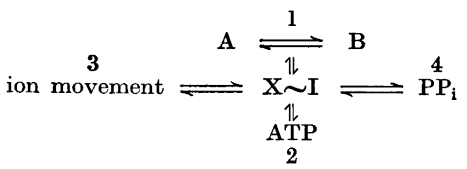

Scheme 2

Scheme 2 shows that, in principal agreement with the argument presented in connection with Scheme 1, PP_i has emerged as a fourth component part to be included in any consideration of the bioevolution of energy transformation in the systems under discussion. The simplicity of the structure of PP_i as compared to that of ATP, as well as the possible occurrence of PP_i containing minerals on the primitive Earth,[1] when taken together with the recently demonstrated metabolic position of PP_i, as shown in Scheme 2, would seem to us to provide sufficient background for a strong focussing of attention on the possibility that PP_i was an early participant in the sequences of energy transforming reactions evolving with time to the metabolically advanced level given in Scheme 2. Thus, for example, very early reactions of energy and phosphate metabolism may well have been those between PP_i and electron transport or between PP_i and ion movement.

In conclusion, while it would appear to be an open question, whether the main direction of early energy transfer between an energy-rich phosphate compound and, for example, electron transport was from electron transport to energy-rich phosphate or the reverse, it seems quite likely that the demonstrated electron transport coupled formation and utilization of PP_i in subcellular energy transfer systems may constitute experimental background for insight into early metabolic processes of major energetic significance for primitive forms of life, *i.e.* that PP_i indeed preceded ATP during the evolution of biological energy transformation.

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Received August 8, 1967.

Thin-layer Chromatography of Bile Acids on Lipophilic Sephadex

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In a previous paper the preparation of a methylated Sephadex derivative was described.[1] As thin-layer gel filtration is now an established technique,[2,3] the possibility of using methylated Sephadex in thin-layer chromatography (TLC) of lipids has been briefly investigated in this laboratory. Because of the volatility of most organic solvents, a closed developing chamber had to be used, in which the thin-layer plate also was kept during the sample application.

Experimental. Thin-layer plates are prepared on glass-plates, 5 × 20 cm, in the following way: 5 g of Sephadex G-25 superfine (Pharmacia, Uppsala, Sweden), methylated as described previously,[1] is mixed with 25 ml of chloroform or dioxane. The slurry is poured on the glass plate and spread into a thin layer with a glass rod. A proper thickness of the layer is obtained by having two stainless steel wires, 0.5 mm diameter, placed across the plate, parallel to and near the two long sides. The glass rod is pressed against the wires while the slurry is spread with a single movement. The plate is then allowed to dry for a few seconds until the gel surface seems dry but still swollen. The stainless steel wires are removed and the plate

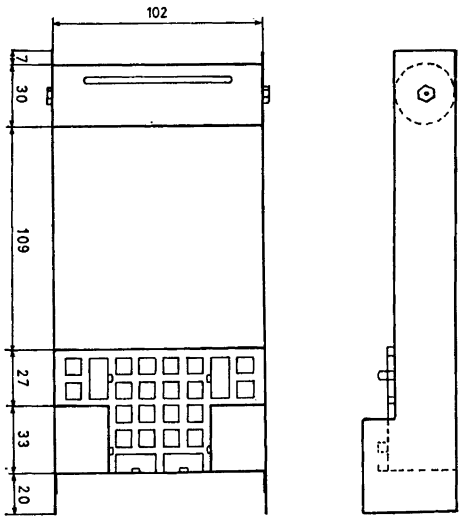

Fig. 1. Stainless steel frame, see text.

placed into the stainless steel frame shown in Fig. 1. At the top of the frame is a cylindrical solvent reservoir filled with the solvent to be used in the chromatography. The reservoir has a narrow slit opening, through which a filter paper strip, serving as a solvent bridge, is inserted. The other end of the strip is folded around the upper part of the thin-layer plate and kept in place with a short piece of teflon tubing, which has been slit open lengthwise. The frame is fitted into a glass jar, 275 × 120 × 55 mm. The front of the jar is covered with a glass plate, held in place with stainless steel clamps. The thin-layer plate is equilibrated for 2 h with descending solvent. The jar is then tilted so that the plate lies in a horizontal position, and the samples are injected with a Hamilton syringe (in 1 – 5 μl) onto the starting line through three silicone rubber stoppers fitted into three 6 mm holes in the covering glass plate. The jar is tilted to the appropriate angle (30° – 80° depending on the solvent used) and the plate is developed by a descending technique. Immediately after the development the damp plate is covered with a filter paper just soaked in chloroform. This paper is pressed against the moist gel layer by means of another glass plate. The paper with the transferred lipids is then sprayed with an appropriate reagent, 5 % phosphomolybdic acid in ethanol being suitable for the detection of the bile acids investigated in this experiment.

