Results of the biliary excretion study imply that the disposition of material excreted into the duodenum *via* the bile does not involve simple direct excretion in the feces. Excretion of large amounts of S^{35} into the duodenum *via* the bile does not lead to fecal excretion of an appreciable quantity of S^{35} during the initial 24 hours after intraperitoneal drug administration. However, about five times as much is excreted in the feces during the first 24 hours when the drug is introduced directly into the gastrointestinal tract (oral dosing). The finding that none of the administered dose is excreted in the bile as metabolite I implies that this metabolite represents further alteration of either metabolite II or III.

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Thin-Layer Radiochromatographic Study of Prochlorperazine Photodeterioration

By SETSUYA SENO, WAYNE V. KESSLER, and JOHN E. CHRISTIAN

Procedures to determine microquantities of photochemical deterioration products of prochlorperazine were developed by using thin-layer chromatography in combination with the radioisotope tracer technique. Up to 11 products were separated on a silica gel thin-layer plate using ethylene dichloride-methanol-ammonia as the solvent. The separated radioactive spots were scraped off the plate, suspended in a Thixcin scintillator gel, and counted. Quantitative results for each product indicated that complex reactions are involved in the photochemical deterioration of prochlorperazine.

PHENOTHIAZINE DERIVATIVES are decomposed by light into various oxidative and cleavage compounds (1–3). They are metabolized by *N*-methyl and side chain cleavage and formation of sulfoxides, phenols, free radicals, and *N*-oxides (4). Several analytical procedures have been used for separating and identifying phenothiazine derivatives and metabolites, especially those of chlorpromazine (4). The most important of these was paper chromatography in combination with spectrophotometry and the radioisotope tracer technique.

Thin-layer chromatography recently has become widely recognized because of many advantages over paper chromatography, such as speed, simplicity, and high sensitivity. The combination of thin-layer chromatography and the radioisotope tracer technique is especially well adapted to very small samples. This combination was reported by Snyder and Stephens (5) in which C^{14} and H^3 labeled lipids were analyzed. The purpose of this study was to use thin-layer chromatography combined with the radioisotope tracer technique for the microquantitative study of the photochemical deterioration of prochlorperazine.

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EXPERIMENTAL

Materials.—Fixed thickness chromatofilm assembly, ascending (model 200-1, Research Specialties Co., Richmond, Calif.) with 8 × 8-in. glass plates was used. Also used were silica gel G (Merck, Germany); prochlorperazine ethanedisulfonate and prochlorperazine-S-35 ethanedisulfonate (Smith Kline and French Laboratories, Philadelphia, Pa.) (the purity of the labeled compound was 99.1%); a Beckman model DB spectrophotometer; a Packard model 314-X Tri-Carb liquid scintillation spectrometer; and solvents and chemicals of reagent grade or equivalent.

Thin-Layer Chromatography.—Aqueous solutions of unlabeled prochlorperazine ethanedisulfonate (0.010, 0.10, and 5.0 mg./ml.) were irradiated by sunlight for 2 hours. The solutions became colored shortly after exposure and darkened further upon continued exposure. The change in the absorption

spectrum is shown in Fig. 1. After irradiation, prochlorperazine lost the characteristic absorption maximum at 255 mμ, became colored, and showed an absorption maximum at 500 mμ. The resultant solutions which were assumed to contain intact prochlorperazine and its deterioration products were analyzed by thin-layer chromatography.

Silica gel G thin-layer plates were prepared in the usual manner. The plates were activated at 105° for 1 hour. They were spotted with 5, 10, and 25-μl. aliquots of the irradiated solutions and were developed. Twelve solvent systems were examined. It took 30 minutes to 2 hours for the solvent front to advance 10 to 15 cm., the time depending on the nature of the solvent and the temperature of the developing chamber. After the solvent had advanced 10 to 15 cm. from the starting point, the plate was removed from the chamber, dried in a hood at room temperature, and subjected to detection of the separated spots. Ultraviolet lamps (dominant wavelengths 2537 and 3660 Å.), iodine solution (1% in methanol), 2′,7′-dichlorofluorescein solution (0.2% in methanol), iodoplatinic acid solution (0.3% aqueous), sulfuric acid (20% aqueous), and antimony trichloride solution (20% in chloroform) were used. Color production by spraying with antimony trichloride solution gave the highest sensitivity. The detection limit was approximately 0.1 mcg. of prochlorperazine ethanedisulfonate. The best separation was obtained with an ethylene dichloride-methanol-ammonia (65:35:5) mixture.

