Biological Disposition of Pentylenetetrazol-10-^{14}C in Rats and Humans

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Abstract □ Female white rats were dosed orally with pentylenetetrazol-10-^{14}C. Total radioactivity in different tissues was determined at five times after dosing. The kidney and liver showed slightly higher levels of radioactivity than did the blood and other tissues analyzed. During the first 24 hr., approximately 73% of the administered ^{14}C was recovered in the urine; less than 1% was recovered in the feces. TLC of urine followed by autoradiography showed the presence of pentylenetetrazol-10-^{14}C and at least five radioactive metabolites. Acetone extracts of the blood were chromatographed and were found to contain pentylenetetrazol-10-^{14}C and four of the five labeled metabolites detected in the urine. TLC of urine collected from humans dosed orally with pentylenetetrazol-10-^{14}C showed the presence of pentylenetetrazol-10-^{14}C and at least four radioactive metabolites. The metabolites in human urine occurred at the same R_f positions as the labeled metabolites of rat urine when cochromatographed, indicating possible similar metabolism by rats and humans.

Keyphrases □ Pentylenetetrazol-10-^{14}C—biological disposition □ Tissue distribution—pentylenetetrazol-10-^{14}C □ Metabolism, pentylenetetrazol-10-^{14}C—rats, humans □ Autoradiograms, TLC—analysis □ Scintillometry—analysis

Subconvulsive oral doses of pentylenetetrazol have been used in the treatment of geriatric patients with symptoms of mental confusion, depression, or arteriosclerosis psychosis. The pharmacological activity and metabolism of pentylenetetrazol have been widely studied. However, the literature contains conflicting reports concerning the biotransformation of the compound and the chemical forms in which it is eliminated from the body. Some authors reported detection of no pentylenetetrazol, or only very low amounts, in the urine of experimental animals dosed with pentylenetetrazol (1–5). Esplin and Woodbury (4) reported that only one labeled compound, chromatographically identical to pentylenetetrazol, was detected in the urine of rats dosed intravenously with ^{14}C-labeled pentylenetetrazol. Stiver (5) reported the presence of pentylenetetrazol and three labeled metabolites in the urine of rats dosed intravenously with pentylenetetrazol-10-^{14}C. The purpose of the work reported here was to study the biological disposition of subconvulsive oral doses of pentylenetetrazol in rats and humans. The study was accomplished by radiotracer techniques, using oral doses of pentylenetetrazol-10-^{14}C.

EXPERIMENTAL

Materials and Methods—Pentylenetetrazol-10-^{14}C, synthesized and purified by the method of Stiver *et al.* (6), was used throughout this study. The radiochemical purity was greater than 99%, as shown by TLC, autoradiography, and liquid scintillation counting techniques. All other chemicals or reagents used were analytical grade. For the animal studies, pentylenetetrazol-10-^{14}C was dissolved in sterile water. Each milliliter of this solution contained 30.4 μc. of ^{14}C and 2.84 mg. of pentylenetetrazol. The pentylenetetrazol-10-^{14}C dosages for humans were reported by Ebert *et al.* (7). Urine collected from that study was used to study urinary metabolites.

Fifteen Sprague-Dawley[1] female rats (152–177 g.) were randomly divided into five groups and fasted for 20 hr. Each was then orally dosed with 12.1 μc./1.13 mg. of pentylenetetrazol-10-^{14}C, an amount well below the convulsant dose (8). The animals were placed individually in metal metabolism cages. Fasting was continued but water was allowed *ad libitum* for the remainder of the study. Urine and feces from each of the three animals in the five groups were collected until the groups were sacrificed at 1, 4, 8, 12, or 24 hr. by decapitation. Samples of blood, portions of abdominal fat, intestinal feces, and muscle from the right rear leg were removed from the carcass as well as the entire heart, brain, lung, spleen, liver, and kidney. Tissues were washed in normal saline solution, and all samples except feces were weighed and frozen until analyzed. Blood was pretreated with sodium citrate. Feces were stored over Drierite, weighed, and pulverized before assay.

Radioanalysis—The radioactivity in all samples was measured at 4° in low potassium vials with a liquid scintillation spectrometer[2] equipped with bialkali phototubes. The scintillator used for all samples consisted of 1 part toluene, 1 part 2-ethoxyethanol, and 0.4% 2,5-diphenyloxazole. All samples were then fortified with toluene-^{14}C internal standard and recounted to determine the counting efficiency.