Results and discussion. Because of the volatility of the solvents used, exact information on the procedure of preparing the plates and transferring the lipids can only be gained by experience. It might be of advantage to check the transfer onto the filter paper by using a coloured substance. As the dry gel surface has a cracked appearance and is extremely fragile, the plate cannot be sprayed directly. By using the transfer technique for revealing the spots, the methylated Sephadex might also be collected and used in another experiment.

Some of the results are given in Table 1 and Fig. 2. As the molecular weights of the bile acids are relatively similar, no effort has been made to demonstrate the presence of a gel filtration effect. Rather, the solvent systems were designed to give a pronounced liquid-gel partition or adsorption effect. Thus, methylated Sephadex is known to swell strongly in solvents of medium polarity, such as dioxane and chloroform, whereas aliphatic hydrocarbons are not incorporated in the gel. A mixture of chloroform and heptane will give a straight phase liquid-gel partition system. Further-

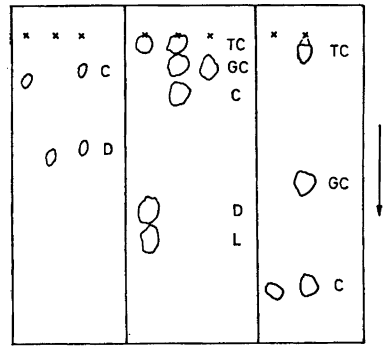

Fig. 2. Descending TLC of bile acids on methylated Sephadex G-25, superfine. *Left chromatogram.* Solvent system: chloroform 32, heptane 16, acetic acid 1 (v/v/v). Developed 2.5 h, angle 80°. *Middle chromatogram.* Solvent system: chloroform 66, 2,2,4-trimethylpentane 33, acetic acid 1. Developed 0.75 h, angle 80°. *Right chromatogram.* Solvent system: dioxane 24, 2,2,4-trimethylpentane 24, acetic acid 1. Developed 3.5 h, angle 30°. The samples, cholic (C), deoxycholic (D), lithocholic (L), glycocholic (GC) and taurocholic (TC) acids were applied in 4 μl of solvent. 4 μg of each substance were used.

Table 1. TLC mobilities of bile acids on methylated Sephadex G-25 superfine in different solvent systems.

Solvent	(v/v)	Angle[a]	Time (h)	TC[b]	Distance in mm from origin			
					GC	C	D	L
Chloroform	2	80°	10.5	—	—	17	67	—
Heptane	1							
Chloroform	33	80°	2.5	—	—	16	50	—
Heptane	16							
Acetic acid	1							
Chloroform	39	80°	0.9	—	12	23	43	58
Heptane	10							
Acetic acid	1							
Chloroform	66	80°	1.0	4	13	25	76	89
TMP[c]	33							
Acetic acid	1							
Chloroform	66	80°	4.0	13	45	89	—	—
TMP	33							
Acetic acid	1							
Dioxane	25	30°	3.5	10	65	109	—	—
TMP	25							
Acetic aci	1							

[a] Angle between the thin-layer plate and the horizontal plane.
[b] TC = taurocholic acid; GC = glycocholic acid; C = cholic acid; D = deoxycholic acid; L = lithocholic acid.
[c] TMP = 2,2,4-trimethylpentane.

more, the presence of adsorption to the gel network is indicated by the greatly increased mobility of polar compounds when small amounts of acetic acid are added to neutral solvent systems.

Solvent systems giving efficient separations of bile acids and bile acid conjugates in TLC on silica gel have been given by Eneroth [4] and Hofman.[5] TLC on lipophilic Sephadex does not seem to offer any advantages over silica gel TLC as far as separation of bile acid isomers is concerned. Nevertheless, TLC on lipophilic Sephadex might be a useful complementary technique. Thus, free bile acids can be chromatographed in neutral solvent systems without tailing. The inert nature of the methylated Sephadex makes it possible to separate labile compounds. Column experiments indicate that recovery of compounds from the thin-layers should be complete. Finally it is suggested that TLC on lipophilic Sephadex could be used as a method for rapid screening of suitable solvent systems to be used in column chromatography.

Acknowledgements. This work was supported by grants from *Karolinska Institutets Reservationsanslag* and from the *Swedish Medical Research Council* (Grant No. 13-X-219).

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Received August 15, 1967.