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Effect of Vehicles on Metabolism of Serotonin and Imipramine

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Abstract □ Effects of different vehicles on excretion and metabolism of serotonin and imipramine were studied. Rats receiving serotonin-^{14}C in dimethyl sulfoxide or propylene glycol excreted radioactivity in the urine slower and in lesser amounts than when water was the vehicle. However, the urinary excretion of radioactivity by rats administered a single dose of imipramine-^{14}C was the same for the three vehicles over a period of 72 hr. Dimethyl sulfoxide and propylene glycol caused not only an increase in the amount of urinary 5-hydroxytryptophol and *N*-acetylserotonin, but also an increase in the conjugated form of these two metabolites as well as that of 5-hydroxyindoleacetic acid. These two nonaqueous vehicles caused a diminished demethylation of imipramine when compared to the water vehicle.

Keyphrases □ Serotonin-^{14}C metabolism—vehicle effect □ Imipramine-^{14}C metabolism—vehicle effect □ Metabolism—serotonin-^{14}C, imipramine-^{14}C □ Paper chromatography—autoradiographic analysis □ Scintillometry—analysis

An aqueous solvent is the most common vehicle for the administration of drugs for pharmacological studies in animals. However, other solvents are also frequently used, such as propylene glycol and dimethyl sulfoxide (DMSO). DMSO is a powerful solvent with a remarkable ability to alter membrane permeability, and its function as a carrier for many substances has been demonstrated by a number of investigators.

Recently, effects of DMSO on the uptake of ^{14}C-pemoline (1, 2), ^{14}C-urea, and ^{14}C-sucrose (3) by the rat brain have been reported. This study is to determine the difference in effects of water and these two organic solvents as vehicles on the metabolism of two ^{14}C-labeled centrally acting agents, serotonin and imipramine.

MATERIALS AND METHODS

Labeled Compounds—Serotonin-2-^{14}C creatinine sulfate[1] and imipramine-^{14}C hydrochloride[2] were used.

Animals and Doses—Male Sprague-Dawley rats, weighing between 180 and 220 g., were used. The compounds were dissolved separately in water, DMSO, and propylene glycol and were administered intraperitoneally to groups of three animals in a dose of 20 mg./kg. (0.5 ml./rat; 1 μc./rat).

Collection of Urine and Fecal Samples—After the administration of the compound, animals were kept in metabolism cages. Urine samples were collected at intervals of 2, 3, 4, 8, 12, and 24 hr. and then at every 24-hr. interval up to 6 days. In some cases the collection was extended beyond 6 days, as indicated in the results. Toluene was added to the collection tube to prevent any bacterial growth. Fecal samples were collected every 24 hr. for several days. All samples were immediately frozen until assayed.

Determination of Radioactivity—Radioactivities were measured with a Nuclear Chicago liquid scintillation spectrometer, model 725. The scintillation fluid was composed of 4 g. of PPO, 50 mg. of POPOP, and 70 g. of naphthalene/l. of toluene. All determinations were performed in duplicate and were corrected to 100% efficiency by the channels ratio method (4) and for recovery of radioactivity.

The urine sample (0.1 ml.) was mixed with 3 ml. of methanol in a counting vial, and the radioactivity was then measured with 15 ml. of the scintillation fluid..

For measuring the radioactivity in feces, 0.1 ml. of 20% water homogenate in a counting vial was treated with 1.0 ml. of 10% hydrogen peroxide in methanol. The mixture was heated at 45° to al-

[1] New England Nuclear Corp.
[2] Nuclear Chicago Corp.

