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Some Geometric Effects observed in Thin-Layer Chromatography of Lipids

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(Figs. 1-3; Table 1)

In lipid biochemistry the samples of interest frequently are labelled with carbon-14, and determination of the distribution of radioactivity among the various components by scanning a developed thin-layer chromatogram is an increasingly common technique. For this purpose it is highly desirable to have all the lipid types well-resolved on a single plate, in order to conserve material and time. An essential requirement for achieving accurate results is that the spots or bands be narrow and that the R*f* values for adjacent spots be sufficiently different to give clear separation between the radioactivity peaks. The same requirements apply for the quantitative analysis of lipid classes on TLC plates by photodensitometry[1,2].

Unfortunately, the significant constituents of natural lipids differ widely in polarities, and in lipids from different sources the various lipid types are present in quite different relative proportions. This first property results in not all the lipid types present in a natural mixture being satisfactorily resolved when a single solvent system is used for development with the usual rectangular channels[3]. In connection with the second characteristic, if sufficient sample is spotted so that all components can be detected the major constituent may be overloaded, leading to a broad band which overlaps adjacent bands and obscures the quantitation of radioactivity. The present paper describes a method of overcoming some of these difficulties through use of channels of different geometric shapes.

EXPERIMENTAL

TLC plates (50×200 mm) were prepared from Silica Gel G (E. Merck AG., Darmstadt) suspended in distilled water of 2.2 times its weight and spread 0.25 mm thick with the standard Desaga applicator. The plates were air-dried for 30 min, then oven-dried at 120°C for an additional 30 min. After cooling they were prewashed, ascending, with chloroform-methanol (4:1 v/v) overnight[3]. Immediately before use the plates were reactivated for another 30 min at 120°C. The desired channel shape was then outlined by a narrow line cleared of adsorbent with a pencil

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or a narrow spatula having a flat end. The trapezoidal shapes were 1 cm wide at one end and 5 cm (the full width of the plate) at the other; *cf.* Fig. 1, A and C. The truncated diamond was 1 cm at either end and 5 cm wide in the center; *cf.* Fig. 3, D. In order to enhance the effect of differential solvent flux for the trapezoids the adsorbent external to the outlined channel was removed with a razor blade from the lower 4 cm (origin end) of the plate before development.

The sample (about 1 mg in 60–70°C petroleum ether, redistilled) was applied with a micropipette in a 1-cm-wide line at 3 cm from the bottom of the plate. The plate was developed ascending, using petroleum ether–diisopropyl ether–acetic acid (87.5:12.5:1 v/v/v), until the solvent front reached to within 1 cm of the top edge of the plate; *i.e.*, the solvent had traveled approximately 16 cm beyond the origin. The developing chamber was lined on one side with a filter paper.

After drying, the plates were scanned with a Radiochromatogram Scanner (Model 7201, Packard Instrument Co. or Aloka TLC–1, Nippon–Musen) for determination of the radioactivity. The lipid components were detected by spraying with 40 % H_2SO_4 and by charring on a hot plate (Model PC–100, Corning Glass Works or Personal Thermo Plate, Nisshin Rika Co.) for 1 to 2 hr at setting 3 (this gave a surface temperature of about 270°C). The charred chromatograms were photographed with a Polaroid copying camera (Model 110 B, Polaroid Corporation).

RESULTS

The results of two series of experiments are presented. Fig. 1 and Table 1 record the separation of an artificial mixture of non-radioactive compounds on TLC plates of three different configurations. In Fig. 2 are shown separations on TLC plates of the same three configurations of lantern fish muscle lipids biosynthesized from sodium acetate–1–^{14}C[4], together with the recordings of the corresponding radioactivity scans. In Fig. 3 still another shape, a truncated diamond (D), is compared with the usual rectangular channel (B), and with the inverted trapezoid (C).

In Fig. 1 the times required for the solvent front to travel to the levels indicated were 32 min for the trapezoid (A), 40 min for the rectangular configuration (B), and 58 min for the reverse trapezoid (STAHL's "wedged-tip" form, refs. 5 and 6). The R*f* values for each component detected on the three plates are given in Table 1.

