saw it as a modification of the rate constant interpreted as an increase with time of catalyst effectiveness. The present treatment shows clearly that the presence of such a function in the rate equations is the normal consequence of competition between the reactions leading to the formation of complexes. It is not required when the reaction medium is saturated with hydrogen. Hence Swicklik *et al.* found $a = 0$ in their experiment No. 1.

Table I shows some inconsistencies in the numerical values obtained by these authors. Their k_{02}/k_{03} ratio in experiment No. 2 indicates a higher reactivity for *trans* in opposition with the accepted view that *cis* is more reactive. This ratio is also appreciably different from that corresponding to experiment No. 3, although experimental conditions differed only in the pressure used. Note that the k_{02}/k_{03} ratios by our values are about equal and acceptable under the above criterion. The value obtained by Swicklik *et al.* for k_{03}, experiment No. 2, is questionable since by making $t = 0$ in their equation (8) one obtains,

$$dC/dt = 100\, k_{03}$$

leading to our own value for this constant.

On the other hand, their statement expressing that k_{01}/k_{04} should equal the *trans-cis* equilibrium ratio appears unwarranted in view of equation (22). It can be demonstrated, through the use of this equation and of relation (18), that k_{01}/k_{04} will only equal the equilibrium ratio in the unlikely event that $k_{02} = k_{03}$.

From relations (5) and (10)

$$k'_A/k'_B = k_{01}k_{02}/k_{03}k_{04}$$

The value of this ratio is 1.135 for experiment No. 2 and 1.04 for experiment No. 3, by our values. The rates of break-down for the complexes are thus approximately equal.

The method proposed for the computation of the constants is undoubtedly less tedious and more expeditive than one by trial and error. The value of k_C, that of the *trans-cis* equilibrium ratio, and those of A and B when B is maximum, constitute the only data needed for its application. A relatively small time lag and zero order kinetic conditions are a prerequisite however. Fortunately this is the general case when conditions similar to those used in industrial practice are used.

It should be noted that equation (22) suggests the occurrence of the *cis-trans* equilibrium described by Feuge *et al.*

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Paper Chromatography of Phospholipides on Silicic Acid Impregnated Glass Fiber Filter Paper[1]

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AS PART OF A PROGRAM on the biosynthesis of the lipides, it was considered desirable to develop a paper chromatography method for resolving phospholipide mixtures and for testing the homogeneity of phospholipide preparations in general. Several methods involving conventional paper chromatography have been described (6, 7, 9). In addition, silicic acid-impregnated cellulose filter paper has been used (8). In most of the above procedures the chromatographed phospholipides were located on the chromatogram by means of functional groups in the phospholipide molecule e.g., amino-, quaternary ammonium-, and phosphate groups. Since the weight percentage of these groups in the phospholipid molecule is often relatively low, there would appear to be a good chance for overlooking phospholipide constituents present in relatively low concentrations in a given phospholipide preparation. Furthermore impurities not having one of the tested functional groups would pass unnoticed.

In an attempt to overcome these difficulties the technique of chromatography on glass paper impregnated with silicic acid (2, 3) has been investigated as a means for separating lysolecithin, sphingomyelin, lecithin, and phosphatidylethanolamine.

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Experimental

Preparation of the Paper and Chromatographic Tank. Silicic acid glass fiber filter paper and the chromatographic tank were prepared essentially according to the method of Dieckert and Reiser (4). But, to insure adequate removal of HCl and KCl, the impregnated glass paper was washed six times with distilled water instead of four times, as reported earlier.

Method. Each phospholipide spotted on the chromatogram was applied in 10 μgm amounts as a 0.1% solution of the lipide. The chromatogram was routinely equilibrated for a period of 30 min. prior to the irrigation step. After equilibration the chromatogram was irrigated by the ascending technique. From 1 to 1½ hrs. were required for the solvent front to travel 10 to 12 in.

Solvent Systems. The five solvent systems investigated were 15, 25, 50, 70, and 90% (V/V) methanol in ethyl ether. A.C.S. grade solvents were used without further purification.