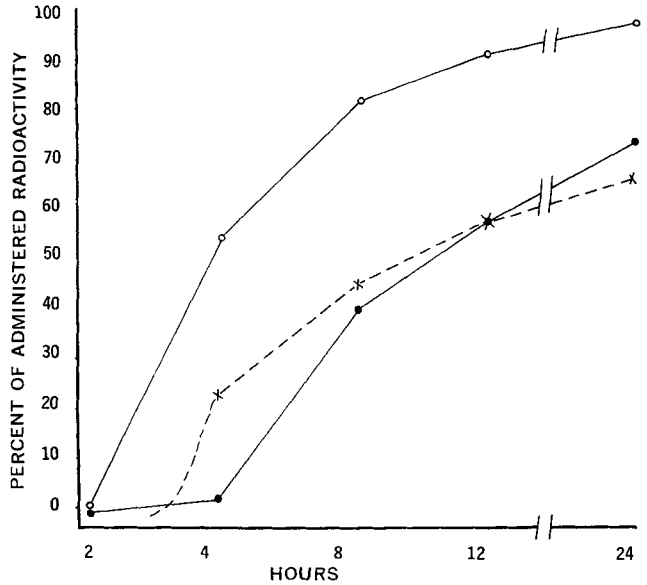

Figure 1—*Cumulative excretion of radioactivity in rat urine following intraperitoneal injection of serotonin-^{14}C. Key: O—O, in water; ●—●, in DMSO; and X--X, in propylene glycol.*

most dryness, treated with 3 ml. of methanol, and 1 hr. later again treated with 15 ml. of the scintillation fluid.

Enzymatic Hydrolysis of Conjugates—Urine was adjusted to pH 5.5 and incubated overnight with β-glucuronidase and sulfatase[3] in sodium acetate buffer, pH 5.5, at 37°.

Chromatography of Urine Sample—Hydrolyzed and unhydrolyzed urine samples from the ^{14}C-serotonin experiment were applied on Whatman 3MM paper and developed in the system of *n*-butanol–acetic acid–water (120:30:50) using the descending technique. The radioactive spots were located by autoradiography of the paper chromatograms with Kodak no-screen X-ray film and eluted by methanol. The eluates were rechromatographed on silica gel G precoated glass plates[4] in the solvent ethyl acetate (5). Serotonin and its metabolites were identified against standards and by their R_f values, UV fluorescence, and color reactions with Ehrlich's reagent. For quantitation, radioactive spots on paper or glass plate were cut out or scraped off and transferred to counting vials; the radioactivity was measured after addition of 3 ml. of methanol and 15 ml. of the scintillation fluid.

Urine samples from the imipramine-^{14}C experiment were chromatographed on silica gel G precoated TLC plates in the solvent system of chloroform–methanol (90:10) (6). Imipramine and desimipramine were detected by spraying the plates with iodoplatinate reagent.[5]

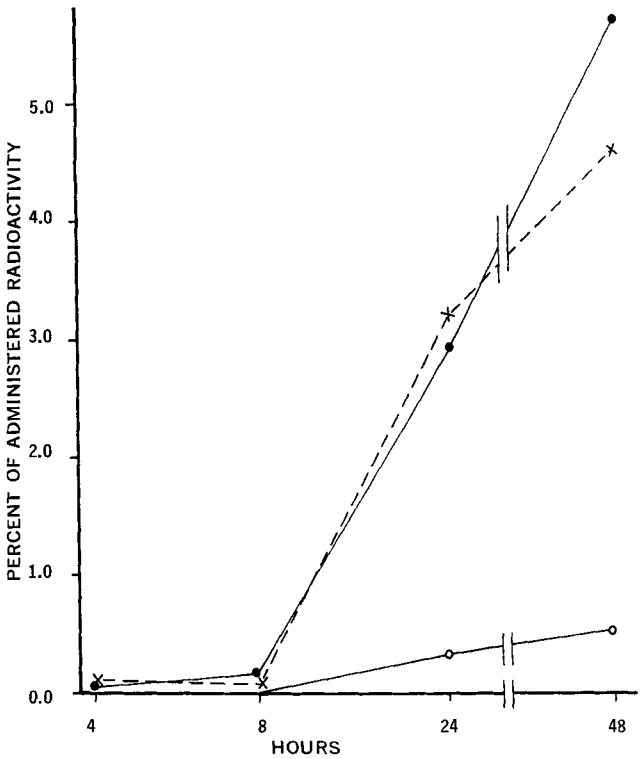

Figure 3—*Cumulative excretion of radioactivity in rat feces following intraperitoneal injection of serotonin-^{14}C. Key: O—O, in water; ●—●, in DMSO; and X--X, in propylene glycol.*

