon ionization detector of the "small Lovelock" type was used. The carrier gas pressure was 12 p.s.i. The samples were analyzed as such and with an added internal standard consisting of methyl myristate, palmitate and stearate.

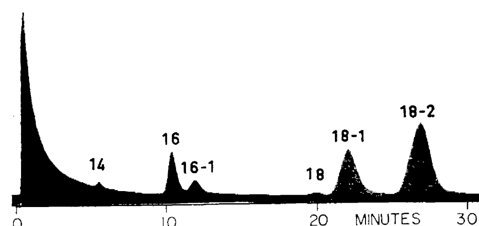

Fig. 2. Fatty acids of the total sterol esters of human serum. Conditions of gas-chromatography: See text.

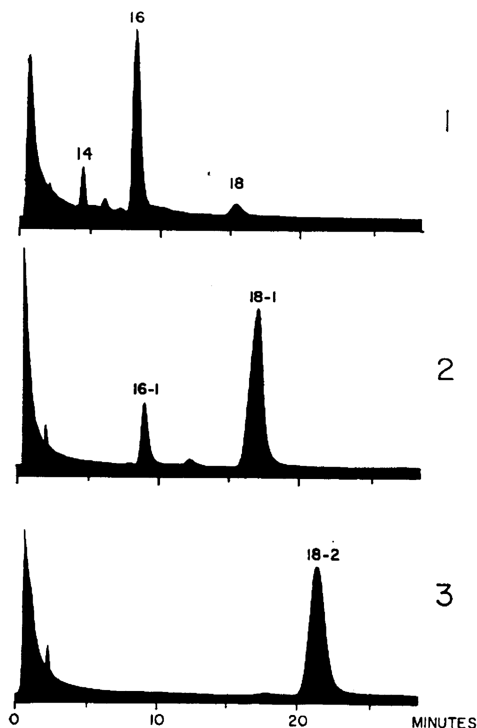

Fig. 3. Fatty acids of isolated serum sterol ester fractions 1, 2 and 3 as illustrated in Fig. 1. Conditions of gaschromatography: See text.

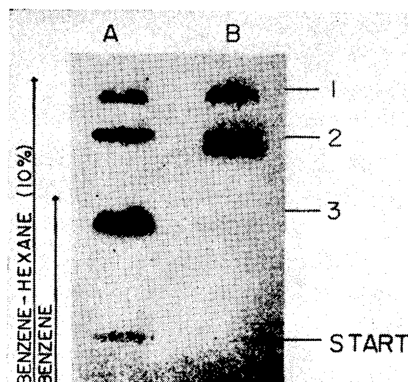

Fig. 4. Thin layer chromatography of serum (A) and skin (B) sterol esters in $AgNO_3$-silica gel.

Fig. 2 shows the gas-chromatogram of fatty acid methyl esters derived from the total serum sterol esters and Fig. 3 of those derived from the fractions 1, 2 and 3 of the $AgNO_3$-silica gel chromatography (Fig. 1). It is clearly seen that fractions 1, 2 and 3 correspond to saturated, monounsaturated and diunsaturated esters, respectively, which are known to be the main sterol ester fractions of serum [8-10]. The order of elution corresponds to that obtained by Klein and Janssen [10] with a specially treated silicic acid. No other polyunsaturated fractions can be detected, which may be due to incomplete elution or decomposition.

2. *Thin layer chromatography on $AgNO_3$-Silica Gel G-plates.* The plates were prepared according to de Vries [2]. After preparation the plates were kept for about 30 min in the dark at room temperature and dried *in vacuo* at 70°C for 2 h. The samples were then rapidly pipetted on the cooled plates and chromatograms were run in the usual manner at room temperature using benzene and 10 % benzene-hexane as solvent. The plates were then dried, sprayed with potassium bichromate-sulphuric acid and treated for 30 min at 125°C. Photographs were taken immediately. (About 10—20 min after the removal of the plates from the oven the surface of the support tends to break). Fig. 4 illustrates the $AgNO_3$-silica gel thin layer chromatographic patterns of about 50 μg of serum sterol esters (Fig. 4 A) and skin surface sterol esters (Fig. 4 B). The fractions obtained from serum sterol esters are identical to those obtained by column chromatography (Fig. 1). The skin surface sterol esters are separated to a saturated (1) and a monounsaturated (2) fraction followed closely by an unknown unsaturated fraction. It differs thus from

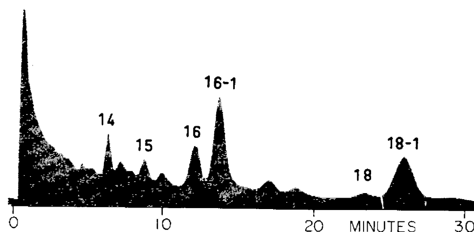

Fig. 5. Fatty acids of skin sterol esters. Compare with Fig. 2. Conditions of gas-chromatography: See text.

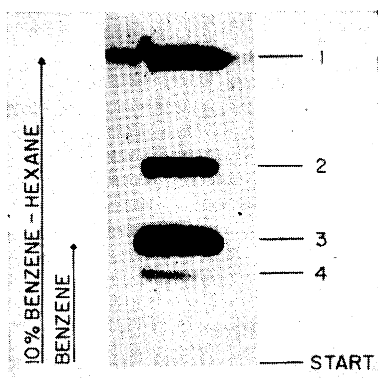

Fig. 6. Thin layer chromatography of skin surface waxes on $AgNO_3$-silica gel.

the serum sterol esters by the complete absence of the diunsaturated fraction. This difference is revealed also by the gas chromatograms of the fatty acids of skin sterol esters (Fig. 5) where no diunsaturated acids are seen. In general the skin sterol fatty acids are similar to other skin surface fatty acids, with pronounced branched chain fatty acid peaks at $C_{14}-C_{16}$[5].

Most of the fatty acids of the skin surface originate from the sebaceous excretion, but the sterol esters are claimed to originate from the keratinizing epidermis [11]. However, it now seems that the sterol ester fatty acids are very similar to those produced mostly by the sebaceous glands.

Fig. 6 illustrates the $AgNO_3$-silica gel thin layer chromatographic pattern of skin surface waxes, where four fractions are seen. The comparison to simultaneous chromatography of authentic straight and branched-chain saturated wax esters as well as mono- and dienoic wax esters prove that these fractions correspond to (1) saturated, (2) mono-, (3) di- and (4) some unknown polyunsaturated waxes.

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