Thin-layer chromatography of tissue lipids without extraction

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SUMMARY Frozen tissue sections were applied directly to silica gel plates, and the lipids were separated by developing the plates in different solvent systems. Quantitation of the lipid classes was achieved by direct transmission densitometry after the plates were treated with a chromic acid–sulfuric acid spray.

SUPPLEMENTARY KEY WORDS histchromatography · brain lipids · adipose tissue · gangliosides · cholesterol · phospholipids · neutral lipids

CHROMATOGRAPHIC separation of free amino acids in animal tissues by direct application of frozen sections on paper was reported by George, Pishawikar, and Scaria in 1958 (1). Recently Strich (2) reported on the separation of tissue lipids by direct TLC. The method consisted of placing frozen sections on plates, coating them with the silica gel slurry, and heating before developing the plates. Although successful to a limited extent, this method lacked reproducibility (3). Honegger (4) separated lipids from brain tissue by direct TLC, but did not report on the quantitative estimation of various components. Curri, Rossi, and Sartorelli (5) and Curri and Levanon (6) have used direct TLC for the separation of lipids. In their method, frozen sections were transferred to a small cover glass (20 mm × 20 mm); this in turn was fixed with agar on a regular TLC plate and then coated with silica gel and dried in a desiccator before developing in the solvent systems. The spots of phospholipids were visualized by exposure of the plates to iodine vapor. These areas of silica gel were scraped off the plate, digested with perchloric acid, and the phosphorus was estimated by colorimetry. Their method, though useful, involves more steps than direct TLC on plates already coated with silica gel. We have found that reproducible separation and quantitation by densitometry of the major classes of lipids can be obtained if the sections are transferred directly to activated silica gel plates which are then developed as usual.

Materials. All tissues were obtained at necropsy and kept frozen at −18°C until used. Tissues from rats, rabbits, and humans were used; however, most of the results reported here were obtained by using normal human brain and adipose tissue. Glass plates of standard size (20 cm × 20 cm), coated with Silica Gel G (250 μ thick) (E. Merck AG, Darmstadt, Germany) and activated at 110°C for 1 hr, were used for TLC. All solvents were analytical grade reagents. Chloroform–methanol–2.5 N ammonium hydroxide 60:40:9 (v/v/v) was used for the separation of gangliosides (7), chloroform–methanol–water 65:25:4 (v/v/v) was used for phospholipids and glycolipids, and petroleum ether–ether–acetic acid 70:30:1 (v/v/v) was used in separation of cholesterol and other neutral lipids and free fatty acids.

Methods. A cylindrical portion of the frozen tissue was punched out using a precooled cork borer (1/8 in. I.D.); the sample was then mounted on the specimen holder of an International Harris model cryostat. To obtain uniform rigid sections most tissues can be cut at the cryostat temperature of −10 to −15°C. For adipose tissue the chamber should be cooled to −25 to −30°C before the sections are cut. For the separation of gangliosides 16 μ thick sections were taken from the cortex of the human brain frontal lobe. Similar sections were taken from the white matter for the separation of phospholipids, glycolipids, neutral lipids, and cholesterol, and from adipose tissue for the separation of neutral lipids and cholesterol. The sections were transferred to the TLC plate by means of a precooled camel's hair brush. For purposes of comparison, a known number of sections were extracted with a mixture of chloroform–methanol 2:1 (v/v) (2 μl/section). These sections were transferred to the chilled capillary end of a tube of 0.5 ml capacity made from the lower portion of a Pasteur pipette. To prevent evaporation, the tube was closed with a silicone rubber stopper, and the solvent mixture was introduced into it by means of a Hamilton microsyringe. The sections were stirred with a stainless steel wire which was passed through the stopper. Similarly, the extract was drawn out with the microsyringe without removing the stopper and was spotted on the TLC plate. It was found that it is better to spot the extracts first when both sections and liquid extracts are used on the same plate. The plates were then lowered into the chromatographic chamber and developed in the appropriate solvent mixture. In preliminary experiments, one, two, three, and four sections were spotted alternately with the extracts from the corresponding number of sections, in order to determine the optimum number of sections to be used for each tissue and solvent mixture. For the detection of gangliosides, the plate was removed from the chamber, dried in air, sprayed with a resorcinol spray (8), covered with a clean glass plate, and heated in an oven at 120–130°C for 20 min. All the other lipid components were visualized by spraying the dried plates with a sulfuric acid–chromic acid spray and heating them in the oven at 180–200°C for 20 min. The various spots were identified by chromatographing standards alongside the sections and extracts. Quantitation (9) was achieved by compar-

Abbreviations: TLC, thin-layer chromatography.

