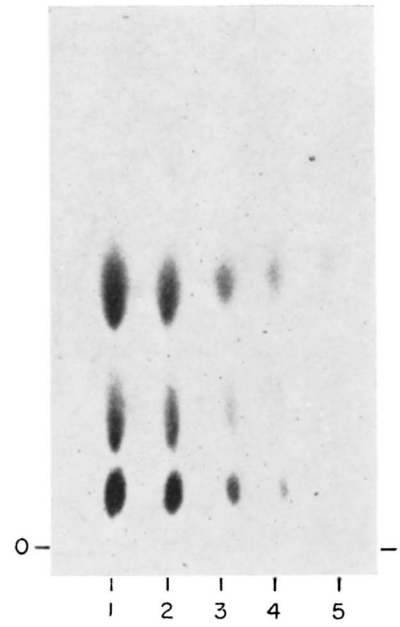

FIG. 1. Lower half of a thin-layer chromatogram on a 5 × 20 cm plate of Silica Gel H developed with chloroform–methanol–2 M ammonia 70:25:3.5. Each lane contains equal amounts of egg lecithin (upper spot), bovine heart sphingomyelin (middle spot), and lysolecithin derived from egg yolk (lower spot). Lanes 1 through 5 contain, respectively, 10, 5, 2, 1, and 0.5 μg of each lipid. A faintly stained spot of each of the lipids was visible to the eye in lane 5 but is barely reproduced photographically. O, origin.

Permanent sensitive stain for choline-containing phospholipids on thin-layer chromatograms

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SUMMARY The phosphomolybdic blue stain for choline of Levine and Chargaff can be used for detection of small amounts of choline-containing phospholipids on thin-layer chromatograms if they are first fixed with polyvinyl propionate to permit a washing step.

KEY WORDS phospholipids · choline-containing · detection · thin-layer chromatography · phosphomolybdic blue · "Neatan" fixing

CHOLINE-CONTAINING phospholipids can be detected on paper chromatograms by the phosphomolybdic blue reaction as described by Levine and Chargaff (1). Because the chromatograms must be washed during the procedure, that technique has not been applicable to thin-layer chromatograms and, instead, the Dragendorff bismuth iodide stain (2) is used. If, however, the thin layer is fixed with a polyvinyl propionate dispersion the Levine and Chargaff stain can be used to detect the choline-containing phospholipids more sensitively, though less specifically, than with the Dragendorff reagent.

Thin-layer chromatograms on silica gel are prepared and developed in the usual manner and sprayed evenly with a dispersion of polyvinyl propionate ("Neatan," E. G. Merck, Darmstadt, Germany). After being thoroughly dried the plate is immersed in water to aid in the release of the film, which is then peeled from the glass with the aid of a razor blade or sharp spatula. The wet film is immersed in a 2% aqueous solution of phosphomolybdic acid for 1 min, washed in running water for 5 min, and dipped in 4% stannous chloride [40% $SnCl_2$ in concentrated HCl (stock solution) diluted 1:10] for about 30 sec. Choline-containing phospholipds give a blue color on a white or faintly blue background. Inadequate washing results in an excessive background color.

This technique will detect about 1 μg of lecithin, lysolecithin, sphingomyelin, or sphingosine phosphoryl choline in a compact spot. It is, thus, more sensitive than the Dragendorff stain and in addition produces a spot of greater contrast, which facilitates photographic reproduction (Fig. 1). With some loss of sensitivity this stain can be applied on top of a rhodamine or ninhydrin stain, or even after a Dragendorff stain. In practice we expose the developed chromatogram first to iodine vapor as a general stain and mark the spots. When the iodine has faded the plate is sprayed with a 0.5% solution of ninhydrin in butanol (bases such as collidine or lutidine cannot be used in the ninhydrin spray in this case, as they lead to an excessively blue background later). The ninhydrin color is developed by warming and by expo-

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TABLE 1 APPROXIMATE LIMITS OF DETECTION FOR SOME LIPIDS NOT CONTAINING CHOLINE

Lipid	Micrograms Detectable
Cholesterol	25
Cholesteryl palmitate	100
Palmitic acid	>100
Dipalmitin	25
Tripalmitin	50
Phosphatidic acid	50
Phosphatidyl ethanolamine	10
Phosphatidyl serine	10
Sphingosine	5

sure of the plate to water vapor. The plate is then fixed and the phosphomolybdate stain is used as described above.

If protected between the leaves of a notebook the stain is relatively stable and moderately dense spots persist for a matter of years. Faint spots may fade within months but may be redeveloped by exposing the film once more to the $SnCl_2$ solution.

The stain, though most sensitive for the choline phospholipids, is relatively nonspecific. Table 1 lists approximate limits of detection for some other lipids.

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