Aliquots of urine in duplicate and radioactive zones on chromatograms were counted in vials containing 15 ml. of the scintillator solution. Samples, duplicate when possible, of tissue and blood (50–80-mg. aliquots) and dried and crushed feces (3.6–90.8-mg. aliquots) were digested for 6–8 hr. at 70° in tightly capped vials. The digesting agent was 0.2 ml. of a mixture of equal amounts of 70% perchloric acid and 2-ethoxyethanol and 0.4 ml. of 30% hydrogen peroxide. When solubilization was complete, the samples were cooled and counted in 50 ml. of scintillator.

TLC and Autoradiography—Metabolites in the blood and urine of rats and the urine of humans were separated chromatographically with methyl ethyl ketone as the solvent. Aliquots of urine, treated urine, or treated plasma were cochromatographed with authentic pentylenetetrazol-10-^{14}C on desiccator-stored, precoated, 250-μ silica gel G plates with fluorescent indicator[3]. Each plate was developed three times in the same solvent system (13 cm. per development). Previous work by Stiver (5) showed that other solvent systems for TLC of pentylenetetrazol did not give as complete a separation as this system.

Autoradiograms were made from the developed chromatograms by first spraying the plates twice[4]. The plates were then exposed to medical X-ray film, in a freezer at −10°, for 7–28 days to allow the detection of spots representing 1% or less of the total ^{14}C applied. The radioactive zones thus detected were further quantitated by liquid scintillation counting of the absorbent scraped from the plates.

To determine whether any conjugates of pentylenetetrazol were present in rat urine, hydrolysis treatments similar to those reported by Ice *et al.* (9) were employed prior to chromatography. Samples of urine were subjected to steam heat, sodium hydroxide, sodium hydroxide plus steam heat, hydrochloric acid, and hydrochloric acid plus steam heat.

Because of the very low levels of activity in human urine samples, the samples were filtered through Whatman No. 1 filter paper to remove precipitated salts; 1-ml. aliquots of the filtrates were lyophilized at 0.2–0.3 mm. Hg, using dry ice and 2-methoxyethanol as a

[1] Sprague-Dawley, Madison, Wis.
[2] Packard Instrument Co., Inc., Downers Grove, Ill.
[3] Brinkmann Instruments, Inc., Des Plaines, Ill.
[4] With a thin coat of Omnispray Intensifier for Autoradiography, New England Nuclear, Boston, Mass.

Table I—Tissue Distribution of ^{14}C at Various Times following Oral Administration of Pentylenetetrazol-10-^{14}C to Rats

Tissue	Hours after Administration: 1	4	8	12	24
Blood	0.72 ± 0.04[a]	0.53 ± 0.01	0.16 ± 0.03	0.14 ± 0.04	0.04 ± 0.00
Brain	0.63 ± 0.01	0.47 ± 0.04	0.18 ± 0.03	0.14 ± 0.04	0.03 ± 0.00
Fat	0.31 ± 0.03	0.21 ± 0.05	0.10 ± 0.07	0.05 ± 0.03	0.01 ± 0.01
Heart	0.75 ± 0.06	0.53 ± 0.03	0.18 ± 0.04	0.15 ± 0.05	0.04 ± 0.00
Kidney	0.99 ± 0.03	0.79 ± 0.01	0.26 ± 0.05	0.20 ± 0.05	0.05 ± 0.00
Leg muscle	0.61 ± 0.05	0.50 ± 0.03	0.19 ± 0.04	0.14 ± 0.04	0.03 ± 0.00
Liver	0.91 ± 0.03	0.69 ± 0.05	0.25 ± 0.05	0.19 ± 0.05	0.04 ± 0.00
Lung	0.74 ± 0.04	0.55 ± 0.03	0.19 ± 0.04	0.15 ± 0.04	0.04 ± 0.00
Spleen	0.71 ± 0.01	0.53 ± 0.01	0.16 ± 0.04	0.14 ± 0.04	0.03 ± 0.00

[a] The mean of three animals ± *SD* is given and expressed as the percent of the administered 12.1-μc. dose per gram of tissue.

coolant until the resulting samples contained about 2% moisture. The lyophilized urine residues were reconstituted in 0.2 ml. of 70% ethanol, and the mixtures were chromatographed.

