notes on methodology

Two-dimensional thin-layer chromatographic isolation of fatty acyl carnitines

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SUMMARY A system of two-dimensional ascending thin-layer chromatography is reported in which palmityl carnitine can be completely separated from all phospholipids on a single chromatogram without the use of the ancillary procedures necessary in previously described systems.

KEY WORDS two-dimensional · thin-layer chromatography · acyl carnitines · isolation

In recent years several communications have appeared dealing with the role of carnitine in fatty acid metabolism (1–8). Several investigators have suggested that acyl carnitines function as carriers of activated fatty acyl groups between intra- and extramitochondrial compartments of the cell (2–5). Accordingly, it has been postulated that palmityl carnitine is an intermediate formed in the initial stages of palmitic acid oxidation (3).

Although palmityl carnitine is soluble in water (3, 4), it can be extracted from tissues only with organic solvents (5, 6), probably because of the formation of palmityl carnitine micelles in the presence of tissue lipids.

Isolation of palmityl carnitine from tissue lipid extracts has been difficult because of the similarity of its chromatographic behavior to that of certain phospholipids (5, 7, 8). For example, although palmityl carnitine can be isolated by thin-layer chromatography (TLC) using the phospholipid separation method of Skipski and coworkers (9), prior removal of phosphatidyl choline with phospholipase A is necessary because the two compounds have the same R_F in the solvent system used (5) (Fig. 1). Similarly, with the method of Friedberg and Bressler (8), palmityl carnitine can be separated from phosphatidyl choline, but palmityl carnitine and sphingomyelin have the same R_F in this system (Fig. 2), and a preliminary removal of sphingomyelin by column chromatography is necessary. A system of two-dimensional ascending TLC is described here in which palmityl carnitine can be

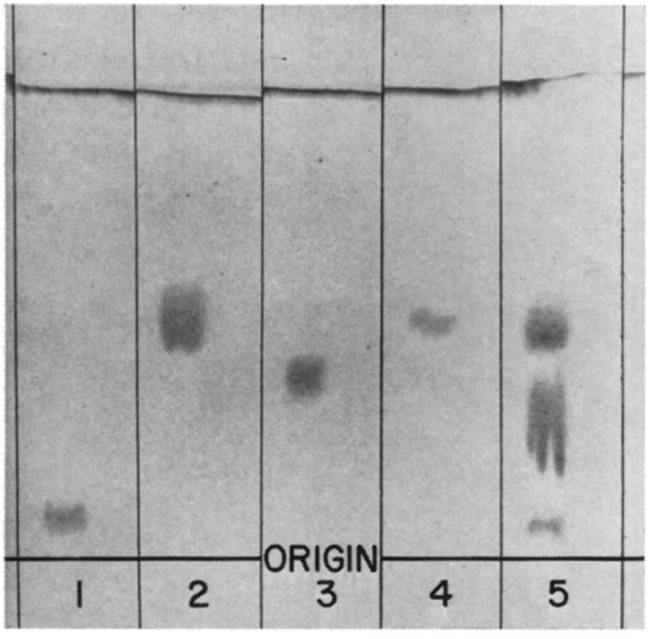

Fig. 1. One-dimensional thin-layer chromatogram of phospholipids and palmityl carnitine developed in chloroform–methanol–glacial acetic acid–water 50:25:8:4. Detection method: iodine vapor. Compounds: *1*, lysophosphatidyl choline; *2*, phosphatidyl choline; *3*, sphingomyelin; *4*, palmityl carnitine; *5*, all four compounds. In this system, palmityl carnitine and phosphatidyl choline overlap.

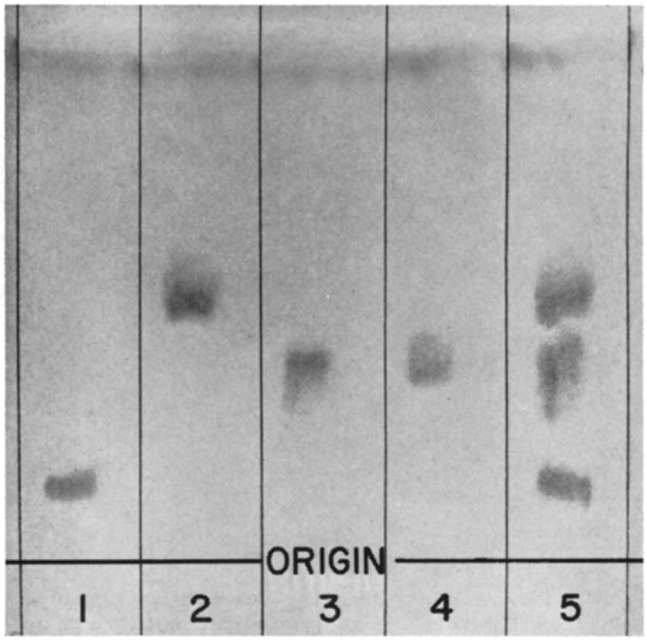

Fig. 2. One-dimensional thin-layer chromatogram of phospholipids and palmityl carnitine developed in chloroform–methanol–ammonia–water 50:35:3:3. Detection method: iodine vapor. Compounds: *1*, lysophosphatidyl choline; *2*, phosphatidyl choline; *3*, sphingomyelin; *4*, palmityl carnitine; *5*, all four compounds. In this system, palmityl carnitine and sphingomyelin overlap.

separated from the phospholipids on a single chromatographic plate without ancillary procedures.

