Absorption, Metabolism, and Excretion of Oxazepam and Its Succinate Half-Ester

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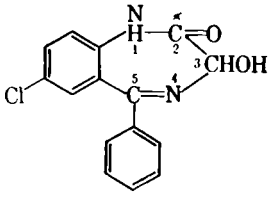
C^{14}-Oxazepam was administered orally to dogs, rats, and pigs. Radioassay of blood, various tissues, and excreta indicated that the drug was well absorbed. A single dose was largely eliminated within 2 days, mostly *via* urine in dogs and pigs and *via* feces in rats. One metabolite, a glucuronide, accounted for more than 95 per cent of the radioactivity of dog and pig urine. The radioactivity of rat urine was distributed among at least seven metabolites. C^{14}-Labeled-Wy-4426 (a succinate half-ester of oxazepam) was found to disappear rapidly from an i.m. injection site in rats and to be distributed uniformly throughout the organs. Exceptions were the gastrointestinal tract, which appeared to be the route favored for excretion in this species, and the liver and kidney, in which somewhat higher than average levels were attained shortly after injection. About two-thirds of the dose was excreted in feces and about one-fifth in urine by the forty-eighth hour after injection. The drug appeared to undergo the same extensive metabolic alteration in rats as oxazepam—at least seven distinct metabolites appearing in urine. After i.v. injection of Wy-4426 in dogs, blood levels peaked within 15 minutes but declined slowly, so that even at 48 hours appreciable levels were found. The blood values from the fourth hour compared closely with those obtained following oral administration of oxazepam. Dogs excreted about two-thirds of the dose in urine and one-third in feces within 72 hours. Three radioactive spots in chromatographed urine of dogs injected with Wy-4426 were identified as unchanged drug, oxazepam, and the glucuronide of oxazepam. Similar spots were obtained when Wy-4426 was administered to pigs. The fate of oxazepam in humans was similar to that in dogs and pigs.

OXAZEPAM,[1] 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepine-2-one, has been characterized pharmacologically in our laboratories as an anticonvulsant and mild central depressant and is currently under clinical investigation as an antianxiety agent.

For oral use in this study we synthesized the drug with C^{14} at the 2-position. Wy-4426, the water-soluble sodium salt of the succinate half-ester, was prepared from C^{14}-oxazepam for parenteral use.



Oxazepam

EXPERIMENTAL

Synthesis of C^{14}-Oxazepam.—2-Amino-5-chlorobenzophenone oxime in acetic acid was treated with a solution of 2-chloroacetyl-1-C^{14} chloride in hexane. The mixture was allowed to stand at room temperature for 48 hours. Methylene chloride and water were then added with mixing, the methylene chloride layer was separated, washed with sodium carbonate solution, and concentrated in a stream of nitrogen. Without isolation the crude 2-chloromethyl-4-phenyl-6-chloroquinazoline-3-oxide was treated with sodium hydroxide in aqueous ethanol, stirred 1 hour, diluted with water, and acidified with HCl. The product, 7-chloro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepine-2-one-4-oxide was separated and recrystallized from aqueous ethanol. Treatment of this material with acetic anhydride in acetic acid at 90–100° afforded crystals of 7-chloro-5-phenyl-3-acetoxy - 1,3 - dihydro - 2*H* - 1,4 - benzodiazepine-2-one after cooling. This solid was then hydrolyzed with sodium hydroxide in aqueous ethanol and oxazepan isolated by dilution with water and acidification with acetic acid. Recrystallization from hot dimethyl formamide gave a product, m.p. 204.5 to 205.5°, specific activity 2970 disintegrations/minute/mcg.

Synthesis of Wy-4426.—C^{14}-Oxazepam and succinic anhydride were heated in pyridine at 95° for 1.5 hours. The mixture was cooled and diluted with ethanol. Addition of water precipitated the pyridinium salt of the succinate half-ester. The pyridinium salt was dissolved in ethanol and treated with sodium hydroxide solution to yield the sodium salt of the half-ester. The Wy-4426 thus obtained was chromatographed and found by radioautography to have a trace of oxazepam.

Colorimetric Assay of Oxazepam and Its Glucuronide.—The following method takes advantage of the fact that 2-amino-5-chlorobenzophenone, the product of acid hydrolysis of oxazepam, is quantitatively extracted from aqueous acid by organic solvent and thereby effectively separated from substances which may interfere in the Bratton-Marshall reaction. The method would appear to be ap-

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[1] Formerly coded Wy-3498.

plicable to the determination of any benzodiazepine, such as chlordiazepoxide, as well as for the metabolites of these drugs, which yield 2-amino-5-chlorobenzophenone on acid hydrolysis.

