The first solvent served to isolate the cholesterol esters and triglycerides in a position near the top of the plate. The second solvent served to separate cholesterol and the two diglyceride isomers. Phospholipids remain at the origin.

A typical separation is shown in Fig. 1.

This technique was used to chromatograph lipid extracts of serum enzyme digests of ^{14}C-labeled lipid preparations. It was thus possible to observe the localization and appearance of reaction products with considerable accuracy.

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they are set free by hydrolysis and then isolated by extraction from the hydrolysate. The samples to be spotted on the plates were prepared in the following way: Urine acidified with HCl (5:1) is hydrolysed for 1 h on a boiling water bath. After filtration an aliquot of the hydrolysate is extracted 3 times by means of a mixture consisting of 4 parts of petrol ether and 1 part of ethyl ether. A minimum of urine pigments pass into the ether layer. A residue containing a small quantity of urine pigments is obtained by evaporation of the solvents, from the bulked extracts on a water bath. To further extracts from 20 ml of urine is added 0.02 ml of an acetone solution containing either 5 μg PNP or 10 μg PNMC. A mixture of 20% acetone and 80% *n*-hexane proved to be the best solvent system for chromatography. Complete separation of PNP and PNMC from urine coextracts was reached at a distance of 15–16 cm.

Detection of the spots is by either ultraviolet light of by the action of ammonia vapour. In the first case PNP and PNMC quench the fluorescence (giving black spots), and in the latter case give lemon-yellow spots. Coextracts from urine give a yellow-brown colour in an ammonical medium and intensely fluorescent spots in ultraviolet light.

Fig. 1 shows a chromatogram in which the spots were detected by ammonia vapour. On the starting line the spots are spotted in the following order: 1 = urine extract with PNP (5 μg); 2 = standard PNP (5 μg); 3 = urine extract; 4 = standard PNMC (10 μg); 5 = urine extract with PNMC (10 μg). It can be seen from Fig. 1 that the spots of PNP and PNMC are well separated from the almost continuous line of coextracts.

In Table I the R_F values of components of the chromatogram detected by ultraviolet light are given. It is obvious from the table that although urine coextracts are a variable mixture of compounds they do not interfere with PNP and PNMC.

However, as can be seen from Fig. 2, PNP and PNMC are difficult to separate from each other in the system used, their R_F values being too close; this has already

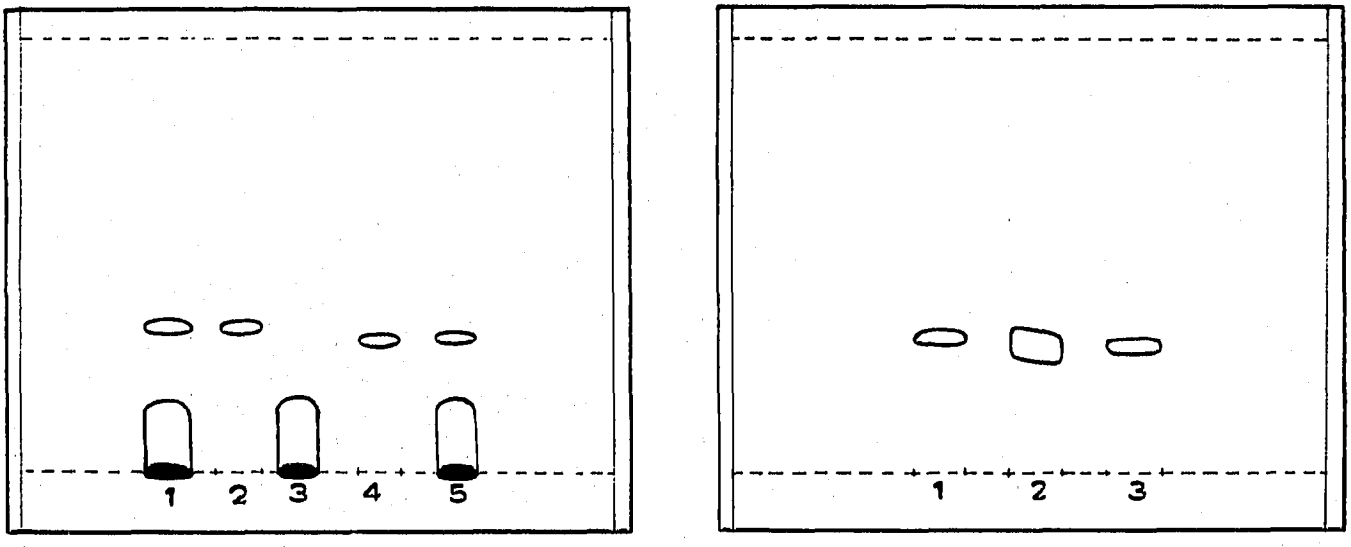

Fig. 1. Separation of PNMC and PNP from urine pigments on a thin layer of silica gel. Solvent: 20% acetone and 80% *n*-hexane. 1 = urine extract and PNP (5 μg); 2 = standard solution PNP (5 μg); 3 = urine extract; 4 = standard PNMC (10 μg); 5 = urine extract and PNMC (10 μg).

Fig. 2. Separation of PNMC and PNP on a thin layer of silica gel. Solvent: 20% acetone and 80% *n*-hexane. 1 = PNP; 2 = mixture of PNP and PNMC; 3 = PNMC.

TABLE I

R_F VALUES OF COMPONENTS DETECTED IN ULTRAVIOLET LIGHT

R_F values of spots					
Extract from urine with PNP	*PNP*	*Extract from urine*	*PNMC*	*Extract from urine with PNMC*	*Colour of fluorescing spot*
origin		origin		origin	yellow
0.03		0.03		0.03	blue
0.05		0.05		0.05	pink
0.08		0.08		0.08	blue
0.12		0.12		0.12	blue
0.33	0.33		0.31	0.31	black
0.84		0.84		0.84	white
front		front		front	bluish

been mentioned by GASPARIČ[14]. The presence of PNP may still be discerned at a level of 0.15 μg and PNMC at 0.25 μg. Similar results are obtained in the analysis of urines of experimental animals given Parathion or Metathion.

The developing systems described by other authors for the separation of phenolic compounds[15–18] did not prove to be successful in our case, *i.e.* for the separation of PNMC and PNP from urine extracts.

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