Spot Test Reagents. After development of the

chromatogram and removal of the solvent, the substances chromatographed were located by spraying the chromatogram with 1:1 sulfuric acid:distilled water followed by heating over a hot plate. With gentle heat cholesterol and related substances appeared as pink spots on a white background. On more drastic heating all substances charred, giving brownish-black spots on a white background. For ease of examination the chromatograms were viewed by transmitted light.

Several other spot test reagents were employed for the identification of functional groups. For the detection of the amino group the chromatogram was sprayed with a 0.1% of ninhydrin in citrate buffer and then heated at 100°C. in a drying oven for 5 min. (5). A blue to violet spot on a white background is a positive test. The quaternary ammonium group was identified with the modified Dragendorf reagent described by Bregoff, Roberts, and Delwiche (1). The choline containing lipides gave a reddish background. One per cent neutral permanganate proved to be useful for locating those lipides containing unsaturated fatty acids (7). A yellow spot on a purple background is a positive test.

Materials. The two lysolecithins, monostearyl-L-α-lecithin and monopalmitoleyl-L-α-lecithin, and the two lecithins, distearyl-L-α-lecithin and dipalmitoleyl-L-α-lecithin, were kindly supplied by D. J. Hanahan. The sphingomyelin was a gift of H. E. Carter and the phosphatidylethanolamine a gift from J. H. Dutton.

Results and Discussion

A mixture of phospholipides containing phosphatidylethanolamine, lecithin, sphingomyelin, and lysolecithin was resolved into its components with 1:1 methanol:ethyl ether as the developing solvent (Table I and Figure 1). With this system reasonably good reproducibility was obtained as measured by the standard deviation about the mean (Table I). No difference was found in the R_f values for a given substance whether it was run singly or in combination with other phospholipides (Table I and Figure 1). No separation of the saturated and unsaturated counterparts of lysolecithin or lecithin was obtained (Table I). In general, an increase in the percentage of methanol in the developing solvent resulted in an increase in the R_f value of a given phospholipide (Table II).

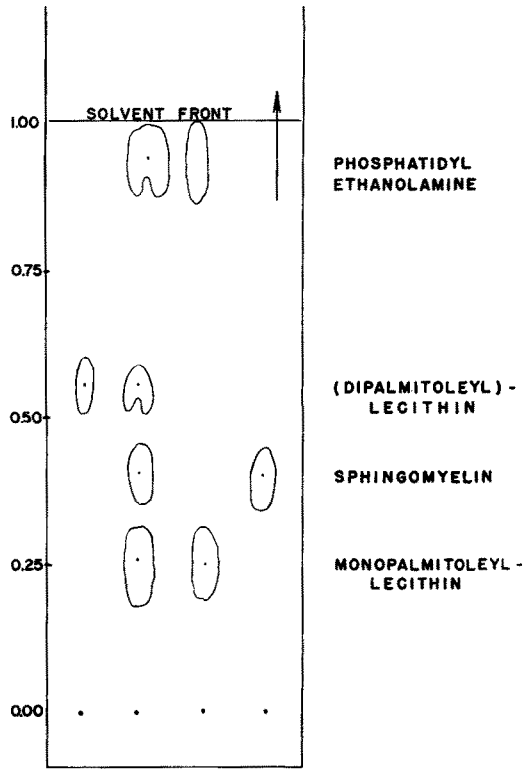
Not all of the spot test reagents listed under experimental were sensitive enough to detect the appropriate functional groups when only 10 μgm of a given phospholipide were involved. The least sensitive spot test proved to be the modified Dragendorf reagent. On the other hand, the most sensitive one was 1:1 sulfuric acid:water. When a 10-μgm sample of the phosphatidylethanolamine preparation or the sphingomyelin preparation was chromatographed, several extra spots appeared, indicating impurities (Table II). Because of its lack of specificity, the sulfuric acid test is useless in most cases for the positive identification of compounds on the chromatogram. This is a point against the test.

TABLE I

R_f Values Obtained in the 50% (V/V) Methanol in Ethyl Ether System

Substance	Mixture	S.D.[a]	Single
Phosphatidylethanolamine	0.94	0.02	0.94
(Distearyl)-L-α-lecithin	0.47	0.04	0.46
(Dipalmitoleyl)-L-α-lecithin	0.52	0.05	0.50
Sphingomyelin	0.37	0.04	0.40
β-Monostearyl-L-α-lecithin	0.21	0.04	0.18
β-Monopalmitoleyl-L-α-lecithin	0.21	0.04	0.23

[a] At least 10 values were used to compute the standard deviation.



