Results. In many cases it was possible to separate 1 cation from numerous metal ions easily and rapidly. Such results are summarized in Table I. Many important and difficult separations have been achieved practically and are given in Table II. Instead of simply giving Rf values of the cation the R_T (rear limit) and R_L (front limit) are given to have clear picture of the spot.

Discussion. It is clear from the results summarized in Tables I and II that titanic tungstate and titanic molybdate are good ion-exchangers. Papers impregnated with these ion-exchangers achieve fast, selective and specific separations of metal ions. Difficult separations: to mention a few Mo^{+6}-V^{+4}-UO_2^{+2}, Pb^{+2}-Sn^{+4}, Mn^{+2}-Zn^{+2}, and Zr^{+4}-Th^{+4} etc. have been achieved clearly and rapidly.

Selective separation of 1 metal ion from numerous metal ions has also been achieved with ease. Very few direct methods are available which separate beryllium from the majority of elements in a single step. Be^{+2} can be easily separated from 41 cations including Ag^+, Al^{+3}, Au^{+3} and Ga^{+3} (which interfere in beryllium determination) on titanic molybdate papers using HCl + *n*-butanol (3:7) as developer. Fast separation of zirconium can also be achieved on these papers from 35 cations (Table I) including Fe^{+3}, Th^{+4}, Sb^{+3}, Mo^{+6}, Ge^{+4}, Cr^{+3} and Ga^{+3}. These are the cations that interfere most in zirconium determination. This is probably the best separation of Zr^{+4} from numerous metal ions yet reported[7].

Zusammenfassung. Mit anorganischen Ionenaustauchern Titanwolframat und -molybdat imprägnierte Papiere wurden zur Chromatographie mehrerer Metallionen in verschiedenen wässrigen, nichtwässrigen und gemischten Lösungsmitteln verwendet. Trennungen einer Anzahl von ternären und binären Gemischen wurden damit erzielt.

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Quantitative Determination of Phospholipids

The quantitative determination of tissue phospholipids after their separation by thin layer chromatography (TLC) has been reported from several laboratories[1-3]. The major differences and similarities of these methods are summarized in Table I. It may be noted that in the methods reported the sensitivity range of phospholipid phosphorus determinations were comparable. There were differences in the choice of absorbent, solvent systems, and detection reagents. The greatest variation is in the time required for digestion of sample, for instance, digested for 20 min by ROUSER et al.[6] as compared with 4 h by PARKER and PETERSON[5].

The existing methods for tissue phospholipids determinations were found to be tedious where a large number of samples had to be determined. This paper describes a simple procedure which allows determination of several samples while still retaining a high degree of sensitivity and accuracy.

Methods. Glass plates (20 × 20 cm) were coated with a 0.3 mm layer of Silica Gel G (Merck) using the apparatus and methods described by CHAHL and KRATZING[9]. Liver lipid solutions usually containing up to 30 µg of phospholipid phosphorus were applied onto each lane by means of the multisample applicator of CHAHL and KRATZING[10]. They were then immediately placed in a light-proof tank

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Table I. Comparisons of methods and conditions for separation and quantitative determination of phospholipids using TLC techniques

Tissue	Silica Gel	Dimension	Detection reagent	Treatment of sample[a]	Digestion time	P in sample (µg)	Reference
Human CSF	G	1	H_2SO_4	D	3 h	0.5–5.0	1
Human serum (same procedure as 1)							2
Rat thymus	G	2	Iodine vapour	Ex	15 min	3.1–6.2	3
Rat liver	H	1	Iodine vapour	E	3 h	0.2–5.0	4
Rat liver	H[b]	1	Iodine vapour	E	4 h	1.85–7.17	5
Beef brain	H	2 × 2	Iodine vapour	D	20 min	0.07–2.40	6

[a] D P/L determined in presence of adsorbent Ex P/L extracted before P determination E P/L eluted before P determination. [b] Adsorbent washed before use.

which was flushed with a continuous stream of dry N_2 to evaporate the solvents. The plates were developed in chloroform:methanol:water, 65:25:4, by volume. The separated components were identified by exposure to iodine vapour. For quantitative estimation, areas of silica gel containing the phospholipids were scraped into 25 ml Kjeldahl flasks and treated according to ROUSER et al.[6].

Aqueous solutions of Na_2HPO_4 (Analar grade) containing 100 µg P/ml, were used as standards. To construct standard curves, aliquots containing 0–30 µg P were delivered into Kjeldahl digestion flasks and treated as experimental samples. The effect of silica gel on standard samples was evaluated by adding 6 cm^2 of the adsorbent from a prepared TLC glass plate into the digestion tube, prior to commencing digestion.

Experiments and results. a) *Separation and identification of phospholipids.* It was found necessary to allow the solvent system to equilibrate for 6 h before use, in order to get discrete separations (Figure 1).