The differences in R*f* values between adjacent spots are an indication of their separation, although, analogous to α values in GLC, they do not provide any measure of the completeness of that separation. From the table we see that for the five components with the highest R*f* values, I–V, this difference, $\triangle$, is greatest for configuration A and least for C, with the rectangular channel B intermediate. However, note that in A the compounds I to III are not as cleanly resolved as they are in B, or even in C. This may be partially caused by overloading. On the other hand, for the more polar components, VIII–X, the sequence is C>B>A. Another measure of the superiority of the reversed trapezoid shape (C) for the separation of

Table 1 Effect of Channel Shape on Rf Values

Configuration	A: Trapezoid		B: Rectangular		C: Reverse Trapezoid	
Components	Rf	△	Rf	△	Rf	△
I Squalene	0.80	0.07	0.87	0.06	0.87	0.03
II Cholesteryl stearate	0.73	0.08	0.81	0.06	0.84	0.05
III Wax esters, satrd.+mono-unsatrd.*	0.65	0.05	0.75	0.04	0.79	0.03
IV Wax esters, polyunsatrd.*	0.60	0.24	0.71	0.13	0.76	0.08
V Unidentified	0.47	0.10	0.58	0.11	0.68	0.09
VI Unidentified	0.37	0.19	0.47	0.22	0.59	0.20
VII Trilaurin	0.18	0.03	0.25	0.04	0.39	0.03
VIII Stearic acid	0.15	0.10	0.21	0.14	0.36	0.22
IX 1-Octadecanol	0.052	0.025	0.068	0.034	0.14	0.06
X Cholesterol	0.027	—	0.034	—	0.083	0.042
XI Dipalmitin	0.00	—	0.00	—	0.041	—
XII Chimyl alcohol+phospho-lipids	0.00		0.00		0.00	

△ = Difference in Rf values between adjacent components.

* Isolated from lantern fish muscle lipids by column chromatography, resolved by preparative TLC, and identified by saponification and GLC of the derived alcohols and fatty acids[7].

the more polar components is the fact that only in this configuration does the spot for dipalmitin (XI) move away from the origin. For compounds of intermediate polarity the Rf differences on all three plates are very similar.

Fig. 2 shows another aspect of the superior separations for components of low Rf values in the reverse trapezoid channel, namely, the improved resolution of the radioactivity peaks observed during scanning. Other than the principal peak (wax esters), which was clearly resolved in all three systems, configuration C, the reverse trapezoid, reveals four additional radioactive components, while in B two peaks are clear with a hint of a third, and in configuration A, the trapezoid, only two additional radioactive components are distinguished.

DISCUSSION

The most important single factor in explaining the observed effects of channel shape is the solvent flow, both amount and direction. After development the total amount of solvent on the plate will be proportional to the area wetted. Ignoring evaporation from the plate, changing the shape of the channel will not change the amount of solvent required to wet the same area, but a change in the width of the wick (the portion of the channel dipping into the bulk solvent) will change the total volume passing through a given element of that wick as well as the time for