Method. Basic silica gel thin-layer chromatographic plates were prepared by the method of Skipski and coworkers (9). The plates were activated at 90° for 10 min. Standard solutions were applied 3 cm in and 3 cm up from one corner of the plate, which was first developed in the system chloroform–methanol–ammonia–water 50:35:3:3 (volume ratios) (8). This resulted in a separation of palmityl carnitine from phosphatidyl choline, but not from sphingomyelin. The plate was then heated again at 90° for 30 min to remove the solvent completely and to reactivate the adsorbent. The plate was developed in the second dimension with chloroform–methanol–acetic acid–water 50:25:8:4 (9). This separated palmityl carnitine from sphingomyelin.

The compounds were made visible by exposure to iodine vapors, which stained both palmityl carnitine and phospholipids (Fig. 3*A*), and by use of the molybdenum blue reagent of Dittmer and Lester (10), which stained the phospholipids but not palmityl carnitine (Fig. 3*B*). The position of each of the compounds on the two-dimensional chromatogram could be ascertained by analysis of their respective positions after development in each of the two solvent systems separately (Figs. 1 and 2). No displacement of R_F values occurred as a result of using the two-dimensional development. Palmityl carnitine was completely separated from the other lipids. In the two-dimensional system phosphatidyl serine and phosphatidyl ethanolamine would be above and to the right of phosphatidyl choline, and triglycerides and fatty acids would be at the front.

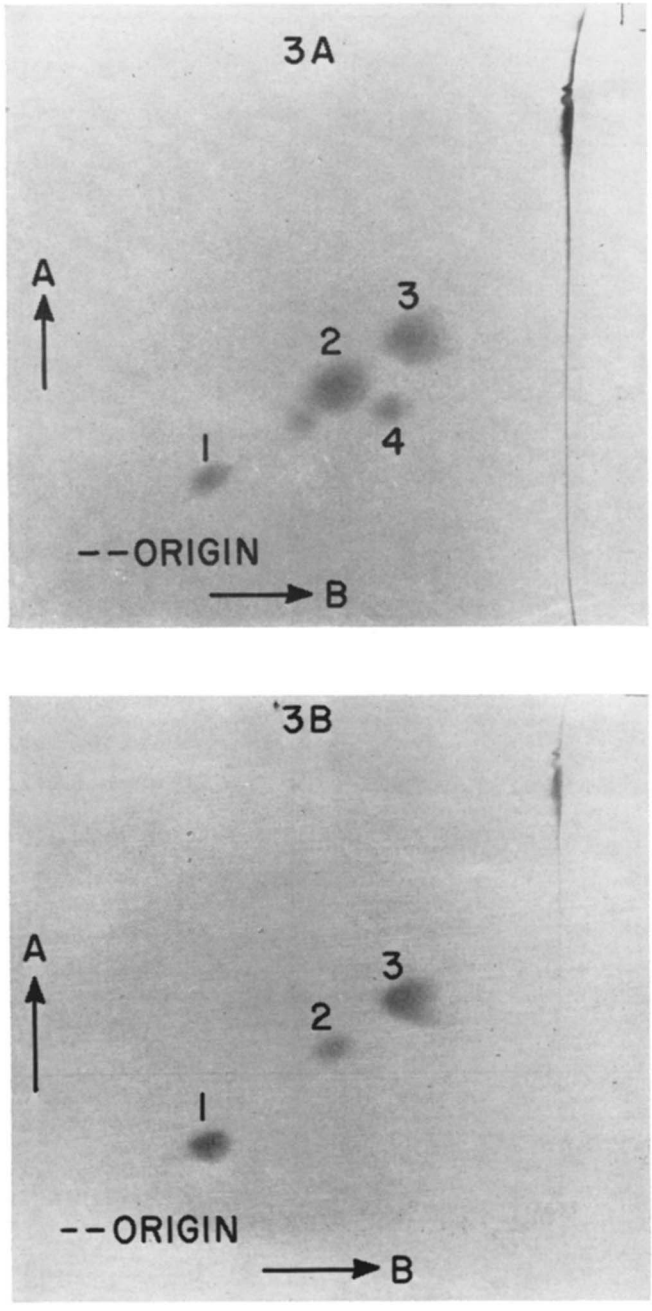

FIG. 3. Two-dimensional thin-layer chromatogram of phospholipids and palmityl carnitine. The plate was first developed in direction *A* in chloroform–methanol–ammonia–water 50:35:3:3, dried, reactivated, and developed in direction *B* in chloroform–methanol–glacial acetic acid–water 50:25:8:4. The plate was exposed to iodine vapor (Fig. 3A), the iodine-stained spots were allowed to fade completely, and the plate was sprayed with the molybdenum blue reagent (Fig. 3B). Compounds: *1*, lysophosphatidyl choline; *2*, sphingomyelin; *3*, phosphatidyl choline; *4*, palmityl carnitine.

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