The separated phospholipid components were identified by reference to published results[11] and by paper chromatography of the glyceryl phosphoryl bases[12]. The liver phospholipid species separated by TLC were eluted from the adsorbent with chloroform:methanol, 2:1, and hydrolyzed to obtain the glyceryl phosphoryl bases[12] before their development on paper chromatograms.

b) *Quantitative determination.* 1. Standard solution. Phosphorus determination with and without silica gel present were identical and were directly proportional to the optical density over the range 0–20 µg (Figure 2).

Table II. Quantitative determination of phospholipids

	Total amount of lipid applied in mg		
	1.25	2.5	5.0
Phosphatidyl ethanolamine	5.5	11.0	22.0
Phosphatidyl choline	11.8	21.5	[b]
Sphingomyelin	3.0	5.0	9.0
Phosphatidyl inositol	3.8	8.5	16.0
Other phospholipids[a]	4.0	9.8	18.0
Total P in sample µg	28.1	55.8	–
Total P in µg/ml of solution[c]	562	558	–

[a] Phospholipids remaining at the origin during TLC separation. [b] Sample too concentrated for determination. [c] Total phosphorus value in lipid sample by addition of values for separated components. Rat liver lipid solution (25 mg/ml of chloroform: methanol, 2:1, by volume) was applied to a TLC plate. Phospholipids were separated, identified and determined quantitatively as described in the text.

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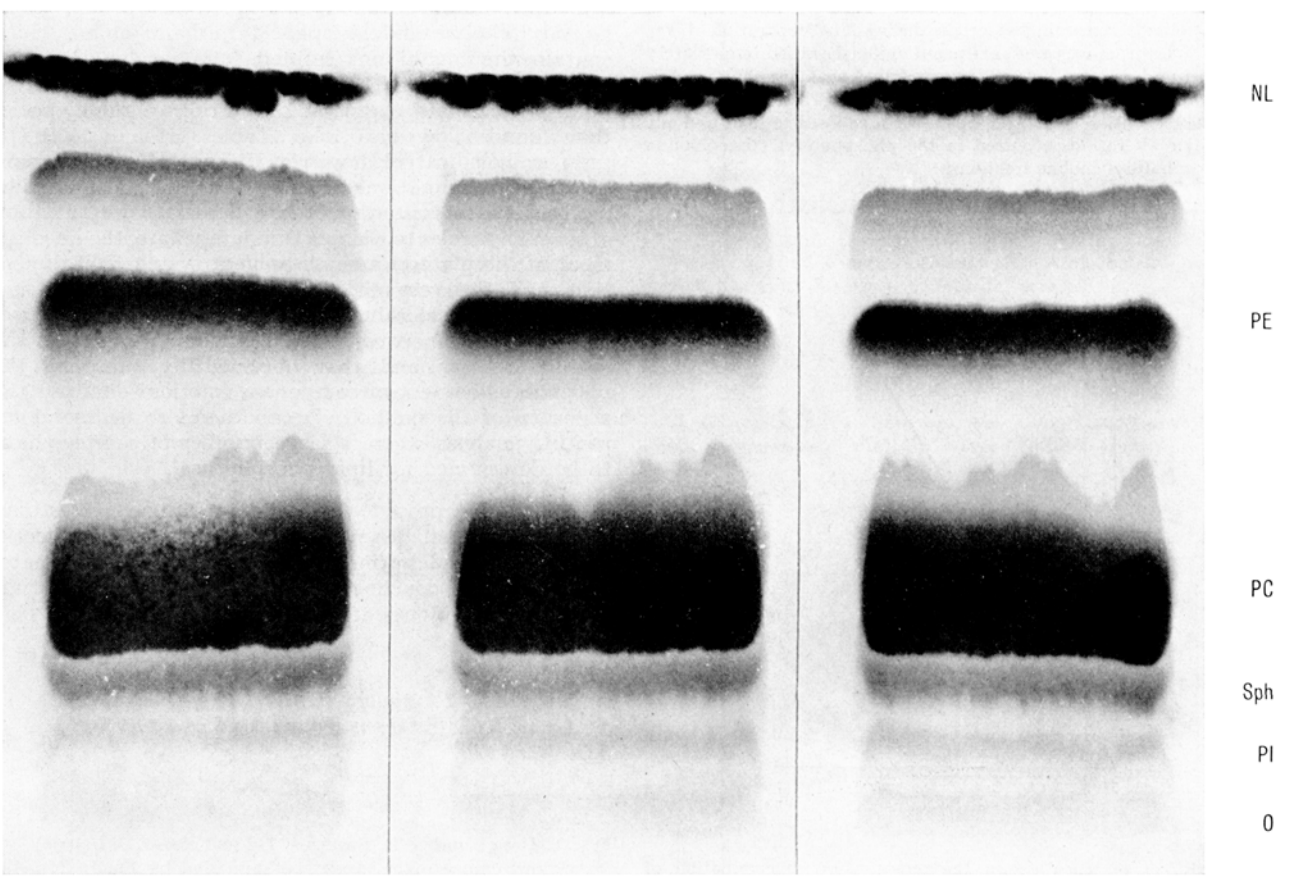

Fig. 1. Liver phospholipid separation. The solvent tank containing the solvent mixture was allowed to equilibrate for 6 h before TLC separation of P/L was commenced. The separation of major P/L species was improved with such treatment. NL, neutral lipids; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; Sph, sphingomyelin; PI, phosphatidyl inositol; O, origin.