To study metabolites in rat plasma, 3-ml. aliquots of whole blood were centrifuged and 1-ml. aliquots of the centrifugates were then lyophilized. Each lyophilized sample was mixed with 0.2 ml. of water in the lyophilization ampul, and then about 0.2 ml. of acetone was added with thorough mixing. The acetone–water supernatant was chromatographed and analyzed for radioactivity.

RESULTS AND DISCUSSION

Tissue Distribution Study—Table I shows that the concentration of pentylenetetrazol-10-^{14}C and/or its labeled metabolites was about equal in the blood, brain, heart, leg muscle, lung, and spleen at the different times studied. The level of ^{14}C detected in the liver was always slightly higher than that detected in the mentioned tissues, and the level of ^{14}C present in the kidney was always slightly higher than that detected in the liver. Fat showed the lowest level of ^{14}C of all tissues assayed. The concentration of ^{14}C in all tissues studied decreased steadily with time.

Table II—Cumulative Urinary and Fecal Excretion of ^{14}C by Rats Dosed Orally with Pentylenetetrazol-10-^{14}C

Hours	Number of Animals	Cumulative ^{14}C: Excreted in Urine, %	Excreted in Feces, %[a]
1	3	2.5 ± 3.5	0.14 ± 0.00[b]
2	12	8.3 ± 8.4	—[c]
4	12	14.2 ± 8.3	0.16 ± 0.06[d]
6	9	32.1 ± 16.1	—[c]
8	9	48.2 ± 14.1	0.21 ± 0.30
10	6	56.1 ± 14.1	—[c]
12	6	63.0 ± 17.0	0.35 ± 0.39
18	3	63.5 ± 9.8	—[c]
24	3	73.4 ± 5.6	0.79 ± 0.22

[a] Feces collected from metabolism cages and feces obtained from the rats' intestines were assayed. Values represent the mean ± *SD* of three animals, except where otherwise noted. [b] Value for one animal only. [c] No animals were sacrificed at this time. [d] Value for two animals only.

Table III—Relative Occurrence of Labeled Compounds in Rat Urine after Oral Administration of Pentylenetetrazol-10-^{14}C

R_f[a]	Time of Urine Sample Collection, hr.: 0–2	2–4	4–6	6–8	8–10	10–12	12–18	18–24
0.00	16.7[b]	18.9	15.8	16.0	17.1	17.2	15.0	16.6
0.15	30.7	18.3	14.2	10.6	7.6	7.6	5.2	8.9
0.62	28.2	37.0	39.4	38.2	35.2	33.7	30.0	26.2
0.73	1.8	4.3	1.9	3.0	3.0	2.7	3.5	3.1
0.78	21.3	22.9	28.0	31.9	36.8	38.5	44.0	43.0

[a] Labeled compounds are specified according to their typical R_f on the thin-layer plates. The compound at R_f 0.78 was identified as pentylenetetrazol on the basis of cochromatography with known pentylenetetrazol-10-^{14}C. The metabolites were not identified. [b] The relative occurrence of each compound is expressed as the average percent of the total ^{14}C in all urine samples collected at each time interval. Values reported through 12 hr. are the average of the same four rats; values beyond 12 hr. are the average of two of these rats. For each time interval, values used for the average did not differ by more than 5%.

The results of the study were in general agreement with those reported previously (3, 4) for the tissue distribution of intravenously administered pentylenetetrazol. The notable exception is that Stiver (5) reported that concentrations of pentylenetetrazol-10-^{14}C and/or its labeled metabolites were about twice as high in kidney tissue as in liver tissue after intravenous administration of pentylenetetrazol-10-^{14}C to male rats.

At the end of 24 hr., approximately 73% of the administered ^{14}C had been excreted in the urine and less than 1% had been excreted in the feces (Table II). These results are in general agreement with those reported by other investigators (4, 5) who studied the excretion of intravenously administered ^{14}C-labeled pentylenetetrazol. Esplin and Woodbury (4) reported a 24-hr. urinary recovery of 63% of the administered ^{14}C and a 48-hr. fecal recovery of 2–5% of the administered ^{14}C. Stiver (5) reported excretion of 66% of the administered ^{14}C in the urine during a 24-hr. study.