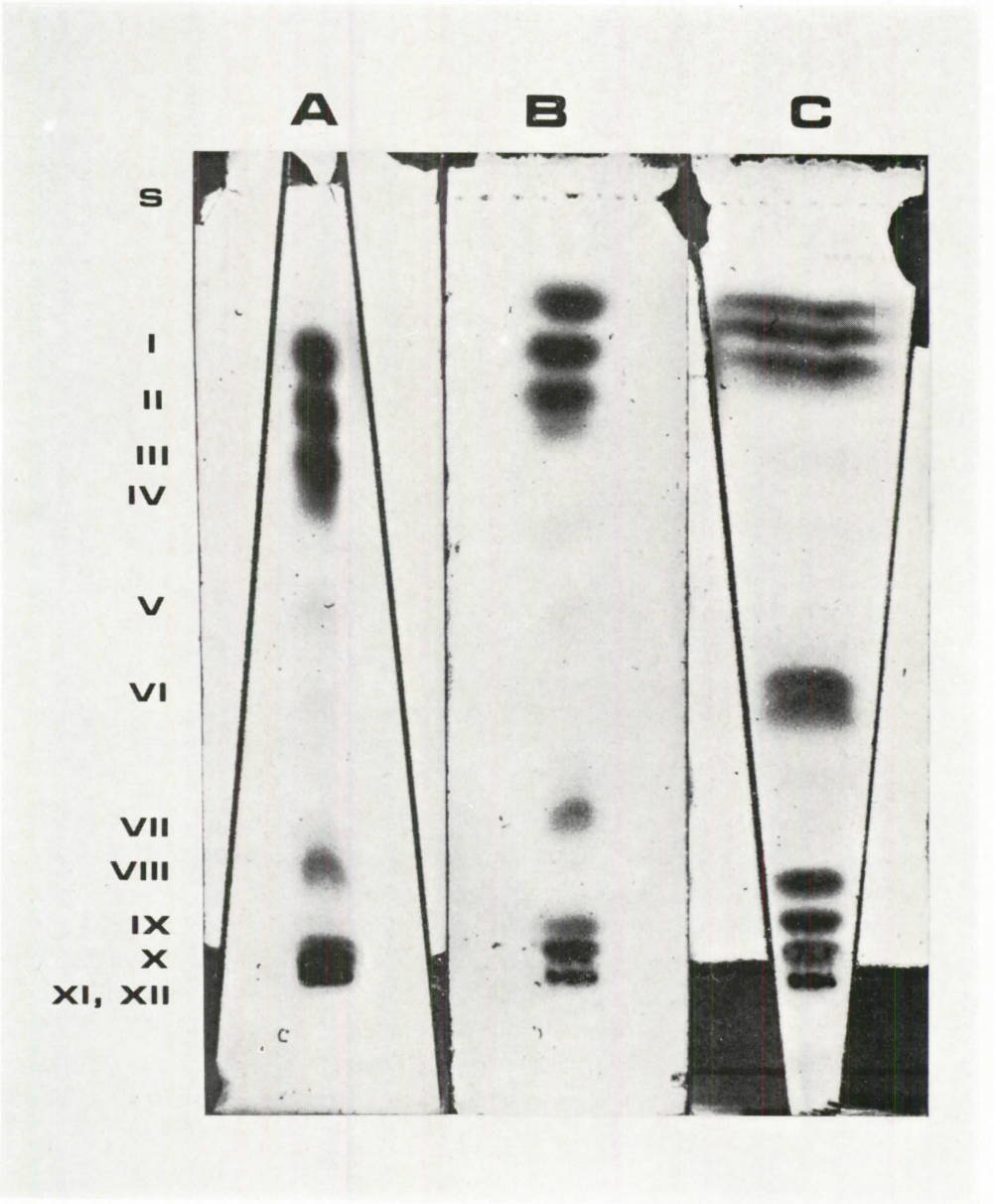

Fig. 1 Effect of channel shape on the separation of a lipid mixture. The components are: I, squalene; II, cholesteryl stearate; III, saturated and monounsaturated C_{30}–C_{38} wax esters[7]; IV, polyunsaturated wax esters C_{34}–C_{44}[7]; V and VI, contaminants; VII, trilaurin; VIII, stearic acid; IX, 1-octadecanol; X, cholesterol; XI, dipalmitin; and XII, hexadecyl glyceryl ether (chimyl alcohol) and phospholipids remaining at the origin. The solvent front reached S. *Cf.* Table 1.