Table III. Quantitative determination of phospholipids in duplicate samples

Phospholipid	P (μg)				Total P/L (%)			
	Rat 1		Rat 2		Rat 1		Rat 2	
Phosphatidyl ethanolamine	4.5	4.5	6.0	6.75	16.6	16.5	16.6	17.9
Phosphatidyl choline	8.75	8.70	10.75	11.0	30.3	29.5	29.2	30.3
Sphingomyelin	4.2	3.5	4.8	5.0	14.5	12.8	12.1	13.8
Phosphatidyl inositol	5.75	6.0	8.75	8.75	19.9	21.1	23.8	21.4
Other phospholipids[a]	5.5	5.5	5.5	5.0	10.0	10.0	17.9	16.6

[a] Phospholipids remaining at origin during TLC separation. Duplicate samples of liver lipids, Rat 1, 1.81 mg and Rat 2, 2.49 mg were separated for phospholipids and quantitatively estimated for phosphorus. Duplicate determination showed good agreement and the percentage distribution of various phospholipid substances in the 2 rats also showed a close agreement.

Table IV. Quantitative determination of phospholipids with minimum oxidation

Phospholipid (% of total)	Rat		
	1	2	3
Phosphatidyl ethanolamine	25.20	26.0	26.60
Phosphatidyl choline	52.10	49.00	51.00
Sphingomyelin	11.25	12.50	11.80
Phosphatidyl inositol	6.51	7.05	5.29
Other phospholipids[a]	4.75	5.77	5.29

[a] Phospholipids remaining at origin during TLC separation. Liver lipid samples from rats were extracted and either used immediately or stored under N_2 at −18 °C. It was found that distribution of P was different from that reported in Table III. This discrepancy is due to deterioration of phospholipids when exposed to light and air. The major alterations occurred in the phosphatidyl ethanol mine and phosphatidyl choline fractions.

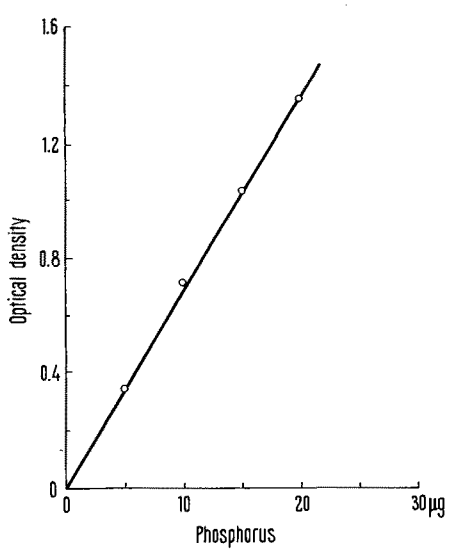

Fig. 2. Effect of silica gel in the quantitative determination of phosphorus. There was a direct proportionality between amounts of P, 0–20 μg, as the Na_2HPO_4, and the optical density of the final solution. This relationship was not affected by the presence of silica gel.

2. Liver phospholipids. A direct proportionality between the amount of lipid material applied on a TLC plate and the amount of phosphorus recovered in the different phospholipid species was demonstrated (Table II). The phosphorus determined in the original solution and that obtained by summing the phosphorus content of individual species after TLC separation agreed to within 96–100%.

In Table IV are shown the proportions of different liver phospholipid species when fresh liver lipid extracts were used. Phosphatidyl choline formed 50% of the total liver phospholipids and phosphatidyl ethanolamine about 25%.

Discussion. It has been reported that in the red blood cells the concentration of phospholipid species, phosphatidyl choline and phosphatidyl ethanolamine, which contain the highly unsaturated fatty acids, decreased when exposed to air[13]. In the present study fresh liver lipid extracts had a greater proportion of these species than found in aged samples (Tables III and IV). The presence of silica gel, however, did not affect the quantitative determination of phosphorus in phospholipids.

The simultaneous, rapid and quantitative application of several samples to a single thin layer plate, the development of the plate in a single solvent system in 1 dimension, and the very short digestion time of 20–30 min resulted in considerable saving of time. The good agreement obtained between total phosphorus values of the original sample and that obtained by summing the phosphorus content of component fractions confirms the accuracy of the method. It can therefore be useful for routine analysis where a large number of samples have to be determined for lipid phosphorus[14].

Résumé. Un procédé facile pour distinguer et estimer quantitativement des phospholipides du foie est décrit. Les phospholipides principaux sont séparés par chromatographie sur une couche mince et, dans la suite, l'on estime les produits toujours adsorbés sur le gel de silice.

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