Rat Urine Metabolite Study—A compound tentatively identified as pentylenetetrazol-10-^{14}C and at least five labeled metabolites were detected in all rat urine samples studied (Table III). The four most prominent metabolites detected in all rat urine samples were seen at the origin and at R_f 0.15, 0.62, and 0.73. Known pentylenetetrazol-10-^{14}C and the compound in the urine tentatively identified as pentylenetetrazol on the basis of cochromatography in one solvent system were both visible on the autoradiograms at R_f 0.78. Three additional minor labeled metabolites were visible on some autoradiograms; these could not be separated for radioassay from neighboring spots because of tailing. None of the labeled metabolites was identified. The number of labeled metabolites detected was greater than that reported by previous investigators (4, 5).

The relative occurrence of pentylenetetrazol in the urine increased steadily from 21% in the 0–2-hr. sample to 43% in the 18–24-hr. sample. The reason for this increase is not clear. The fluctuations in relative occurrence of the various labeled compounds in the urine with time were similar in general to those reported by Stiver (5) who analyzed urine collected from male rats dosed intravenously with pentylenetetrazol-10-^{14}C.

Table IV—Relative Occurrence of Pentylenetetrazol-10-^{14}C and Its Labeled Metabolites in Rat Urine Subjected to Various Hydrolysis Treatments

R_f[a]	Treatment: None	Heat	HCl	HCl plus Heat	NaOH	NaOH plus Heat
0.00	15.3[b]	15.5	2.3	2.0	25.4	26.5
0.08	2.8	2.6	32.2	31.3	0.0[c]	0.0[c]
0.15	17.6	17.4	0.0[c]	0.0[c]	10.7	9.5
0.37	1.3	1.4	0.0[c]	0.0[c]	0.9	1.1
0.62	36.7	37.2	38.1	39.1	37.3	37.2
0.73	2.3	2.1	2.4	2.2	2.3	2.3
0.78	24.0	23.9	25.0	25.5	23.4	23.3

[a] Labeled compounds are specified according to their typical R_f on the thin-layer plates. The compound at R_f 0.78 was identified as pentylenetetrazol on the basis of cochromatography with known pentylenetetrazol-10-^{14}C. The metabolites were not identified. [b] The relative occurrence of each compound is expressed as the percent of the total ^{14}C in each sample analyzed. Rat urine collected from one rat 2–6 hr. after oral administration of pentylenetetrazol-10-^{14}C was used. [c] No darkening of the autoradiogram was observed at this R_f, indicating that a negligible amount of ^{14}C was present.

Table V—Relative Occurrence of Labeled Compounds in Rat Plasma after Oral Administration of Pentylenetetrazol-10-^{14}C

Sacrifice Time, hr.	R_f	Labeled Compounds, %[a]
1	0.00[b]	1.5 ± 0.53
	0.08	1.7 ± 0.76
	0.62	11.1 ± 1.36
	0.73	2.2 ± 0.79
	0.78	83.4 ± 2.14
8	0.00	3.4 ± 1.36
	0.08	1.9 ± 2.37
	0.62	15.6 ± 0.59
	0.73	4.0 ± 1.25
	0.78	75.1 ± 2.28

[a] The relative occurrence of each compound is expressed as the percent of the total ^{14}C in each sample and is given as the mean of three samples ± *SD*. [b] Labeled compounds are specified according to their typical R_f on the thin-layer plates. The compound at R_f 0.78 was identified as pentylenetetrazol on the basis of cochromatography with known pentylenetetrazol-10-^{14}C. The metabolites were not identified.

The relative occurrence of pentylenetetrazol-10-^{14}C and its labeled metabolites in urine subjected to various hydrolysis treatments is given in Table IV. No new labeled compounds were detected in any of the treated urine samples. If any conjugates of pentylenetetrazol were originally present in the urine, they were not hydrolyzed under the conditions of the treatment employed. However, the data given in Table IV suggest that the metabolites seen at R_f 0.00, 0.15, and 0.37 in untreated urine are acid-hydrolyzable conjugates of the metabolite at R_f 0.08, and that the compounds at R_f 0.08 and 0.15 are base-hydrolyzable conjugates of the metabolite(s) remaining at the origin. Because the NaOH treatment only partially hydrolyzed the material at R_f 0.15, probably at least two labeled metabolites have an R_f of 0.15.