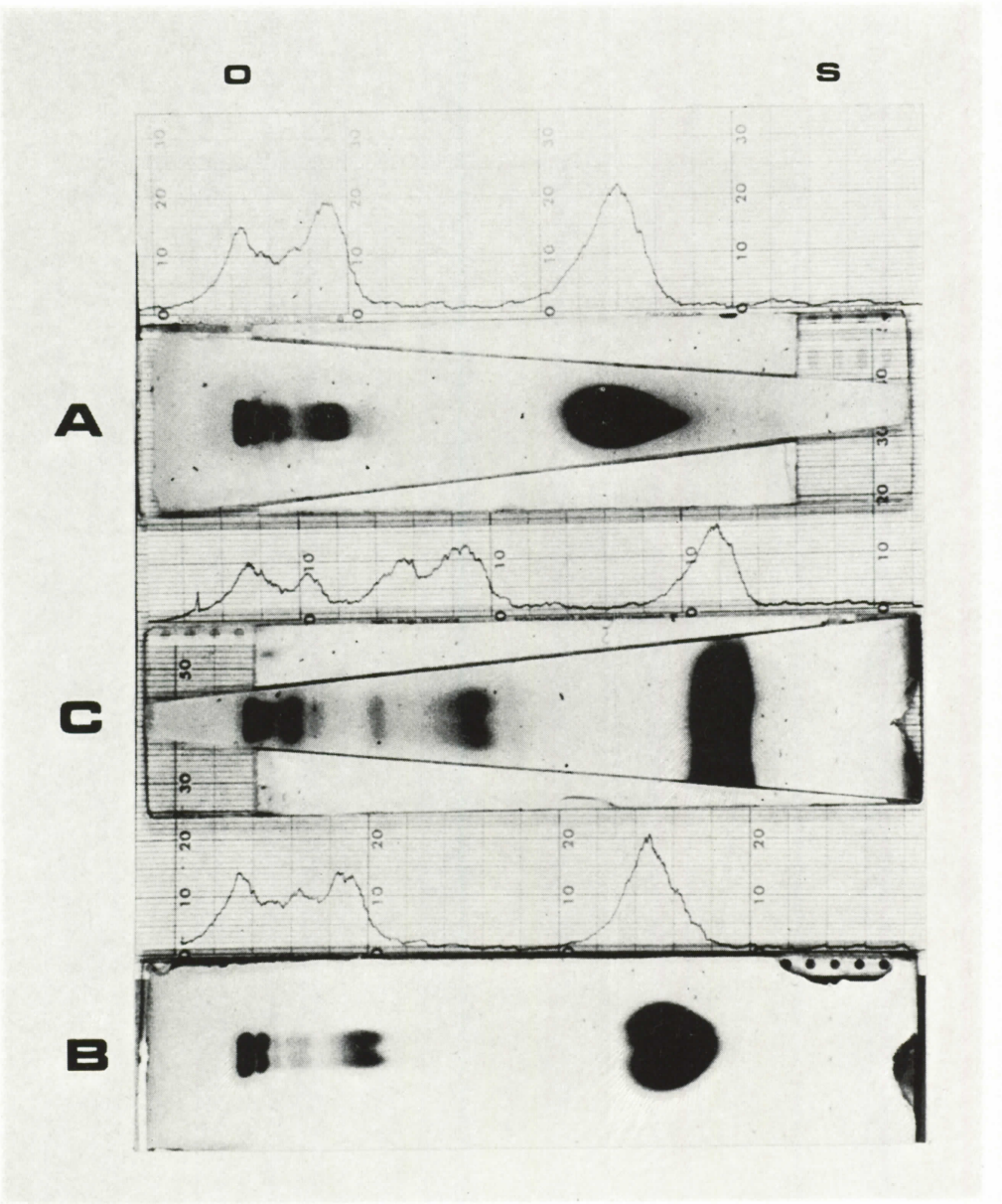

Fig. 2 Effect of channel shape on the resolution of radioactivity measured by scanning. Above each plate is the recorder trace of the scan of that plate made with the Packard Radiochromatogram Scanner. The origin is at O; the solvent front reached S. The sample is the total lipid biosynthesized from sodium acetate-1-^{14}C by the lantern fish, *Lampanyctus ritteri*[4]; the main component is wax esters.