Five-milliliter aliquots of aqueous prochlorperazine ethanedisulfonate solution (1.0 mg./ml.) were placed in test tubes. The test tubes were plugged with Saran wrapped corks, placed under an ultraviolet lamp (dominant wavelength 3660 Å.) 40 cm. from the filter, and irradiated for 1 to 30 hours. Irradiation caused changes in their color and absorption spectra. Changes in absorbance values at 255 and 500 mμ are shown in Fig. 2. Five-microliter aliquots of the original and irradiated solutions were spotted on a thin-layer plate and chromatographed using the ethylene dichloride-methanol-ammonia solvent. From one to eight separate spots were detected on the thin-layer plate by the color reaction with antimony trichloride. The chromatogram is shown in Fig. 3. The spots with the highest R_f value (R_f = 0.83) correspond to intact prochlorperazine. Others are those of deterioration products. They were not identified in this study.

Thin-Layer Radiochromatography and Scintillation Counting.—Prochlorperazine-S-35 ethanedisulfonate was dissolved in water to yield a concentration of 1.188 mg./ml. (specific activity 0.839 μc./ml.). Aliquots of this solution, each measuring 3.5 ml., were placed in test tubes and irradiated with ultraviolet light for 1 to 33 hours in the same manner as the unlabeled prochlorperazine. Five-microliter aliquots of the original and irradiated solutions were then chromatographed. An autoradiogram of the chromatogram was made by placing a sheet of X-ray film (Kodak No-screen) directly in contact with the silica gel layer and exposing for 80 hours. The developed autoradiogram is shown in Fig. 4.

The radioactivity of each spot located by autoradiography was measured with a liquid scintillation spectrometer. Since silica gel is insoluble and tends to settle in ordinary scintillator solutions and since the complete extraction of adsorbed radioactive material is uncertain, it was desirable to use a scintillator gel system for the radioactivity measurements. A Thixcin gel scintillator similar to that reported by White and Helf (6) was used. The gel consisted of 2.5% Thixcin R in a toluene scintillator

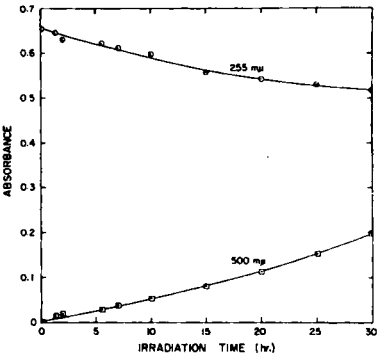

Fig. 2.—Effect of ultraviolet irradiation (dominant wavelength 3600 Å.) on the absorption spectra of prochlorperazine.

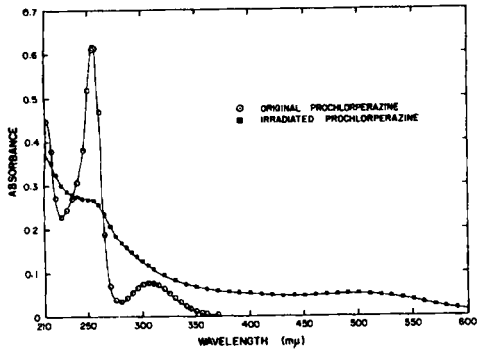

Fig. 1.—Change in absorption spectrum of prochlorperazine upon exposure to sunlight. Concentration of prochlorperazine ethanedisulfonate was 0.010 mg./ml.

