4 R. M. BOCK AND N. S. LING, *Anal. Chem.*, 26 (1954) 1543.
5 C. W. PARR, *Biochem. J.*, 56 (1954) xxvii.
6 B. DRAKE, *Arkiv. Kemi*, 8 (1955) 1.
7 E. A. PETERSON AND H. A. SOBER, *Anal. Chem.*, 31 (1959) 857.
8 E. A. PETERSON AND J. ROWLAND, *J. Chromatog.*, 5 (1961) 330.
9 J. J. WREN, *J. Chromatog.*, 12 (1963) 32.
10 P. LEBRETON, *Bull. Soc. Chim.*, (1960) 2188.
11 *Pat. Appl.*, 17030 (1966).

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An improved spray reagent for detecting lipids on thin-layer chromatograms

During the separations of lipids on thin-layer plates using silica gel a number of reagent sprays have been examined, both destructive (sulphuric acid, chromic acid, phosphoric acid, iodine) and non-destructive (Rhodamine B, 2′,7′-dichlorofluorescein, 2′,7′-dibromofluorescein, bromthymol blue, water). As the analysis of fatty acids of

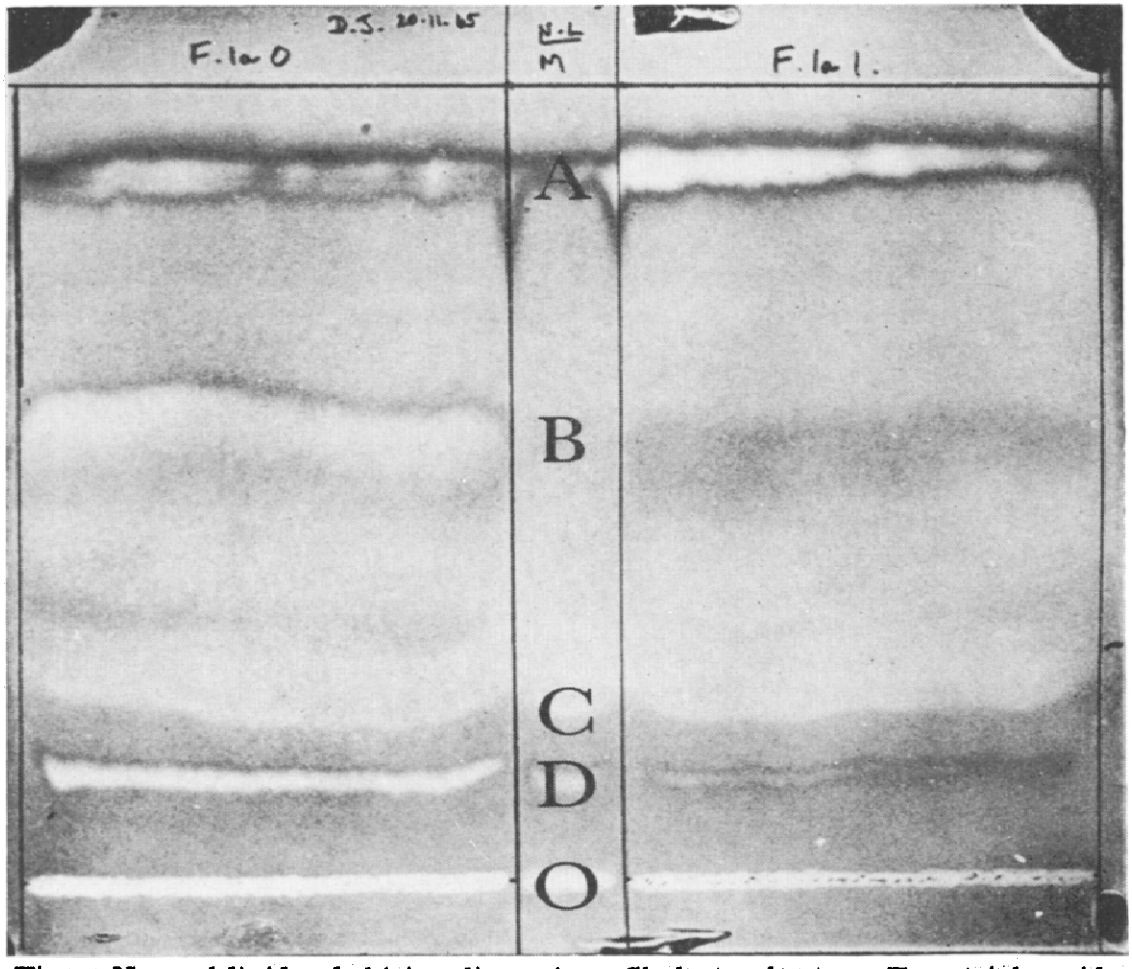

Fig. 1. Neutral lipids of chicken liver. A = Cholesterol esters; B = triglycerides; C = free fatty acids; D = cholesterol; O = origin (containing phospholipids).