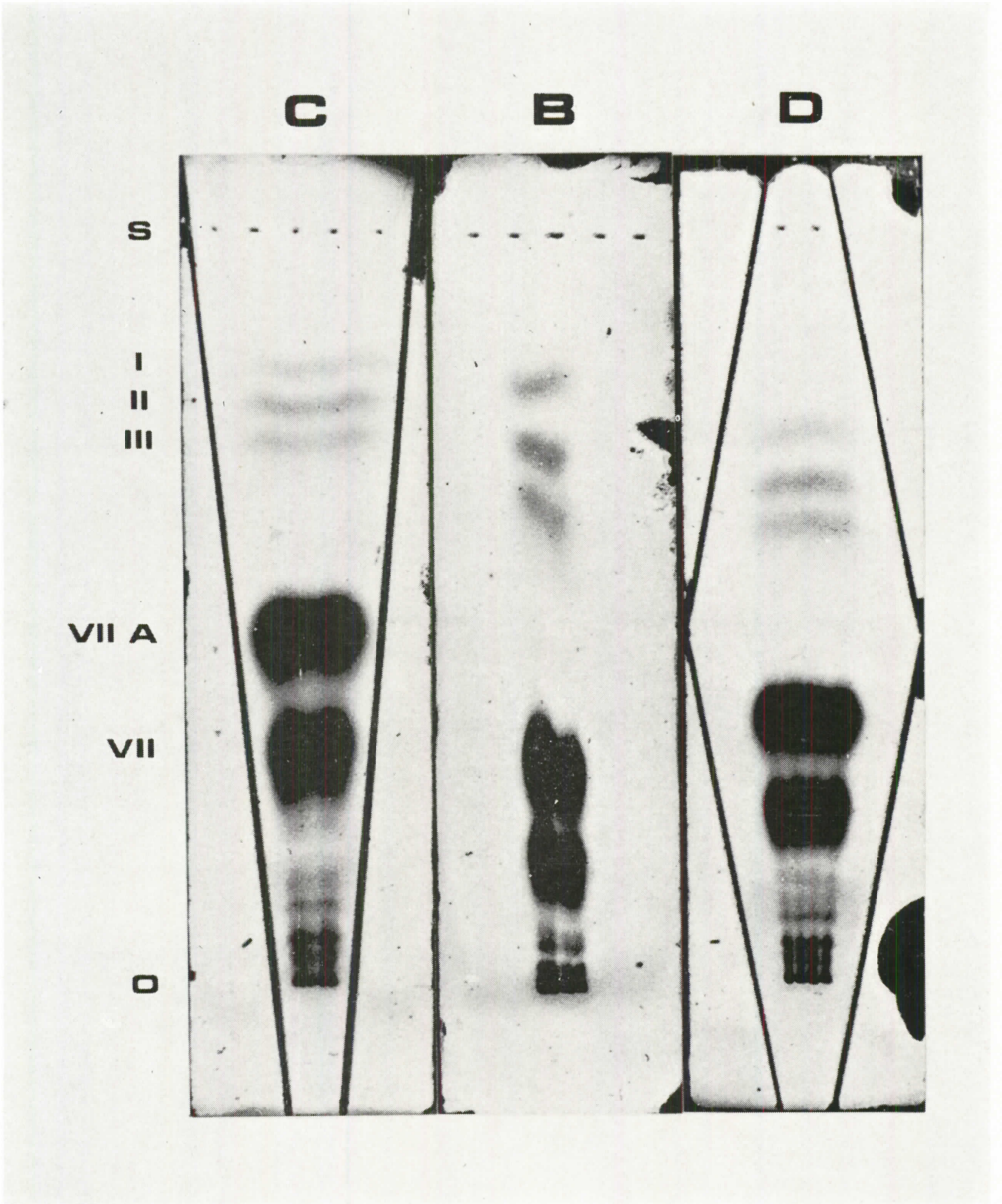

Fig. 3 Effect of channel shape on the separation of lipids. The components are: I, squalene; II, cholesteryl stearate; III, C_{30}–C_{38} wax esters; VII A, diacyl glyceryl ethers from dogfish liver oil; and VII, triglycerides from dogfish liver oil. The origin is at O; the solvent front reached S.

the front to reach a given height. With a solvent of constant composition, the distance that a particular substance moves in TLC will be directly dependent on the volume of solvent passing through it. This phenomenon accounts qualitatively for the observed order of Rf values (configuration A<B<C for all components), as we can see from the following calculations. The amount of solvent which has passed through the origin in each configuration is proportional to the area of the developed plate above 3.0 cm; these values are 44.8, 80.0, and 51.2 cm^2 for A, B, and C, respectively. The corresponding widths of the channels at this height are 4.40, 5.00, and 1.60 cm. Dividing the area by the width we obtain a relative measure of the total flow of solvent through a 1-cm wide element at the origin for each shape: A, 10.2; B, 16.0; and C, 32.1. The greater the flow of solvent the farther a component will move, and since the Rf values of Table 1 were obtained for a fixed vertical distance of solvent migration, the predicted Rf values for all components are as observed, namely: A<B<C.

