Application of Thin-Layer Chromatographic Zonal 
14C-Profile Scans to the Analysis of Urinary 
Constituents Derived from Lipids

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Thin-layer chromatography (TLC) has been a useful tool in the analysis of numerous urinary constituents; as in most applications of adsorption TLC, it is best used in conjunction with other chromatographic processes. The application of TLC to the resolution of polar urinary products derived from lipids is shown for the metabolism of a 14C-labeled fatty acid and a 14C-labeled glyceryl thioether. An automatic system, including fraction collection of adsorbent, pipetting, data transmission, and computer calculations, was used to obtain the TLC zonal 14C profiles.

THIN-LAYER CHROMATOGRAPHY (TLC) is a useful tool in the analysis of many urinary constituents such as steroids, amino acids, porphyrins, and bile acids (1-9). Urinary analysis by TLC is most effective when done in conjunction with other chromatographic processes, so that minor metabolites can be detected and resolved completely free of other components. The purpose of this paper is to describe recent improvements in an automated TLC zonal analysis system (10) developed in our laboratory for lipids and to show how this system can be used for urine analyses. Although lipids are not normally found in urine, we detected polar 14C products derived from a fatty acid and a glyceryl thioether fed to rats.

Methods

Animal Experiments

Twenty microcuries of a 14C-labeled glyceryl thioether (thio-[1-14C-hexadecyl]-2,3 propanediol, 2.01 μc./mg.) or 50 μc. of palmitic-14C acid (25.4 μc./mg.) were administered by stomach intubation to female

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Charles River strain rats. All animals were placed individually in rat metabolism cages (4–½” × 8 × 4–½” in., Model AC-5062, Acme Metal Products, Inc.), which collected urine free of fecal contamination for a 24-hr. period. Some urine samples were extracted 3 times with diethyl ether or butanol before chromatography and radioassay.

**Thin-Layer Chromatography and Zonal Analysis System**

All TLC was carried out on 250-μ layers of silica gel G. Urine samples (5 μl.) were applied directly to the chromatoplates and chromatographed immediately. A standard mixture of several short-chain acids (formic, acetic, propionic), glycerol, and citric acid was also chromatographed to check on the resolution attainable in the solvent systems used. The radiopurity of the 14C standards of the mixture and of the ingested labeled lipids was > 99% as determined by TLC zonal profile analyses (10). Our chromatograms were developed at ambient temperature in equilibrated solvent chambers containing either ethanol: NH₄OH: H₂O (80:4:16, v/v/v) or butanol:water (90:10, v/v). Resolved components were visualized with iodine vapor, after which adjacent 2-mm. zones of a chromatographic lane from below the origin to above the solvent front were collected with an automatic zonal scraper (Fig. 1) developed in our laboratory (11).

The scraper system has now been made more useful by the development of an automatic pipetting device (Fig. 1) whose turntable is compatible with the sample-holder trays from the zonal scraper and which dispenses scintillation fluids into the vials already containing TLC adsorbent. The design of the turntable transport mechanism is identical to that of the zonal scraper (11, 12). The volume of fluid dispensed (0.2–22.0 ml.) is arbitrarily set with a calibrated screw that determines the precise stroke of a Teflon-stainless steel piston displacing the desired volume of fluid from a stainless steel cylinder.

Fig. 1. Automatic zonal scraper (A) for 1-, 2-, or 5-mm. collecting of adsorbent layers from thin-layer chromatograms; and an automatic pipetting device (B) for dispensing 0.2–22.0 ml. of scintillation solution.
piston-cylinder unit, including 2 stainless-steel solenoid check valves, is connected to the fluid reservoir by Teflon tubing.

The turntables from the zonal scraper fit not only the pipetting device just described, but also the Packard 4000 scintillation counter, where quantitative measurements of both radioactivity and chemical mass may be made. The values for chemical mass are based on the color quenching of a standard scintillation source by a charred sample (13). The scintillation spectrometers are connected to a computer by a dedicated telephone line involving a commercially available data phone as seen in Fig. 2 (Western Electric 401A). An interface scanner (Fig. 2), developed by our Technical Services Department (14), determines instrumentation priority and also includes a manual digital input for transmission of identification symbols of data groups. The data receiving and processing system include a data receiver (Western Electric 401J), a computer, and a Benson-Lehner electroplotter. From this entire system we receive zonal profile scans (Fig. 3 and 4) and a data sheet of calculations as directed by a computer program in Fortran. The area percentage under each peak of the zonal profile is printed out on the data sheets, in addition to statistics, specific activities, and other calculations desired. The excellent portrayal of resolution obtainable with this 2-mm. fractionation of adsorbent layers is evident from the

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**Fig. 2.** Automatic data transfer system for transmitting digital read-out from liquid scintillation spectrometers: data phone (A), interface scanner (B), and liquid scintillation spectrometers (C).
\( ^{14}\)C-profile scans of the standard mixture illustrated in Fig. 3. Chromatographic resolution of the 5 peaks shown in Fig. 3 did require double development in the butanol:water solvent system (90:10 v/v); single development yielded only 4 peaks. The TLC zonal scanning sys-

![Diagram](https://via.placeholder.com/150)

**Fig. 3.** Zonal \(^{14}\)C-profile scan of a standard mixture of \(^{14}\)C compounds resolved on 250-μ layers of silica gel G. Solvent development was carried out twice in a solvent system of butanol:water (90:10 v/v). Peak numbers refer to citric acid (1), acetic acid (2), formic acid (3), propionic acid (4), and glycerol (5).

tem may be likened to collecting infinite samples of a column eluate for analysis or to continuous column monitoring; it has recently been reviewed in depth for the analysis of labeled lipids (13, 15).

**Results and Discussion**

We applied the TLC system just described to the analysis of minor constituents of urine originating from the metabolism of lipids. These animal experiments were directed merely at illustrating the usefulness of TLC and zonal scanning in urine analysis, and Fig. 4B clearly shows
the sensitivity of our system for the products derived from palmitic-1-$^{14}$C acid. Less than 0.82% of the $^{14}$C administered was found in a 24-hr. urine collection; none of this was extractable with diethyl ether. TLC resolved a single peak of activity and 3 closely related activity peaks which differed from each other by less than 100 disintegrations per minute, yet were easily detected as separate components.

Our experiment with the glycercyl thioether demonstrated the advantage of isolating metabolites by TLC-adsorption chromatography.

![Graph A](image)

**Fig. 4.** Zonal $^{14}$C-profile scans of urinary components isolated from rats fed A glycercyl 1-$^{14}$C hexadecyl thioether or B palmitic-1-$^{14}$C acid. Solvent development for chromatography was: A carried out twice in butanol:H$_2$O (90:10 v/v); B ethanol:NH$_4$OH:H$_2$O (80:4:16 v/v/v).
before anion-exchange chromatography. Unlike O-ethers and other lipids, glyceryl 1-14C hexadecyl thioether was metabolized to produce a major nonlipid 14C product (45% of the total 14C activity fed). The 14C constituent originating from the glyceryl thioether could not be extracted with diethyl ether or butanol. However, the urinary 14C in the aqueous phase was isolated as a single component (Fig. 4A) on 250-µ silica gel G layers in a solvent system of ethanol:ammonium hydroxide: water (80:4:16 v/v/v). Chromatography by ion-exchange chromatography resolved this single 14C component into 2 major and 3 minor 14C fractions. In this particular instance, TLC preferentially concentrated the urinary 14C components before subsequent chromatography.

References