Rat Plasma Metabolite Study—TLC of acetone-extracted blood plasma samples showed the presence of pentylenetetrazol-10-^{14}C at R_f 0.78 and at least four radioactive metabolites: at R_f positions 0.00, 0.08, 0.62, and 0.73. On some autoradiograms, a fifth minor labeled metabolite, which appeared as a very faint spot at R_f 0.50, was detected. None of the labeled metabolites was identified. The five labeled metabolites detected in the plasma extracts occurred at the same R_f's as five of the labeled metabolites detected in the urine sample chromatographed on the same plates.

Table V shows that the relative occurrence of the various metabolites was greater in the plasma extracts of animals sacrificed at 8 hr. than in those rats sacrificed at 1 hr. The major metabolite, at R_f 0.62, increased from 11% at 1 hr. to 16% at 8 hr. Pentylenetetrazol represented 83% of the total ^{14}C in the 1-hr. plasma extract but only 75% of the ^{14}C in the 8-hr. plasma extract.

Stiver (5) detected only pentylenetetrazol-10-^{14}C and no labeled metabolites in the plasma of male rats dosed intravenously with pentylenetetrazol-10-^{14}C. However, the detection technique reported by Stiver (5) was less sensitive than the one reported here.

Human Urine Metabolite Study—TLC of urine collected from one human during the period extending from 24 to 36 hr. after the first of three 100-mg. doses of pentylenetetrazol-10-^{14}C, which contained 22.8% of the administered radioactivity, was analyzed. Chromatographs showed the presence of a compound tentatively identified as pentylenetetrazol at R_f 0.78 and at least three unidentified labeled metabolites at R_f positions 0.00, 0.25, and 0.62. Pentylenetetrazol, tentatively identified on the basis of cochromatography with known pentylenetetrazol-10-^{14}C, accounted for about 24% of the ^{14}C in the sample.

TLC of urine collected from four humans during the period extending from 6 to 9 hr. after a single 100-mg. oral dose of pentylenetetrazol-10-^{14}C showed the presence of pentylenetetrazol at R_f 0.78 and at least four labeled metabolites at R_f 0.00, 0.25, 0.62, and 0.73. The labeled metabolites were not identified. Three of the labeled metabolites detected in human urine occurred at the same R_f's as three metabolites detected in rat urine at R_f 0.00, 0.62, and 0.73.

Table VI—Relative Occurrence of Labeled Compounds in the Urine of Four Human Subjects 6–9 Hr. after Oral Administration of Pentylenetetrazol-10-^{14}C

R_f	Labeled Compounds, %[a]
0.00[b]	22.6 ± 3.0
0.25	6.4 ± 3.1
0.62	55.5 ± 4.8
0.73	5.7 ± 2.0
0.78	9.9 ± 3.3

[a] The relative occurrence of each compound is expressed as the percent of the total ^{14}C in each urine sample. [b] Labeled compounds are specified in terms of their typical R_f on the thin-layer plate. Pentylenetetrazol was identified on the basis of cochromatography with known pentylenetetrazol-10-^{14}C. The labeled metabolites were not identified.

The relative occurrence of pentylenetetrazol-10-^{14}C and its labeled metabolites in the four human urine samples analyzed is given in Table VI. Pentylenetetrazol accounted for an average of 9.9% of the ^{14}C in the samples, and the major metabolite at R_f 0.62 accounted for 55%. The proportion of pentylenetetrazol in the 6–9-hr. human urine (9.9%) was well below that detected in rat urine at any time during a 24-hr. study—*viz.*, 21–44%.

The metabolism of orally administered pentylenetetrazol-10-^{14}C by rats and humans was similar in that both species excreted unchanged pentylenetetrazol and at least three labeled metabolites which were chromatographically similar. Pentylenetetrazol and the three similar metabolites detected in the urine of both species accounted for 94% of the ^{14}C in human urine collected during the 6–9-hr. period and for 89% of the ^{14}C present in rat urine collected during the 6–8-hr. period after dosing. The metabolism of pentylenetetrazol-10-^{14}C by the two species differed in that the human urine contained one labeled metabolite not detected in rat urine, and some rat urine samples contained four minor labeled metabolites which were not detected in human urine.

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