RESULTS AND DISCUSSION

Excretion—Urine levels of radioactivity after the intraperitoneal administration of serotonin-^{14}C were less when the compound was administered in DMSO or propylene glycol than when administered in water. After 24 hr., 98% of the given dose was excreted when the aqueous solution was used, whereas only 75 and 67% of the injected radioactivity were recovered respectively when DMSO and propylene glycol were the vehicles (Fig. 1). However, in rats injected

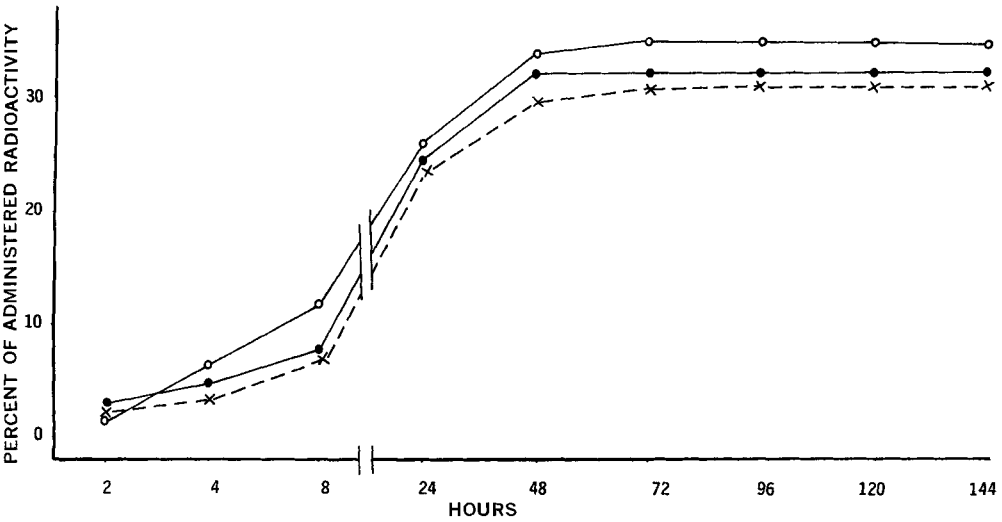

Figure 2—*Cumulative excretion of radioactivity in rat urine following intraperitoneal injection of imipramine-^{14}C. Key: O—O, in water; ●—●, in DMSO; and X--X, in propylene glycol.*

[3] Glusulase, Endo Laboratories, Inc.
[4] Brinkmann Co.

[5] This reagent was found to be useful in detecting imipramine and desimipramine in less than 1-mcg. quantities, giving a pinkish color.

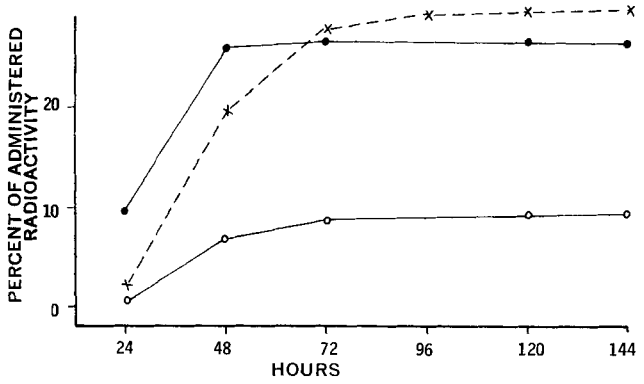

Figure 4—*Cumulative excretion of radioactivity in rat feces following intraperitoneal injection of imipramine-^{14}C. Key: O—O, in water; ●—●, in DMSO; and ×--×, in propylene glycol.*