TRACING OF A CHROMATOGRAM SHOWING SEPARATION OF THE PHOSPHOLIPIDES USING 50% (V/V) METHANOL IN ETHYL ETHER

FIG. 1

Summary

1. A reasonably reproducible method has been developed for separating lysolecithin, lecithin,, sphingomyelin, and phosphatidylethanolamine, using glass fiber filter paper impregnated with silicic acid as the chromatographic medium and 1:1 methanol:ethyl ether as the developing solvent.

2. Hot sulfuric acid was used to locate the chromatographed compounds on the chromatogram. The advantages and disadvantages of this reagent and the other spot test reagents used were discussed.

3. No separation between the unsaturated and saturated counterparts of lysolecithin or lecithin was obtained.

TABLE II

R_f Values with Increasing Levels of Methanol in Ethyl Ether

Substance	% Methanol (V/V)				
	15%	25%	50%	70%	90%
Phosphatidylethanolamine	0.43	0.74[a]	0.94	0.96	0.95
(Distearyl)-L-α-lecithin	0.03	0.11	0.47	0.70	0.78
Sphingomyelin	0.02	0.06	0.37[b]	0.58	0.69
β-Monostearyl-L-α-lecithin	0.00	0.00	0.21	0.36	0.52

[a] Impurity having R_f 0.91 gave positive sterol test with the sulfuric acid:water reagent.

[b] Impurity having R_f 0.93 gave charring with the sulfuric acid:water test.

4. In general, an increase in the percentage of methanol in the irrigating solvent resulted in an increase in the R_f value of a given phospholipide.

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Letter to Editor

July 2, 1956

I have read with interest the paper on "Determination of Soaps by Ion Exchange Resin" by J. W. Jenkins, published in the May 1956 (p. 225) issue of your esteemed journal. The method developed is no doubt interesting and is apt to be adopted for routine analysis in soap factories in the near future.

In this connection I draw your attention to a paper on the same subject published by me and my student in January 1955 (p. 187) issue of the *Indian Soap Journal*, which maintains an exchange relationship with your journal.

B. K. MUKHERJI
Honorary Editor
Indian Soap Journal
Calcutta, India

ABSTRACTS . . . . . R. A. Reiners, Editor

• Oils and Fats

S. S. Chang, Abstractor
Sin'itiro Kawamura, Abstractor
Dorothy M. Rathmann, Abstractor

Chemistry of the phosphatides. E. Baer(Univ. Toronto). *Ann. Rev. Biochem.* **24**, 135–56(1955). A review of three years' work. (*C. A.* **50**, 6538)

The coconut-like flavor defect of milk fat. I. Isolation of the flavor compound from butter oil and its identification as δ-decalactone. P. G. Keeney and S. Patton(Dept. of Dairy Sci., Penn. Agr. Exp. Station, Univ. Park). *J. Dairy Sci.* **39**, 1104–1113(1956). The coconut-like off-flavor compound, which develops in butter oil during storage or when butter oil has been heated, was isolated and identified as δ-decalactone(lactone of 5-hydroxy decanoic acid). Infrared spectroscopy and paper chromatography were used in establishing flavor compound's coidentity with δ-decalactone. Paper chromatographic methods are presented whereby a homologous series of gamma lactones from nonalactone to dodecalactone can be resolved. Evidence that a similar series of delta lactones can be separated in a like manner was obtained.

The coconut-like flavor defect of milk fat. II. Demonstration of δ-decalactone in dried cream, dry whole milk, and evaporated milk. *Ibid.* **39**, 1114–1119(1956). Coconut-like flavor extracts were obtained from dried cream, dry whole milk and evaporated milk. The presence of δ-decalactone in these flavor extracts was established by paper chromatography, and the characteristic coconut-like flavor defect of these products is attributed to this lactone.

