AN IMPROVED METHOD FOR THE DETECTION OF LIPID ON PAPER CHROMATOGRAMS*

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To date, the most satisfactory method for the detection of lipid on chromatograms has involved staining the chromatogram with one of the rhodamine dyes and viewing, while wet, under ultraviolet light (Marinetti, et al., 1957). Rhodamine stain on paper, however, precludes further color test to identify components, requires extensive decolorization if the spots are to be eluted, and does not permit visualization after the paper has dried. This communication describes an improved technique for the detection of lipids employing a dilute acid solution of protoporphyrin.

An affinity of acid hematin for lipid was demonstrated by Baker (1946). He observed that when applied to tissue sections, acid hematin would combine with lipid components. By employing a process of pyridine extraction, it was noted that acid hematin could be used as a specific test for phospholipid (Baker, 1947; Casselman, 1952). Cain (1947) conducted an examination of Baker's test and concluded that the specificity of the test depended on the relatively greater affinity of the phospholipid for the acid hematin than the other lipids. In the present communication it is shown that protoporphyrin, though structurally similar to hematin, is not selective for phospholipid but is bound by neutral fats, cholesterol and many fatty acids as well.

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EXPERIMENTAL

Twenty five mg. of protoporphyrin (Mann, C.P.) was dissolved in 5 ml. of 5 N hydrochloric acid. The solution was made to 500 ml. with water and filtered through Whatman no. 41 filter paper. This preparation remains stable for at least one year at room temperature. Chromatograms were dipped into the protoporphyrin solution and allowed to remain there for five minutes. After washing ten minutes in distilled water, the chromatograms were allowed to drain and viewed under an ultraviolet lamp (long wavelength).

RESULTS AND DISCUSSION

The lipids appear as brilliant red spots on a blue background. The red fluorescence fades within several hours after the papers are dry and for maximum sensitivity should be viewed while the papers are still damp. Under these conditions, less than one microgram of lecithin may be detected on paper whereas when rhodamine is used as a stain, 2 to 5 micrograms of lecithin are required (Marinetti et al., 1957). Furthermore, the protoporphyrin procedure results in very low background and color tests may, therefore, be carried out on the same chromatogram.

The affinity of protoporphyrin for lipid was first observed by Kosaki, et al. (1957) who noted, by fluoromicroscopy, a marked cellular affinity for protoporphyrin. The myelin sheath of nerves was particularly involved (Kosaki, Kotani, Nakagawa and Saka, 1957). This binding was further investigated by fractionating beef brain lipid and testing each component for its ability to bind protoporphyrin. Under the conditions, spingomyelin and spingomyelin-containing lipids were the only fractions having this binding capacity (Kosaki, Ikeda et al., 1957). Using the acid solution of protoporphyrin and applying it to lipids purified by paper chromatography, we have not been able to demonstrate any specific binding of protoporphyrin by phospholipid. On the contrary, protoporphyrin is an extremely sensitive substance in the detection of all phospholipids and many other lipids as well.

TABLE 1

Affinity of Protoporphyrin for Various Compounds*

Compound	Reaction	Compound	Reaction
lecithin [1]	pos	tributyrin [2]	pos
lysolecithin [1]	pos	monobutyrin [2]	pos
choline chloride [2]	neg	butyric acid [2]	neg
phosphoryl choline chloride [2]	neg	caproic acid [4]	neg
sphingomyelin [1]	pos	caprylic acid [6]	pos
inositol phosphatide [1]	pos	capric acid [6]	pos
cholesterol [4]	pos	lauric acid [6]	pos
cholesterol acetate [2]	pos	myristic acid [2]	pos
cortisone [3]	neg	palmitic acid [2]	pos
hydrocortisone [3]	neg	stearic acid [2]	pos
testosterone [3]	neg	oleic acid [5]	pos
triolein [2]	pos	n-octyl alcohol [2]	neg
tristearin [2]	pos	α-tocopherol [5]	neg
tripalmitin [2]	pos	α-tocopherol succinate [7]	pos
trimyristin [2]	pos		

* These compounds were dissolved in appropriate solvents, applied to filter paper in small spots, dried in a hot air oven and treated with protoporphyrin as described. [1] Blood lipids purified by paper chromatography, [2] Eastman Kodak, [3] Sigma, [4] Matheson, [5] merck, [6] Nutritional Biochemicals, [7] Mann.

A number of substances were tested with protoporphyrin to determine its selectivity. The results of some of these tests are presented in Table 1.

Certain structural characteristics appear to be necessary for staining by protoporphyrin. It is notable that fatty acids below eight carbons give a negative test as does octyl alcohol making it appear that a carboxyl group in aliphatic compound of at

least eight carbon atoms is necessary. However, it is also interesting to note that monobutyrin which contains seven carbon atoms in an ester linkage is positive. Among the polynuclear compounds tested, cholesterol and its acetate are positive whereas steroids without the isooctane side chain are negative. The structural requirements of this staining test will be further explored.

SUMMARY

A simple technique is described whereby, using an acid solution of protoporphyrin, as little as one microgram of lipid may be detected on paper chromatograms. This substance is bound to certain lipids and fluoresces in ultraviolet light. The protoporphyrin does not stain the paper and thereby permits color testing on the same chromatograms.

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