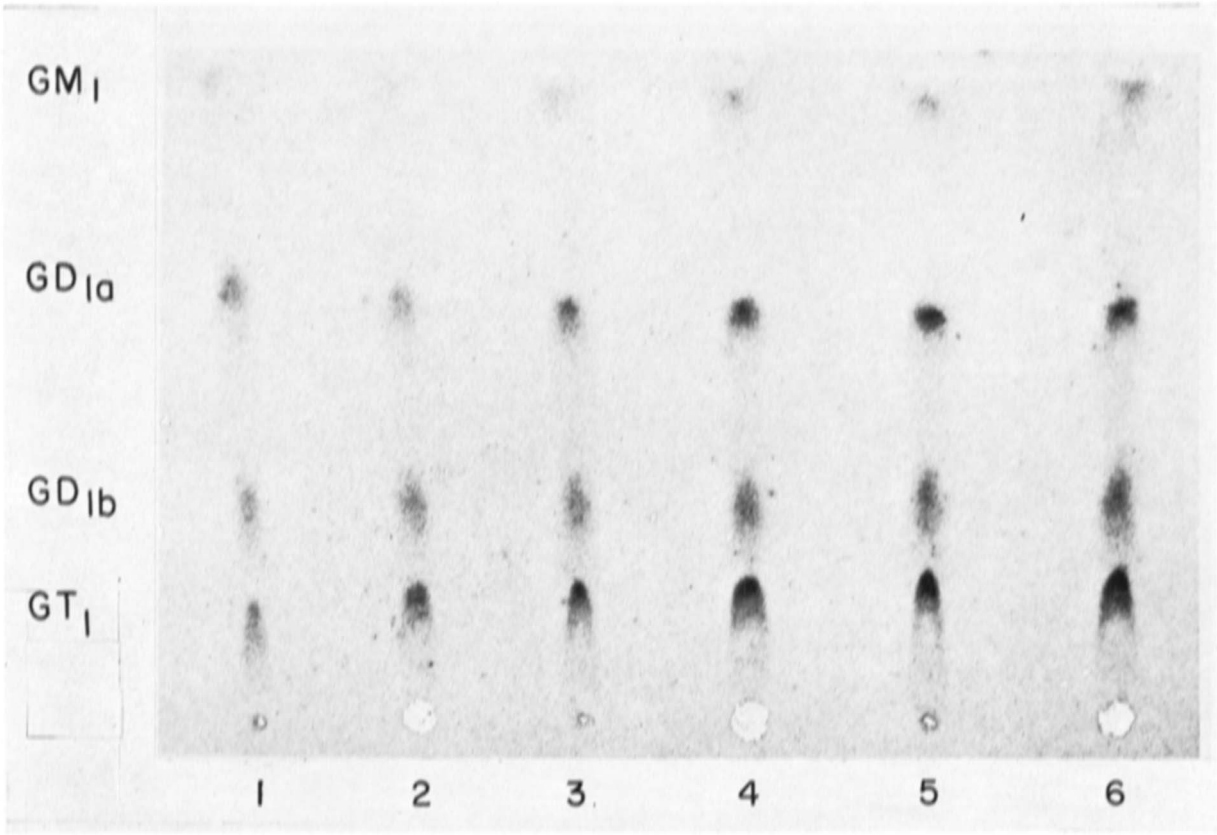

FIG. 1. Separation of gangliosides from extracts and sections of normal human cerebral cortex. *1*, extract of one section; *2*, one section; *3* and *5*, extracts of two sections; *4* and *6*, two sections. GM_1, monosialogangliosides-1; GD_{1a}, disialogangliosides-a; GD_{1b}, disialogangliosides-b; GT_1, trisialogangliosides-1. Nomenclature is according to Svennerholm (10). Adsorbent, Silica Gel G. Solvent, chloroform–methanol–2.5 N ammonia 60:40:9 (v/v/v). Detection, resorcinol spray (8).

ing the optical density of the spots from the sections and corresponding extracts by means of a Photovolt Densitometer with an automatic recorder and integrator.

