PRELIMINARY STUDIES OF THE METABOLISM OF LYSERGIC ACID DIETHYLAMIDE USING RADIOACTIVE CARBON-MARKED MOLECULES[1, 2, 3]

E. S. BOYD, E. ROTHLIN, J. F. BONNER, I. H. SLATER,[4] AND H. C. HODGE

Radioactive LSD-25 as prepared by Stoll, Rutschmann, and Hofmann (4) was used in these experiments. This compound contained radioactive carbon in the alpha position of each of the ethyl groups of the amide part of the molecule. The activity was such that 50 mg. of the crystalline material contained 270 microcuries. Since this is approximately 12,000 cpm./μg. and since the background of our counter is about 120 cpm., accurate counts could be obtained on samples as small as 0.01 μg. of LSD-25.

Rats were given 1 mg./kg. body weight of the labeled LSD-25 intraperitoneally and placed in a metabolism cage. The air entering the cage was dried and the carbon dioxide removed. The air leaving the cage was led through a series of carbon dioxide absorbers (potassium hydroxide solutions) arranged in such a way that the absorbers could be sampled at any desired time interval. Urine and feces were also collected. Rats were sacrificed at time intervals from 1½ to 12 hours after administration of the LSD-25, and the tissues were analyzed for radioactivity.

About 3½ per cent of the administered radioactivity was found in the expired air at the end of 12 hours (1). By this time the rate of expiration of labeled carbon had decreased to an insignificant value. The ratio of carbon 14 activity to milligrams of carbon dioxide (specific activity) in the various carbon dioxide traps indicated that all of the radioactivity exhaled was in the form of carbon dioxide. By the end of 12 hours about 3 per cent of the administered radioactive carbon had been excreted in the urine and only about 1 per cent in the feces. It should be noted that these figures and the following data on distribution in the tissues refer only to radioactive carbon and not to LSD-25. Other data, however, indicate that most of the radioactivity in all samples except expired air represents LSD-25 or metabolites not greatly altered chemically from the original molecule.

The distribution of radioactivity in most of the tissues of the rat in the period from 1½ hours to 12 hours after administration is fairly uniform; most of the tissues contain relatively similar, small concentrations of radioactivity. These concentrations seem to be interdependent and decrease slowly and proportionally over the 12-hour interval. Of the tissues analyzed, the liver and kidney tended to have higher concentrations and the brain and adipose tissue lower concentrations. The gut and its contents were the exception; together they contained 70 to 80 per cent of the dose at 3 and at 12 hours after administration.

These data give a clear picture of the over-all distribution. After intraperitoneal administration LSD-25 is picked up by the blood stream and is carried primarily to the liver where the LSD-25 and/or its metabolites are rapidly excreted into the bile. Only a fraction of the dose (probably 10 to 20 per cent) ever reached the systemic circulation in the rat. The LSD-25 and its metabolites which do reach the systemic circulation are fairly evenly distributed throughout the body. Some enterohepatic circulation of the LSD-25 and its metabolites between the liver and the gut would account for the fact that the level of radioactivity in the liver decreases only slowly from 1½ to 12 hours after administration.

A number of experiments was performed in which the bile ducts of female white rats were cannulated according to the method of Siperstein and Chaikoff (3). The rats were

[1] Read before the Tenth Annual Meeting, Society of Biological Psychiatry, Chicago, June 11–12, 1955.

[2] From the Division of Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, New York, and the Sandoz Company, Switzerland.

[3] This investigation was supported in part by a grant from the Geschickter Fund for Medical Research Inc.

[4] Present address: Eli Lilly and Company, Indianapolis, Indiana.

then given 1 mg./kg. body weight of the C^{14}-labeled LSD-25 intravenously, and the bile was collected for 3 hours. Two experiments in which the total radioactivity in the bile was determined showed an average of 64 per cent of the administered dose in the bile and 12 per cent in the contents of the gastrointestinal tract.

Several experiments were performed in which either the bile itself or an alkaline alcohol (2 per cent of 15 N ammonium hydroxide in absolute alcohol) extract of lyophylized bile was chromatographed on Whatman no. 1 paper in a n-butanol-acetic acid-water (6:1:5) system. The papers were then examined under ultraviolet light, and the fluorescent areas outlined, sprayed with Ehrlich's reagent (6), and counted in an apparatus similar to that described by Jones (2).

The chromatograms showed two distinct compounds with Rf values of 0.23 and 0.15 which were labeled M-1 and M-2 for reference purposes. There was also a spread of radioactivity in the region of Rf 0.6 to 0.9. The number of compounds represented in this spread is as yet uncertain, but it seems likely that at least one of them is unchanged LSD-25. The compounds M-1 and M-2 represent roughly 60 per cent of the total radioactivity in the bile. The infrared spectra of M-1 and M-2, as determined by the method of Toribara and DiStefano (5), are distinct from each other and from those of LSD-25, LAE (the monoethylamide), and lysergic acid. The compounds are compared in table 1 with LSD-25 on the bases of fluorescence, colors with Ehrlich's reagent, and Rf values.

The fluorescence of the metabolites and the colors with Ehrlich's reagent indicate that most of the ring structure of the parent compound is still intact. The radioactivity indicates that at least part of the diethyl side chain is still present. In addition, the decreased Rf values, in the system used, indicate an increased polarity in both of the compounds.

TABLE 1.—Comparison of LSD-25 with Two of its Metabolites

Compound	Fluorescence	Color with Ehrlich's Reagent	Rf Value in Butanol-Acetic Acid-Water
LSD-25	blue	violet	0.83
M-1	blue	violet	0.23
M-2	blue	blue	0.15

A tentative identification of M-1 and M-2 in the urine of rats given 1 mg./kg. of LSD-25 intraperitoneally indicates that these metabolites may be distributed widely throughout the body.

The biosynthesis of radioactive ergot alkaloids was attempted. Rye plants were grown, and the flowers were inoculated with ergot spores. Thereafter the rye was placed in an atmosphere containing radioactive carbon dioxide while the ergot corms developed. The sclerotia exhibited a slight radioactivity, but the alkaloid content was extremely low. Grown in this way ergot alkaloids presumably would have random carbon 14 marking, both in the nucleus and in the side chain. Such molecules would provide the opportunity for an important comparison with the distribution of radioactivity following the administration of LSD with the radiocarbon in the side chain.

BIBLIOGRAPHY

1. Boyd, E. S., Rothlin, E., Bonner, J. F., Slater, I. H., and Hodge, H. C.: *J. Pharm. Exper. Therap., 113:* 6, 1955.
2. Jones, R. A.: *Anal. Chem., 24:* 1055, 1952.
3. Siperstein, M. D., and Chaikoff, I. L.: *J. Biol. Chem., 198:* 93, 1952.
4. Stoll, A., Rutschmann, J., and Hofmann, A.: *Helv. Chim. Acta, 37:* 820, 1954.
5. Toribara, T. Y., and DiStefano, V.: *Anal. Chem., 26:* 1519, 1954.
6. Weller, L. E., Wittwer, S. H., and Sell, H. M.: *J. Am. Chem. Soc., 76:* 629, 1954.