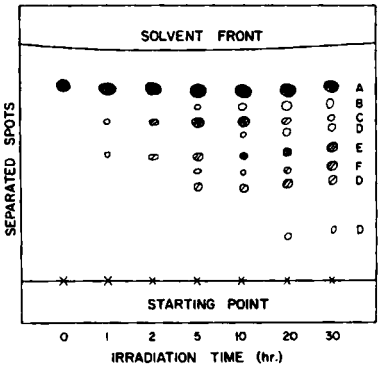

Fig. 3.—Thin-layer chromatogram of photodeterioration products of prochlorperazine. Solvent front averaged 13.3 cm. from the starting point. Color of spots (using antimony trichloride reagent) were: A, pink; B, faint pink; C, purple; D, faint violet; E, dark blue; F, violet.

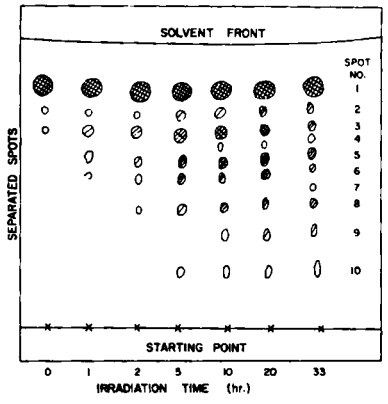

Fig. 4.—Autoradiogram of thin-layer chromatogram of photodeterioration products of prochlorperazine-S-35. Spot 1 corresponds to prochlorperazine.

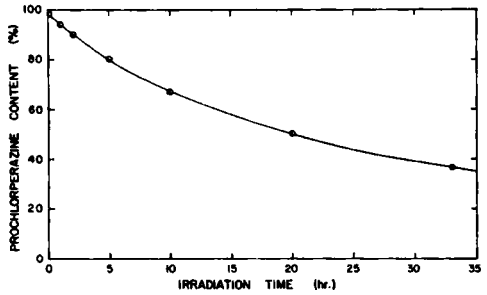

Fig. 5.—Decrease in prochlorperazine content of a sample upon ultraviolet irradiation.

containing 0.5% PPO (2,5-diphenyloxazole) and 0.03% POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene].

The adsorbed spots on the thin-layer chromatogram were scraped quantitatively into a counting vial. The silica gel powder was then suspended in 15 ml. of the scintillator gel. A slight yellow coloration of the gel caused some depression of the counting efficiency. This coloration was prevented by adding 50 μl. of dimethylformamide. All samples counted with the same efficiency. The quantity of material in a spot at each irradiation time was calculated as the per cent of the total count rate at that irradiation time.

The decrease in the prochlorperazine content of the samples upon irradiation is shown in Fig. 5. The curve is that of a first-order reaction during the initial 15 hours, but it deviates thereafter. The calculated first-order rate constant is 0.037 hour^{-1}.

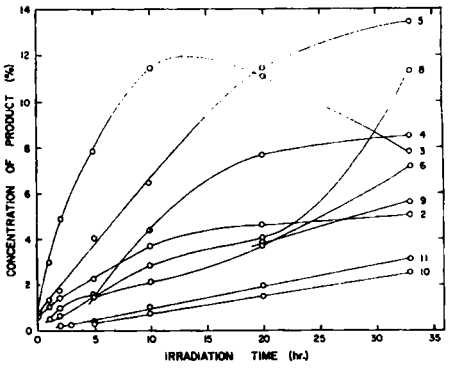

Fig. 6.—Relationships between the concentration of the deterioration products of prochlorperazine and irradiation time. Numbers on curves refer to spot numbers in Fig. 4. Spot 7 is included with spot 8 at 33 hour. Spot 11 refers to starting point.

Figure 6 shows the concentration changes of the deterioration products detected. These curves show that progressive changes of the products into new ones are occurring in the photochemical deterioration process.

CONCLUSIONS

The detection sensitivity of the separated spots was greater in the autoradiographic procedure (Fig. 4) than in the chemical procedure (Fig. 3) in which antimony trichloride was used. This was shown by the greater number of spots detected in the autoradiographic procedure. The detection sensitivity could be increased by increasing the exposure time of the autoradiogram and the specific activity of the sample.

The technique in which thin-layer chromatography is combined with autoradiography and scintillation counting is useful in the study of drug stability, especially in the early stage of deterioration. The sensitivity of the technique would be generally much greater than that of other methods of analysis.

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