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Chromatographic one step extraction and purification of lipids from microvolumes of plasma*

Existing procedures of lipid extraction from plasma vary in efficacy[1,2] but they all consist of several steps such as dispersion in the solvent, filtration, evaporation, etc. Where lipid purity is of essence further procedural steps are usually required[3,4]. For these reasons, perhaps, we encountered considerable lipid losses in dealing with rabbit plasma volumes of under 100 μl. We have, therefore, devised and used successfully for several years a simple one step procedure reported briefly before[5] of extraction and purification of lipids from small volumes of plasma. It is based on the same principle as our previously described method for the removal of non-lipid contaminants from lipid extracts[6]. We now report the procedure in detail as applied to rabbit plasma lipids. We demonstrate good recovery in comparison with BLOOR's method of extraction[7] and purity of the extract.

Materials

Chromatographically pure *o*-phosphoethanolamine was obtained from Calbiochem, Los Angeles, Calif.: DL-*o*-phosphoserine from Mann Research Inc., New York; silicic acid (Bio-Sil Ha, minus 325 mesh) from Bio Rad Labs, Richmond, Calif. Reagents were of analytical grade (Fisher Scientific, N.Y.) but not redistilled.

Methods

Silicated Whatman No. 40 papers, 14 in. × 4 1/2 in. were prepared as described before[6]. Since paper batches differ it may be advantageous to prerun the strips in the same paper chromatographic system as that used for the extraction procedure (see below). After the prerun the eluate contained no phosphorus, no cholesterol and about 50 μg of glyceride (subtracted in calculations).

Venous blood was obtained from White New Zealand female rabbits kept on standard pellets and anticoagulated with ethylenediaminetetraacetate disodiumsalt (EDTA), 5 mg/1 ml blood. Plasma separated by centrifugation for 30 min at 3000 r.p.m. (1500 G) was subjected to lipid extraction immediately or it was kept frozen at —20° until extraction.

Procedure

One half ml of rabbit plasma was streaked in a narrow line across each paper 1 in. below the syphon rod fold. The paper was allowed to dry in air and it was then placed in a glass jar 12 in. × 12 in. × 24 in. as for descending paper chromatography. A small beaker was placed under the tapered end of the paper. The trough was filled with 20% methanol in chloroform which was also used for saturating the atmosphere of the jar. The chromatogram was run overnight. Pure lipids were eluted into the beaker in 10–30 ml of the solvent. Other plasma constituents remained adsorbed to the paper. Analyses performed on the lipid eluates were: phosphorus[8], total cholesterol[9], non-phospholipid glycerol[10], acid hydrolysis for amino acid residues and inorganic

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salts[6]. Fatty acid analysis of total lipid extracts by gas–liquid chromatography was kindly performed by Dr. AARON MARCUS of the Veterans' Administration Hospital, New York City, N.Y.

BLOOR's extract[7] was obtained by dropwise addition of 2 ml plasma to 40 ml ethyl alcohol: diethyl ether (3:1) followed by agitation at 40° for 20 min. The filtrate was brought to dryness in a rotating evaporator and taken up in chloroform.

Recovery

It can be seen from Table I that lipid recovery by the chromatographic procedure was equal to, or better than, that by BLOOR's method. The significantly higher cholesterol values were confirmed by direct cholesterol determination on plasma. Significant fatty acid degenerative changes were absent (Table II).

TABLE I

RABBIT PLASMA LIPIDS

	Bloor	*Chrom.*	*Diff.*	*Probability**
Lipid P	23.2 ± 0.9**	24.5 ± 0.9	+ 1.3	$0.2 > P > 0.1$
Total cholesterol	326.6 ± 23.0	373.4 ± 26.5	+46.8	$P < 0.001$
Non-phospholipid glycerol	246.8 ± 17.8	260.8 ± 22.0	+14.0	$0.4 > P > 0.3$

* For paired observations.
** All values in μg/ml plasma ± S.E.M. Averages from 4 paper and 2 BLOOR extractions.

TABLE II

FATTY ACIDS OF RABBIT PLASMA TOTAL LIPID EXTRACTS*

Fatty acid	*Bloor*	*Chrom.*	*Diff.*	*Fatty acid*	*Bloor*	*Chrom.*	*Diff.*
12:0	0.11**	0.17	+0.06	17:0	0.71	0.56	−0.15
14:0	1.67	1.96	+0.29	?	0.00	0.21	+0.21
15:0	0.43	0.64	+0.21	18:0	6.39	5.84	−0.55
16:0 DMA	0.23	0.30	+0.07	18:1	25.30	24.60	−0.70
16:0	43.50	43.80	+0.30	18:2	14.63	14.07	−0.56
16:1	4.95	5.31	+0.36	18:3	1.51	2.01	+0.50
				20:4	0.63	0.62	−0.01

* Determined by Dr. A. MARCUS, Veterans' Administration Hospital, New York City, N.Y.
** Values (in %) are averages of same three plasmas.