Results. Satisfactory separation of gangliosides into four main components were obtained by using sections from human cerebral cortex (Fig. 1). No attempt was made to quantitate the spots by densitometry. The size and intensity of the spots from the sections and extracts appeared to be comparable. The chromatographic separation of phospholipids and cholesterol is shown in Fig. 2. Results of the densitometric readings are in Table 1. There were no significant differences between the optical densities of the spots from the sections and the extracts. Cholesterol and phosphatidylserine were not estimated by densitometry; these spots were too close to the solvent front and the origin, respectively. However, there appeared to be close similarity between the section and the extract (Fig. 2). Direct TLC of the neutral lipids from the adipose tissue and brain white matter is shown in Fig. 3. Comparison of the densitometric readings of cholesterol and triglycerides (Table 2) indicates that the spots from direct TLC of the sections as well as the extracts gave essentially the same results. The differences between the analyses were not statistically significant.

TABLE 1 QUANTITATION OF GLYCOLIPIDS AND PHOSPHOLIPIDS*

Fraction	Direct TLC (5)	TLC of Extract (5)
Cerebrosides	55 ± 1.2	56 ± 2.9
Phosphatidylethanolamine	48 ± 3.7	45 ± 4.8
Phosphatidylcholine and cerebroside sulfates	40 ± 2.7	38 ± 3.3
Sphingomyelin	20 ± 2.5	19 ± 2.3

* Values are expressed as densitometric readings ±SD. The figures in parentheses indicate the number of determinations. Two sections (each 16 μ in thickness) of human brain white matter or the equivalent extract was used for each spot.

TABLE 2 QUANTITATION OF TRIGLYCERIDES AND CHOLESTEROL*

Fraction	Direct TLC (4)	TLC of Extract (4)
Adipose tissue		
Triglycerides	111 ± 6.4	108 ± 11.6
Cholesterol	9.8 ± 0.5	10.0 ± 2.5
Brain white matter		
Cholesterol	29 ± 4.1	31 ± 2.4

* Values are expressed as densitometric readings ±SD. The figures in parentheses indicate the number of determinations. One section (8 μ in thickness) or the equivalent extract was used for each spot.