the eluted lipids by gas chromatography was required[1], destructive reagents could not be used; many of the non-destructive reagents did not localise lipids sufficiently accurately, or cause sufficient contrast with background, for accurate photographic records. For this reason a mixed spray of a non-destructive nature has been developed which gives excellent contrast for both colour and black-and-white photography. This spray has proved superior to any so far described. Its composition is as follows:

Rhodamine B	100 mg
2′,7′-dichlorofluorescein	35 mg
diethyl ether	150 ml
95% ethanol	70 ml
water	16 ml

Chromatograms are sprayed and allowed to dry before examination under ultra-violet light (366Å). Lipids fluoresce orange or orange-purple on a green background (Figs. 1–3).

These colours appear as the chromatogram dries. A high proportion of ether is therefore added to the reagent to assist rapid evaporation of the solvent. It is essential to have water in the spray reagent otherwise colours are not intense; colours can be further intensified by heavy spraying. Plates to separate phospholipids

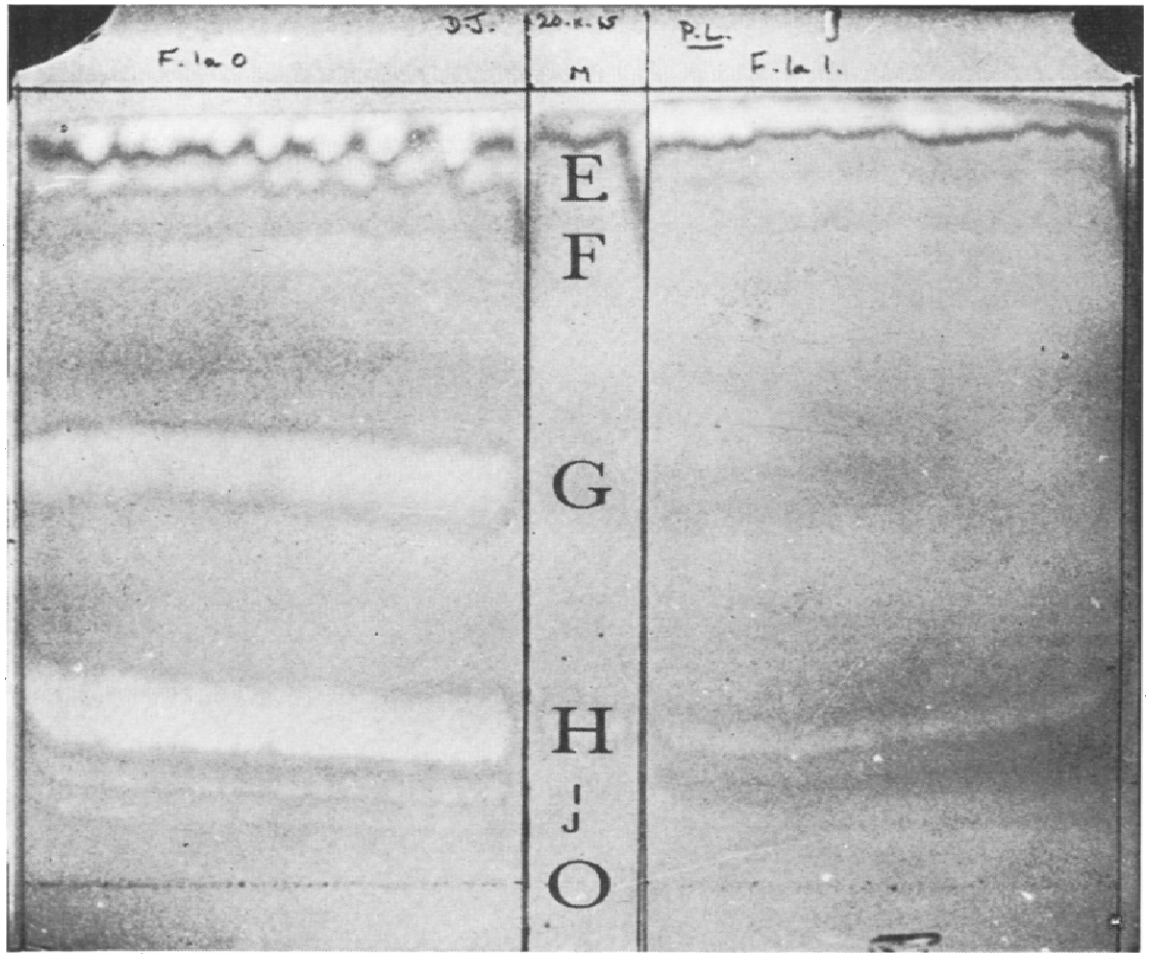

Fig. 2. Phospholipids of chicken liver. E = Neutral lipids; F = free fatty acids; G = phosphatidyl ethanolamine; H = phosphatidyl choline; I = sphingomyelin; J = lysolecithins; O = origin.

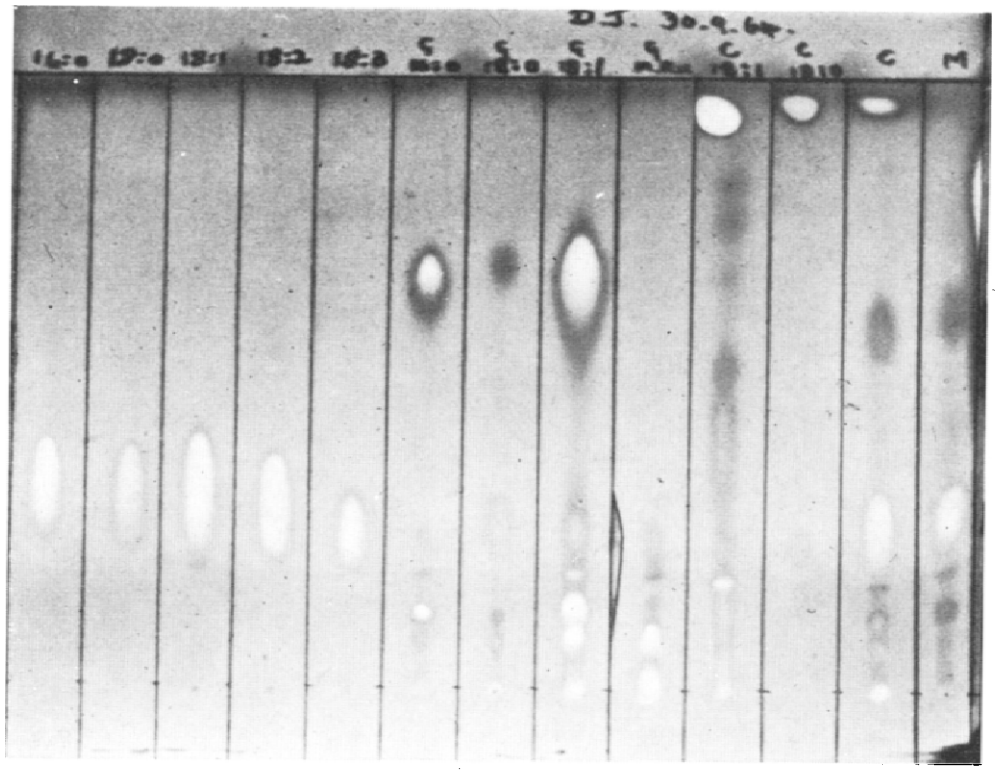

Fig. 3. Various neutral lipids (ex British Drug Houses). Columns 1–5: free fatty acids; columns 6–8: triglycerides; column 9: glyceryl monoricinoleate; columns 10–11; cholesterol esters; column 12: cholesterol; column 13: marker. These lipids were as received.

developed in chloroform–methanol–water (60:25:4) show colours within one or two minutes. Plates to separate neutral lipids developed in petrol–ether–acetic acid (85:15:1) are somewhat slower to show colour, for the acetic acid, which evaporates slowly, suppresses colour formation.

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1 D. E. BOWYER, W. F. M. LEAT, A. N. HOWARD AND G. A. GRESHAM, *Biochim. Biophys. Acta*, 70 (1963) 423.

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