We used the procedure successfully in preparation of lipids for quantitation of rabbit[11] and human[12] plasma phospholipids. Good total phospholipid recovery was further confirmed by addition and complete recovery of 100,000 c.p.m. of ^{32}P phospholipids in plasma. Good recovery of other lipid classes was confirmed by thin layer chromatography of non-polar lipids[13].

Purification

Inorganic salt analysis on extracts from 1 ml of plasma showed absence of phosphate, chloride and potassium. Sodium was present to the extent of 2.3 μg. Amino acid paper chromatography of rabbit and human plasma lipid hydrolysates showed only presence of serine, ethanolamine and their phosphoderivatives (Fig. 1). Total lipid extracts which migrated with the solvent front were the only ninhydrin staining spots.

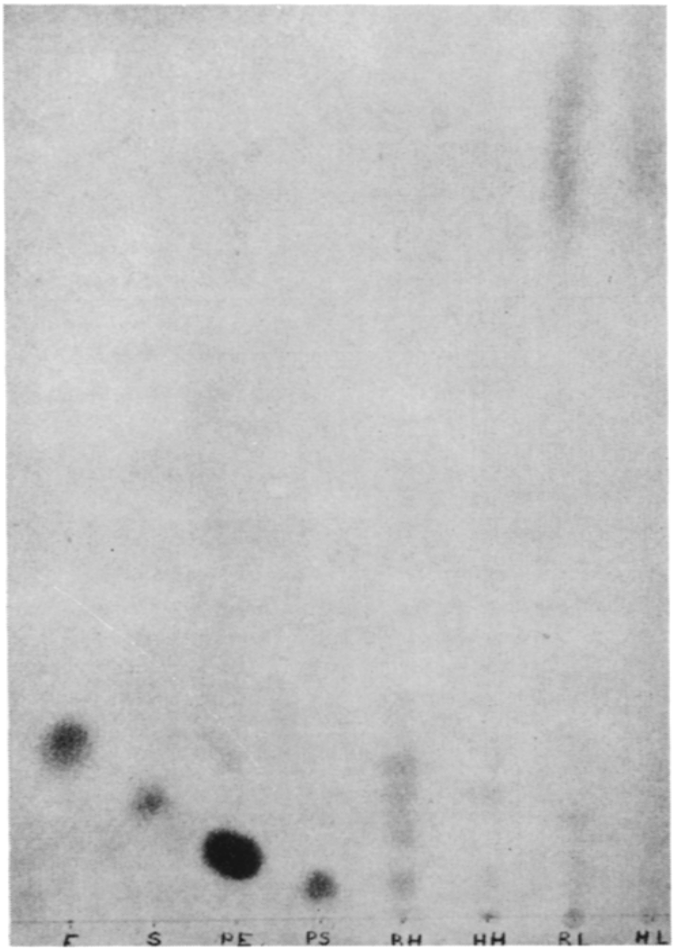

Fig. 1. Amino-acid paper chromatography of lipid extracts and their hydrolysates stained with ninhydrin. (E) = Ethanolamine; (S) = serine; (PE) = phosphoethanolamine; (PS) = phosphoserine; (RH) = hydrolysate of rabbit plasma lipids; (HH) = hydrolysate of human plasma lipids; (RL) = rabbit plasma lipids; (HL) = human plasma lipids. Extracts of 1 ml plasma applied.

Removal of non-lipid radioactivity was checked by addition to plasma of ^{32}P phosphate and of ^{14}C glycerol. No radioactivity was found in the lipid eluate of 0.5 ml of plasma to which 500,000 c.p.m. of ^{32}P phosphate were added. Five per cent of added 100,000 counts of ^{14}C glycerol was found in the eluate.

Comment

The method offers several distinctive features. The greatest asset appears to be good recovery from micro-volumes of plasma. By using narrower strips of paper of the same length we extracted as little as 10 μl of plasma without loss. On the other hand,

by using the more absorbent S & S No. 589 Green Ribbon C paper, we successfully extracted 2 ml of plasma from each 14 in. × 41/2 in. strip. Another advantage is economy of technician's time in case of multiple samples. As many as 36 narrow strips can be easily placed in one jar. The inconvenience of paper silication is only real when the procedure is seldom used.

If collection beakers are tared determination of total lipid by weighing may be conveniently done by evaporation of solvent without further transfers. This may be particularly useful clinically in pediatric work.

We were not able to detect any significant lipid degradation but a more detailed analysis might have revealed some alterations such as transesterification. On the other hand drying of samples before extraction and an essentially unhydrous atmosphere of the jar would tend to reduce degradatory process. The lipid extract did not seem to contain non-lipid plasma elements but minute amounts of material extractable from paper itselfa ppeared in the eluate. This was practically eliminated by the prerun.

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Biochemistry of sphingolipids

XXI. Separation of dinitrophenyl derivatives of long-chain bases by reaction paper chromatography

Thin-layer and paper chromatographic separations of DNP (dinitrophenyl)-derivatives of long-chain bases and their degradation products* originating from different hydrolytic conditions have been reported in our previous papers[1–5].

* The names of the long-chain bases and their degradation products are presented in the form of the semi-systematic nomenclature used up to the present time, and also in the terms proposed by IUPAC-IUB Commission on Biochemical Nomenclature[12].