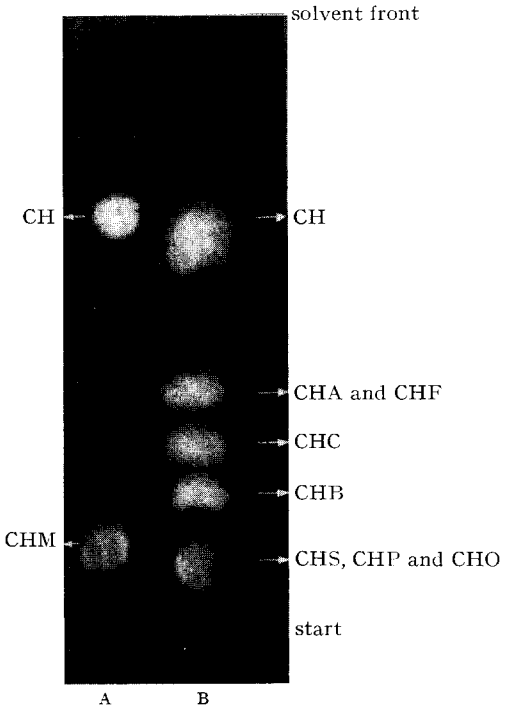
The nature of cholesterol esters in human blood serum

In a previous study we described a chromatographic method for separating cholesterol and its esters on paper[1]. In the present paper we report about our further experiments and applications of this method with biological material.

The nature of cholesterol esters of human blood serum was investigated by several authors and it was shown that they contain cholesterol combined with higher fatty acids, *e.g.* palmitic oleic and linoleic acid[2].

Paper chromatography

A strip of Whatman's No. 3 paper (16 × 24 cm) was dipped into a solution of 10% paraffin oil in ether (v/v). After the evaporation of the solute 200 γ of cholesterol and its esters in chloroform were applied at the starting line (2 cm from one end of the paper). The chromatography was carried by ascending technique using the system, acetic acid–chloroform–paraffin oil, 65:25:10 (v/v), as mobile phase. After 12–15 h at about 18–20° C the mobile phase ran on to the bottom of the paper. The chromatogram was then removed and dried at 70–100° C.



Detection

(1) The chromatogram was dipped into 10% (w/v) phosphotungstic acid in ethanol and heated at about 100–110° C until the purple spots developed (about 5 min).

(2) The chromatogram was immersed into 20% (v/v) sulphuric acid in acetic anhydride between two glass plates. In about 20 min the green spots developed.

Fig. 1. Paper chromatogram of cholesterol and cholesterol esters. Ascending chromatography as described. A: Blood serum: CHM, mixture of cholesterol esters with higher fatty acids; CH, free cholesterol. B: Standards: CHS, CHP, CHO, cholesterol stearate, cholesterol palmitate, cholesterol oleate; CHC, cholesterol capronate; CHB, cholesterol butyrate; CHA, CHF, cholesterol acetate, cholesterol formate; CH, cholesterol.

TABLE I

R_F VALUES

Time: 15 h; solvent front: 20.4 cm; temperature: 18–20° C

Cholesterol	0.65	Cholesterol capronate	0.22
Cholesterol formate	0.40	Cholesterol palmitate	0.10
Cholesterol acetate	0.40	Cholesterol stearate	0.10
Cholesterol butyrate	0.30	Cholesterol oleate	0.11

Cholesterol esters were prepared according to PAGE AND RUDY[3] or SWELL AND TREDWELL[4].

Isolation of cholesterol and its esters from blood serum

2 ml of serum were mixed with 20 ml of ethanol/ether 3:1 (v/v). The filtered extract was evaporated on a water bath to dryness and the lipids were reextracted 4 times with 5 ml quantities of light petroleum (b.p. 40–60°). After the evaporation of light petroleum the residue was dissolved in chloroform (0.2 ml). For the paper chromatography 0.02 ml of this extract was applied to the paper.

Elution of cholesterol and esters from paper chromatograms

After the separation the dried chromatogram was out into strips 1 cm wide. The strips were eluated with chloroform. After standing for 24 h at room temperature with occasional stirring the Liebermann-Burchard reaction was carried out in the eluates. From the values obtained it is possible to calculate the ratio of free cholesterol/esters. The results are in good agreement with those obtained in the quantitative methods[5] (Fig. 2).

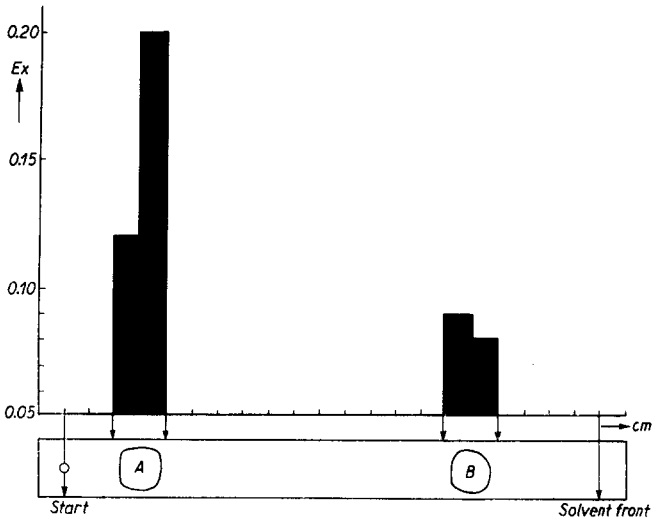


Fig. 2. Elution of cholesterol esters and free cholesterol of human blood serum from paper chromatogram. A: cholesterol esters; B: free cholesterol.

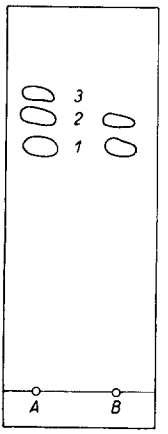


Fig. 3. Paper chromatogram of higher fatty acids in cholesterol esters of serum. Ascending chromatography as described. A: Standards (from bottom of scheme upwards): 1, palmitic and oleic acid; 2, linoleic; 3, linolenic. B: Higher acids of cholesterol esters of human blood serum: 1, palmitic and oleic acid; 2, linoleic.

Paper chromatography of higher fatty acids isolated from cholesterol esters was carried out on Whatman's paper No. 3 impregnated with 10% paraffin oil in ether (v/v) and acetic acid as mobile phase. The spots are developed by the method of KAUFMANN AND NITSCH[6]. As shown in Fig. 3 it can be postulated that cholesterol esters of human blood serum probably contain palmitic, oleic and linoleic acid.

Further experiments are in progress and will be published later.

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Reversible changes in bacteriochlorophyll in purple bacteria upon illumination

Illumination was found to bring about a reversible change in the absorption spectrum of a suspension of purple bacteria. The change was measured by means of a sensitive differential spectrophotometer. We constructed this spectrophotometer—previously described[1]—for the measurement of small changes in the absorption spectrum of light-scattering suspensions upon illumination.

The absorption vessel was a glass cylinder of 5 cm length and 2 cm diameter, sealed at