Modification of the refractive index method for the detection of foreign fats in dairy products. V. R. Bhalerao and F. A. Kummerow(Dept. of Food Tech., Univ. of Ill., Urbana, Ill.). *J. Dairy Sci.* **39**, 947–955(1956). A method based on the glyceride structure of butterfat was worked out to detect adulteration of the butterfat by a foreign fat at a 10% level. The suspected sample of butterfat was separated into alcohol-soluble and insoluble triglycerides at 20° in order to increase concentration of the adulterant in one of these fractions. The refractive index of the alcohol-soluble fraction of butterfat was found to vary from 1.4538 to 1.4541, whereas that of the insoluble fraction was found to vary from 1.4539 to 1.4544. The increase in the refractive index of the alcohol-insoluble fraction indicated the presence of vegetable or animal fat. This fraction was further fractionated from acetone at 0°. The acetone-soluble fraction was iodinated with Wijs solution, and refractive index of the iodinated fraction was determined. The refractive index of the iodinated fraction of butterfat was found to vary from 1.4713 to 1.4732. The addition of 10% of foreign fat other than coconut oil increased the refractive index significantly, enabling the detection with a fair degree of accuracy.

The methods of examination of fats and fat products. Committee reports. Tomotaro Tsuchiya *et al. J. Japan Oil Chemists' Soc.* **5**, 47–56(1956). This is the concluding report for chemical tests including acid no., neutralization no., saponification no., ester no., thiocyanogen no., determination of conjugated unsaturated fatty acids (spectroscopic method), determination of unsaponifiable matter, determination of solid fatty acids, and ether-insoluble chloriodide.

Separation and determination of fatty acids. XVIII. Paper chromatography of fatty acids as their acetol ester derivatives. Yoshiyuki Inoue, Osamu Hirayama, and Manjiro Noda(Saikyô Univ., Kyoto). *J. Japan Oil Chemists' Soc.* **5**, 16–18(1956). Acetol esters, $RCOOCH_2COCH_3$, were synthesized from 10 saturated fatty acids from acetic to arachidic by reacting with monobromoacetone. They were converted to their 2,4-dinitrophenylhydrazones and thiosemicarbazones, which were chromatographed on paper by the inverse phase method and the R_f values were determined. The solvents used included methanol-decalin (8:1), methanol-ethyl acetate-decaline (40:3:7), methanol-acetic acid-decalin (50:2:7), and 90% ethanol-acetic acid-decalin (30:5:3). This method is applicable for the separation of mixed fatty acids.

Segregation of soybean fatty acids by urea complex with particular reference to the concentration of urea solution. Hiroshi Sakurai and Masao Fujiwara(Osaka Univ., Sakai). *J. Chem. Soc. Japan, Ind. Chem. Sect.* **59**, 33-6(1956). The optimum temperatures of the segregation of soybean fatty acids by urea complex were in the range 10–25°. The optimum concentration of urea solution was found to be at relative saturation degree of 0.9423, i.e. 43.3% at 10°, 49.0% at 20°, and 51.6% at 25°.

Fatty oils of aquatic invertebrates. X. Fatty oils of Stichopus japonicus, Astriclypeus manni, Clypeaster japonicus, and Gorgonocephalus caryi. Yoshiyuki Toyama and Toru Takagi(Nagoya Univ.). *J. Chem. Soc. Japan, Pure Chem. Sect.* **77**, 102–5 (1956). *S. japonicus, A. manni, C. japonicus,* and *G. caryi* contained, respectively, 2.5, 1.1, 1.7, 2.3% ether extract, and the oils had, respectively, n_D^{30} 1.4686, 1.4776, 1.4758, 1.4702 (at 30°); acid no. 95.2, 41.9, 28.5, 96.0; saponification no. 149.3, 157.1, 164.6, 146.9; iodine no. 106.2, 158.6, 190.3, 91.6; unsaponifiable matter 16.97, 18.97, 14.75, 29.58%; solid fatty acids 27.91, —, 17.30, —%; sterol content of the unsaponifiable matter 7.45, 64.7, —, 62.4% highly unsaturated acid contents (%), dienoic 3.6, —, —, 1.0, —; trienoic 5.3, —, —, 0.9, —; tetraenoic 7.7, —, 17.2, —; pentaenoic 7.5, —, 34.3, —; hexaenoic 5.7, —, 6.5, —; their sterols were chiefly Δ^7-sterol, cholesterol, cholesterol, and Δ^5-sterol, respectively. *S. japonicus* seemed to contain batyl alcohol and selachyl alcohol.