It should be possible to account quantitatively for the observed effects if an accurate expression for the solvent flow through an advancing component spot on the plate were derived, taking into account horizontal as well as vertical movement into and out of a particular element of area. In the case where the substance moves only a short distance away from the origin (low Rf value) we can ignore the horizontal movement of solvent. Then the values calculated above for the solvent flow through the origin can be considered as the height of a rectangular channel of the same total area and having the width of the specific channel at the origin line. From this we can calculate an adjusted Rf value, dividing the observed distance moved by a component in any shaped channel by the equivalent (rectangular) height of the solvent in that configuration. Calculated in this way the Rf values should be independent of the channel shape. For example, the adjusted Rf values for component IX of Table 1 are: A, 0.081; B, 0.068; and C, 0.070 — more nearly constant than the values of Table 1 of 0.052, 0.068, and 0.14, respectively. For components of higher Rf value this simplification is not satisfactory.

An additional useful property of the trapezoid configuration (A) is the focusing effect. This results from the net horizontal vector of solvent flow directed inward as the solvent and components more upward in a channel constantly becoming narrower. Consequently the spots are kept more compact than they would be in a rectangular channel; that is, this solvent movement opposes the horizontal diffusion which in rectangular channels causes an increase in the diameter of the spot as it is "irrigated" by solvent. In Fig. 1 note that the samples were spotted on a 1-cm-wide line at the origin; nevertheless, in A the spots of the three components of highest Rf (I–III) are nearly round. This phenomenon can be used to decrease the limits of detectability of components, which was determined in our work by the contrast between the charred area and the background. Everything else being equal, the same amount of a given compound adsorbed onto a smaller area will give a greater density of color and hence greater contrast. Therefore, since the spots in configuration A are kept more compact than they would be in B, a smaller total

quantity of a given compound will be just detectable in configuration A than in the rectangular channel, B.

Finally, note that in configuration C the compounds eventually developed as lines, not as arcs. This is in contrast to the "wedged-tip" configurations described by STAHL[6], where, using a much narrower wick and samples adsorbed on a single spot at the origin, the zones developed as arcs of circles, centered on the origin. In our configuration, with a wick no less than a fifth the width of the channel at its widest, a channel with gradually tapering sides (the exterior angle at the base in C is 84°18′), and the sample spread over a 1 cm line at the origin, apparently the net horizontal flow of solvent directed outward was sufficient to reinforce the diffusion of the components into thin lines. These lines were horizontal, reflecting the vertical direction of the opposing forces of capillarity and gravity.

SUMMARY

Some components of complex lipid mixtures were resolved better in trapezoidal, reverse trapezoidal, or diamond-shaped channels than in the usual rectangular TLC channel. This superiority was apparent from both the observed R*f* values and the increased number of radioactive peaks detected by scanning. The results are explained qualitatively in terms of the amount and direction of solvent flow in the different configurations.

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脂質の薄層クロマトグラフィーで観察された二・三の幾何学的効果

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最近脂質の生化学的研究において，薄層クロマトグラフィー（TLC）を用いた成分の定性・定量的および放射能の分布測定が重要な分析手段となっている．本報では脂質の成分クラスの分離に関し，TLCに応用可能な図形の検討を行なった．

一般に用いられている長方形のTLCと比較し，梯形・逆梯形あるいはダイヤモンド形TLCチャンネルにおいて，複雑な脂質混合系のある成分をよりよく分離検出することができた．このことはTLC上で観察されたRf値およびラジオ薄層クロマトグラムのスキャンニングによる放射活性ピークの検出数から明らかであった．

これらのTLCでみられる幾何学的効果は，異なる図形へ流れ込む溶媒の量と方向性によって，定性的に説明されることを考察した．