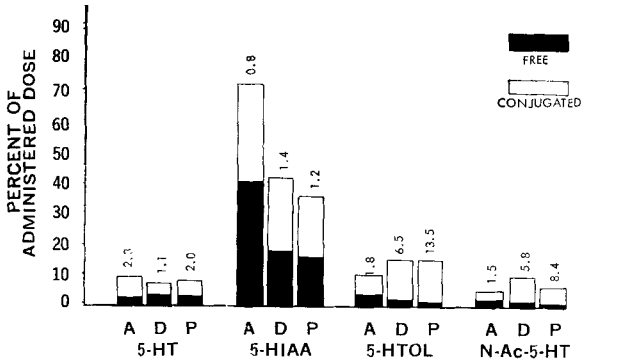

Figure 5—*Distribution of metabolites in 24-hr. rat urine following intraperitoneal injection of serotonin-^{14}C. Key: A, in water; D, in DMSO; P, in propylene glycol; 5-HT, unchanged serotonin recovered; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HTOL, 5-hydroxytryptophol; and N-Ac-5-HT, N-acetylserotonin. Values shown at the top of each column represent ratio of the conjugated form to the free form for each metabolite.*

with imipramine-^{14}C, urinary excretion of radioactivity was the same (approximately 33%) over a period of 72 hr., regardless of which of the three vehicles was used (Fig. 2).

Rats receiving serotonin or imipramine in the two nonaqueous solvents showed higher excretion of radioactivity in feces than when the aqueous solution was used (Figs. 3 and 4).

Metabolism—The urinary metabolites of serotonin-^{14}C recovered in the first 24 hr. after administration are shown in Fig. 5. Both DMSO and propylene glycol doubled the urinary excretion of 5-hydroxytryptophol and *N*-acetylserotonin over that with the aqueous vehicle (Fig. 5 and Table I). A similar alternation of metabolic pathways of serotonin by ingestion of ethanol has been reported (7).

In this study, the cause for the increased urinary 5-hydroxytryptophol and *N*-acetylserotonin remains to be determined. The latter could have resulted from a blockage of the oxidative pathway by DMSO and propylene glycol; these two solvents were found to inhibit monoamine oxidase *in vitro*.[6] It has been postulated that DMSO could alter secondary and tertiary structures of proteins by substituting the protein-bound water, and this could cause a partial loss of enzyme activity (8, 9).

[6] During the assay for inhibitors of monoamine oxidase, the solvents DMSO and propylene glycol were found to be weak inhibitors of bovine liver monoamine oxidase with I_{55} equal to 1.12 and 0.41 *M*, respectively.

Table I—Effects of Vehicles on Percent[a] Distribution of Metabolites in 24-hr. Urine of Rats Administered Intraperitoneal Dose (20 mg./kg.) of Serotonin-^{14}C

Vehicle	Percentage[a] Urinary Metabolites of Serotonin			
	5-HT[b]	5-HIAA[b]	5-HTOL[b]	N-Ac-5-HT[b]
Water	12.6	70.8	10.6	5.9
DMSO	8.3	63.6	18.0	10.0
Propylene glycol	13.7	53.1	23.0	10.1

[a] For a direct comparison of the distribution of serotonin metabolites in the 24-hr. urine with the three vehicles, the total excreted radioactivity in the individual case was corrected to 100%. [b] Key: 5-HT, unchanged serotonin recovered; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HTOL, 5-hydroxytryptophol; and N-Ac-5-HT, *N*-acetylserotonin.

Table II—Effect of Vehicles on the Ratio of Desimipramine to Imipramine in Urine of Rats Administered Imipramine-^{14}C (20 mg./kg. i.p.)

Urine Collection, hr.	Ratio of Desimipramine to Imipramine		
	Water	DMSO	Propylene Glycol
0–4	11.33	2.73	5.19
4–8	9.57	6.66	6.57
8–24	10.08	9.25	11.96

An increase in the conjugated form of the three metabolites, especially 5-hydroxytryptophol and *N*-acetylserotonin, was also observed with both DMSO and propylene glycol (Fig. 5). In this aspect, the latter solvent was more effective than the former.

Table II lists the ratio of desimipramine to imipramine in the urinary excretion. When water was the vehicle, this ratio was held constant throughout the intervals of 4, 8, and 24 hr. With DMSO and propylene glycol as the two vehicles, the low ratios in the first 4 hr. indicated a diminished demethylation of imipramine, particularly for DMSO. It is possible that the two nonaqueous solvents inhibit the demethylation enzyme, and this is currently under study.

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