J. Lipid Research, April 1963
Volume 4, Number 2

Notes on Methodology

Assay of the radioactivity of the aldehydogenic moiety of plasmalogens*

EDGAR M. NEPTUNE, JR., and R. S. IDE
*Naval Medical Research Institute,
National Naval Medical Center,
Bethesda, Maryland*

[Manuscript received September 4, 1962; accepted November 30, 1962.]

» Although a reliable and sensitive technique exists for the chemical determination of aldehydes derived from phosphatides (1), the routine assay of their specific radioactivity is a very laborious procedure (2). In the chemical measurement, a hydrazone of the aldehyde is formed and is finally dissolved in ethanol. When tracer palmitic acid, labeled with carbon-14, was added to the first step in the aldehyde assay, a large percentage of the radioactivity appeared in the final solution of hydrazone. Therefore, if any radioactive fatty acid ester is cleaved during formation of the *p*-nitrophenylhydrazone, some of the radioactivity would appear in the final solution. Thus, the assay of radioactivity of the final ethanolic solution cannot be used for determining the specific activity of the hydrazone.

Authentic samples of palmitic acid and palmitaldehyde-hydrazone were placed on thin-layer silicic acid plates (3). Of the various solvent systems tried, *n*-hexane–ether–acetic acid 75:25:2 appeared to yield the best separation. The *n*-hexane was distilled over aqueous potassium permanganate; the distilled hexane was kept in the receiving vessel for 24 hr and was then decanted with care so that the small amount of water remained in the original receiving vessel. The hexane was dried over anhydrous sodium sulfate. The ethyl ether was anhydrous. The acetic acid was glacial.

With this system, an approximate R_f of 0.63 was observed for the free acid and 0.54 for the hydrazone. Although the spots are close, they are distinctly separate. 2′,7′-Dichlorofluorescein (0.2% in absolute ethanol) was used as an indicator spray; with this dye palmitic acid fluoresces, but the hydrazone quenches ultraviolet light. This technique will detect 20 μg of either compound. The two spots can be scraped off the plate and collected in tubes, and the lipids eluted with methanol. An aliquot of the methanol can then be assayed for radioactivity in a gas flow counter.

When the chemical concentration is very low, unlabeled hydrazone and acid may be added as carrier to permit visualization of the spots. Any remaining phosphatide in the final ethanol solution will remain at the origin of the chromatogram. In this laboratory, neutral lipids are routinely separated from phospholipids before commencing further separations and analyses (4). This separation must be made initially because cholesterol and diglyceride have the same R_f as the hydrazone and may contribute radioactivity to the hydrazone spot.

This method is simple and rapid. Multiple samples can be run simultaneously. Recovery of 100 μg of authentic palmitic acid-1-C^{14} or 100 μg of authentic palmitaldehyde-1-C^{14} hydrazone was 92–95% of the applied radioactivity. This was determined by using one 20-μl pipette, which was rinsed in chloroform and dried before each sample was taken. One aliquot of the standard was pipetted directly into a planchet, dried, and counted in a proportional gas flow counter; a second aliquot was pipetted onto the chromatographic plate and was developed, eluted, and counted as described above. Radioassay by scintillation technique might present difficulties due to quenching.

Although better separations might be obtained with subtle modifications of the reagents or technique, the method as described has given satisfactory resolution and reproducible results in our laboratory.

* The opinions or assertions expressed herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.

REFERENCES

1. Wittenberg, J. B., S. R. Korey, and F. H. Swenson. *J. Biol. Chem.* **219**: 39, 1956.
2. Korey, S. R., and M. Orchen. *Arch. Biochem. Biophys.* **83**: 381, 1959.
3. Stahl, E. *Chemiker Zeit.* **82**: 323, 1958.
4. Colodzin, M., E. M. Neptune, Jr., and H. C. Sudduth. *J. Lipid Res.* **3**: 234, 1962.

Downloaded from www.jlr.org by guest, on June 19, 2012