Preservation and photography of rhodamine 6g stained lipid spots*

CHARLES M. GROSSMAN and ALAN M. MACEWAN†

Department of Chemistry,
University of Portland,
Portland, Oregon

[Received for publication March 15, 1960]

► In recent years Rhodamine 6G and ultraviolet fluorescence have been widely used for identification of lipid spots on paper. The technique has been particularly useful in identifying phosphatides separated by chromatography on silicic acid-impregnated paper (1). Marinetti *et al.* (2) have successfully used Rhodamine 6G for identifying radioactive phosphatide spots for counting. The chief disadvantage has been that stained strips must be examined while wet. Upon drying, both the fluorescence and the colors change significantly. To compare strips prepared on different days, the investigator must depend upon a subjective diagrammatic representation. Reports in the literature have also contained such diagrams. Although a photograph of a Rhodamine 6G-stained chromatogram was published recently by Troup *et al.* (3), the spots were accentuated by encircling them and some of the spots within the circles were not clearly visible.

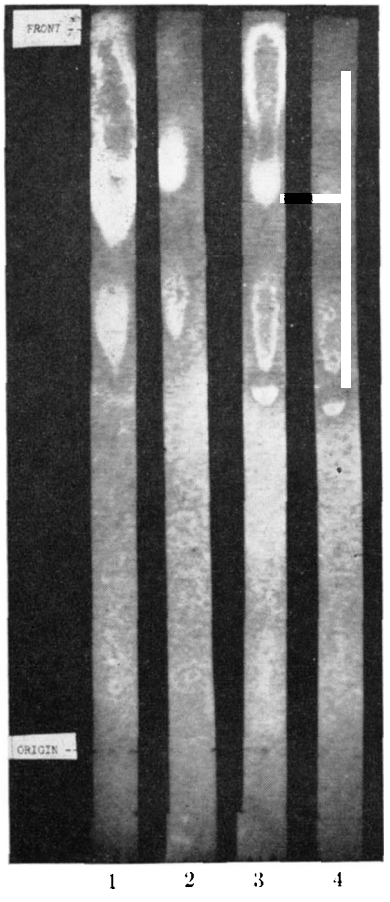
The original characteristics of the fluorescent spots have been easily preserved for months simply by wrapping the strips in Saran Wrap.[1] This maintains the necessary amount of moisture and permits observation in ultraviolet light without unwrapping the strips. In some of our chromatograms of plasma on silicic acid-impregnated glass paper according to the method of Brown *et al.* (4), it has been noticed that the lysolecithin spot faded within 24 hours. Except for this, all other areas remained constant in appearance for many months after wrapping the strips.

To avoid diagraming the spots, photography was attempted. For reproduction of the ultraviolet fluorescence, black-and-white film suffices. Figure 1 represents such a reproduction of chromatograms of plasma and red blood cell phosphatides from a normal male. The strips were stained with a freshly prepared 0.001%

* This work was supported by Research Grant H-4826 from the National Heart Institute and by a grant from the A. H. Robins Co., Richmond, Va.

† Present address: Department of Biology, University of Arizona, Tucson, Ariz.

[1] Dow Chemical Co., Midland, Mich.



1. Plasma total lipid
2. Plasma phosphatide
3. Red cell lipid
4. Red cell phosphatide

FIG. 1. Rhodamine 6G stain and ultraviolet fluorescence of plasma and red cell whole lipid and precipitated phospholipid from a 50-year-old normal male. Strips *1* and *3* have lipid superimposed upon the phosphatidylethanolamine area. A suggestive inositol phosphatide spot is seen in the red cell strips. On the original strips the colors were as follows: *a*, dark blue surrounded by yellow; *b*, pale yellow; *c*, bright yellow; *d*, yellow; *e*, yellow center in purple; *f*, light purple in a darker purple background.

aqueous Rhodamine 6G solution for 2 minutes, followed by a 1-minute wash with running tap water. The ultraviolet source was a Hanovia black Woods light[2] with a wave length of 3600 to 3700A. The wet strips were wrapped in Saran Wrap and photographed, using a high contrast film (Contrast Process Ortho) and a double thickness of a Wratten 2 B gelatin filter, which absorbs the reflected ultraviolet light. With two lamps 16 inches from the strips at angles of approximately 45° each, a 45-second exposure and f/11 aperture were used. The film was developed with D 11 developer. Since some reflections occurred with the Saran Wrap, it was found desirable to remove this for the photographic exposure, following which the strips were again wrapped.

[2] "Inspecto-lite," Engelhard Industries, Inc., Elmhurst, N. Y.

The chromatograms presented in Figure 1 are original uncut strips from each of four different runs done in triplicate. An area of glass paper about 5 mm wide and extending the entire length except for 1 cm at the bottom and top was cut out to separate the individual strips. The strips were chosen pretty much at random and selection of the best chromatograms was deliberately avoided. Our purpose is to show the results of photography primarily, since reproduction by photography in published reports should permit a more ready comparison of chromatograms from different laboratories. The identification of the phosphatides presented was achieved by appropriate stains, by comparisons with simultaneous radioautograms, and by comparing R_f values with synthetic compounds or purified biological materials (except in the case of the inositol phosphatide). We have not as yet identified a phosphatidylserine spot in red cell chromatograms.

These techniques should be helpful in preserving and recording other "wet" stained chromatograms and might be applicable to the protoporphyrin stain and ultraviolet fluorescence of lipids recently reported by Sulya and Smith (5).

Since this manuscript was submitted, Vogel and Zieve (6) have published some photographs of dry chromatograms viewed in ultraviolet light.

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