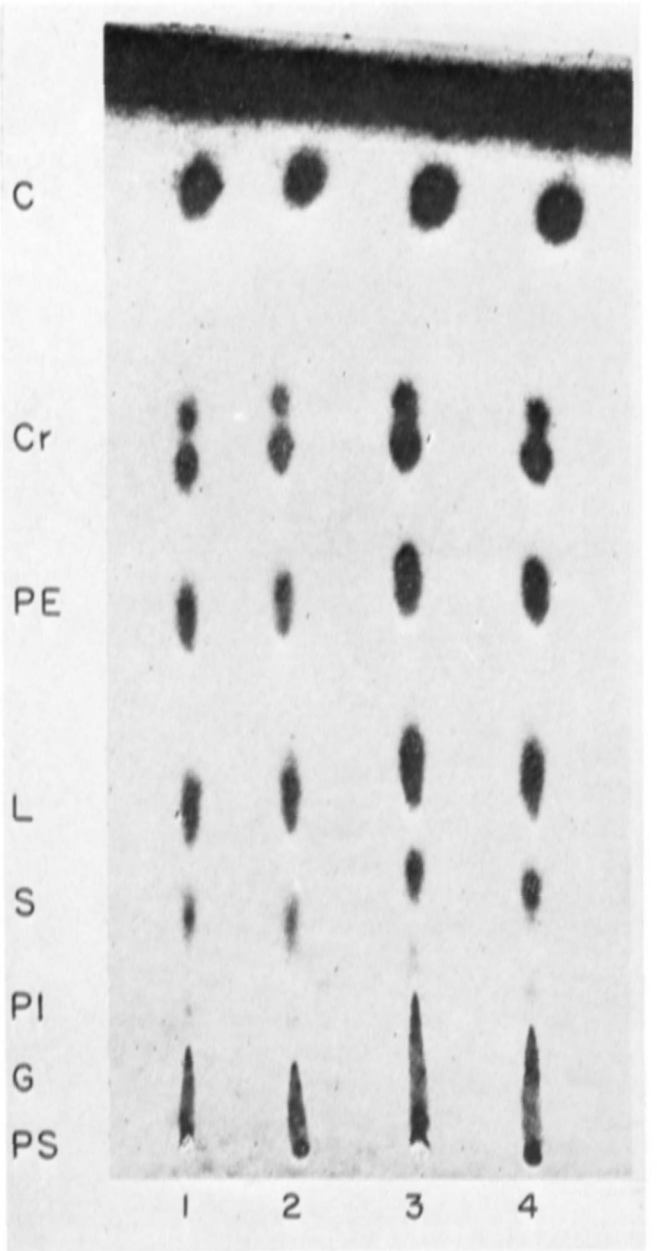

FIG. 2. TLC of frozen sections and extracts from the white matter of normal human brain cerebrum. *1*, one section; *2*, extract of one section; *3*, two sections; *4*, extract of two sections. *C*, cholesterol; *Cr*, cerebrosides; *PE*, phosphatidylethanolamine; *L*, lecithin plus cerebroside sulfates; *S*, sphingomyelin; *PI*, phosphatidylinositol; *G*, gangliosides; *PS*, phosphatidylserine. Adsorbent, Silica Gel G. Solvent, chloroform–methanol–water 65:25:4 (v/v/v). Detection, sulfuric acid–chromic acid spray.

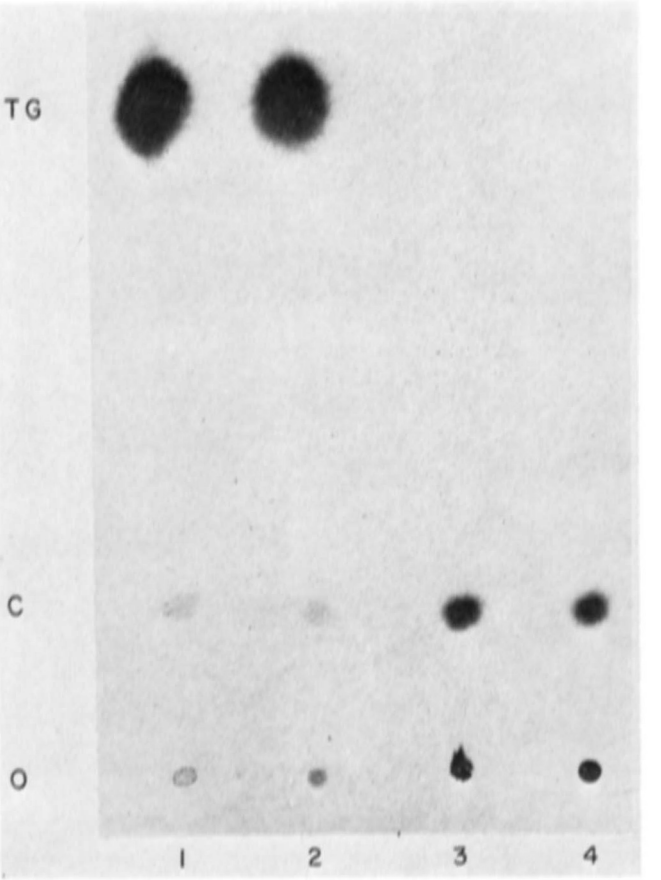

FIG. 3. TLC of adipose tissue and brain. Lanes *1* and *2*, one section of normal human adipose tissue and extract of one section, respectively; lanes *3* and *4*, one section of normal human brain white matter, and extract of one section, respectively. *TG*, triglycerides; *C*, cholesterol; *O*, origin. Adsorbent, Silica Gel G. Solvent, petroleum ether–diethyl ether–acetic acid 70:30:1 (v/v/v). Detection, sulfuric acid–chromic acid spray.

Discussion. Direct TLC in conjunction with densitometry has been shown to be a good method for the rapid estimation of the lipids from brain and adipose tissue. This method can be used not only for the quick identification and estimation of the lipid components, but also for the comparison between identical regions of different samples or different portions or layers of the same sample without extraction. Microextraction has the disadvantage that, in spite of precautions, there is the possibility of change in the concentrations of the extract due to evaporation of the solvent. The present method has the limitation that poorly separated spots can be quantitated as one spot. However, this limitation could be overcome by selecting a suitable solvent system. Lipid analyses from the various other tissues of the rat like liver, kidney, pancreas, heart, and skeletal muscles were also successfully carried out by this method; however, no attempt was made to quantitate the results. Free amino acids from brain sections were also separated using standard procedures. Other tissue constituents, which lend themselves to separation from tissue extracts, could possibly be separated from sections as well by the proper choice of adsorbents and solvents. This method, therefore, has great advantages in obtaining an analysis from a very small sample of an organ (eg. biopsy material). By using adjacent sections for TLC analysis and histochemistry, complementary information can be obtained as to the chemical constituents of tissues. This method may be-

come useful in tissue matching studies involving cardiac, kidney, and other organ transplant operations when rapid analyses are of great importance.

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