Oxazepam.—Two milliliters of plasma, urine, or homogenates of feces or tissue was shaken in glass or polyethylene-stoppered centrifuge tubes with 10 ml. of ethylene dichloride for 30 minutes on a mechanical shaker. One milliliter of aqueous phase (A) was removed for subsequent determination of oxazepam glucuronide. Eight milliliters of the organic phase was transferred to a clean 40-ml. centrifuge tube containing 4 ml. 6 *N* HCl and agitated for 1 minute on a Vortex, Jr. mixer. The tube was centrifuged; 3 ml. of the aqueous phase was transferred to a clean 40-ml. centrifuge tube which was then heated for 1 hour in a boiling water bath. The tube was stoppered after temperature equilibration. After removal from the bath and cooling, 15 ml. of H_2O was added, followed by 10 ml. of CCl_4. The tube was stoppered and shaken mechanically for 30 minutes. Following centrifugation and removal of the aqueous phase, 8 ml. of the organic layer was transferred to a clean 40-ml. tube and extracted with a mixture of 2 ml. 1 *N* HCl and 1 ml. freshly prepared 0.1% $NaNO_2$ by agitation for 2 minutes on a Vortex, Jr. mixer. Two milliliters of the aqueous phase was transferred to a test tube containing 0.5 ml. of 0.5% ammonium sulfamate. After mixing and allowing 2 minutes for complete destruction of nitrite, 0.5 ml. of 0.1% *N*-(1-naphthyl)ethylenediamine·2HCl was added, and the contents of the tube mixed. After standing for 10 minutes, the sample was read at 550 mμ in a Bausch & Lomb spectronic 20 colorimeter.

Standards were prepared by shaking 2-ml. portions of appropriate biological control samples with 10-ml. ethylene dichloride solutions containing known amounts of oxazepam. When assayed, a 0.5 mcg./ml. ethylene dichloride solution of oxazepam gave a reading of 0.05 absorbance units.

Oxazepam Glucuronide.—Since no sample of feces from species investigated—dog, rat, pig, or man—contained this metabolite, this part of the assay of feces is unnecessary. To 1 ml. of aqueous phase A (from which oxazepam was removed by extraction with ethylene dichloride) in a clean 40-ml. centrifuge tube was added 1 ml. concentrated HCl. The tube was heated for 1 hour in a boiling water bath (stoppered after temperature equilibration). Following the addition of 10 ml. of H_2O to the cooled tube, the procedure employed was the same as that for oxazepam following its hydrolysis.

Standards were prepared by addition of 1-ml. solutions of oxazepam in 6 *N* HCl to 1 ml. ethylene dichloride-extracted biological samples from untreated animals. After addition of 1 ml. concentrated HCl to this mixture, hydrolysis and subsequent treatment followed the procedure for oxazepam. Treated in this manner, a 5-mcg. solution of oxazepam gave a reading of 0.08 absorbance units, which was 70% of the value obtained by direct coupling of an equivalent amount of 2-amino-5-chlorobenzophenone. The absorbance-concentration curve was linear up to 30 mcg. of oxazepam, and replicate samples agreed within 5%. Because of negligible blanks, it was possible to detect as little as 0.25 mcg. of oxazepam in the assay for glucuronide. Lower concentrations were determined by increasing the sample size; there was no concomitant increase in the blank.

Fluorometric Assay of Oxazepam in Urine and Serum.—Three milliliters of urine or serum was added to 0.5 ml. of capryl alcohol in a 12-ml. centrifuge tube equipped with a standard taper joint and polyethylene stopper. The liquids were mixed thoroughly by a Vortex mixer. Six milliliters of ethylene dichloride was added, and the phases were thoroughly mixed by shaking. The tube was centrifuged and the aqueous layer removed by aspiration. Four milliliters of the organic layer was transferred to a 40-ml. centrifuge tube containing 20 ml. of 0.1 *M* KH_2PO_4; the mixture was shaken. After centrifugation, the aqueous layer was removed by aspiration, 20 ml. of fresh phosphate solution was added, and again the mixture was shaken, centrifuged, and the aqueous layer removed. Three milliliters of the ethylene dichloride layer was transferred to another tube containing 3.0 ml. 3 *N* HCl. The mixture was then shaken and centrifuged and the acid layer transferred to a quartz cell.

The fluorescence was measured on a Photovolt fluorometer equipped with a high-pressure mercury lamp, a 200–400 mμ broad-band primary filter, and a B 540 (520–660 mμ) broad-band secondary filter.

Standards were prepared by substituting measured volumes of a standard solution of oxazepam in ethylene dichloride for an equivalent volume of the pure solvent. This preparation was placed in a tube containing control urine or serum and was carried through the procedure.

The method described will detect as little as 0.5 mcg. oxazepam/ml. urine or serum. As in all fluorescence analysis, standards used should be fairly close in concentration to that of the sample being determined. Low blank values were obtained in urine and serum, and none of the metabolites interfered with the test for unchanged drug. Duplicate samples agreed within 5%.

Paper Chromatography.—Samples of urine spotted directly and extracts of feces were chromatographed on Whatman No. 1 paper in butanol 17, ethanol 3, water 20 (upper phase) and butanol 6, pyridine 4, water 3.

After development and drying of the chromatograms, they were either placed in contact with Kodak No Screen X-Ray Film for radioautography or were sectioned for radioanalysis in a thin-window gas flow counter (Nuclear-Chicago Ultrascaler).

Radioassay of Biological Samples.—Feces—cecum and small intestine—were homogenized and an aliquot was taken for Schoeniger combustion; a 10% ethanolamine solution was used as absorbent. An aliquot of this solution was added to scintillator fluid in a counting vial. Urine was added directly to counting vials containing scintillator made up in dioxane-naphthalene-ethanol. Tissue samples, other than small intestine and cecum, were dissolved in hyamine hydroxide prior to addition of scintillator fluid. The samples were then assayed in a Packard TriCarb scintillation spectrometer.

Administration of Drug.—C^{14}-Oxazepam was made up in gelatin capsules for oral administration to three fasted female dogs at a dose of approximately 2 mg./Kg. Plasma samples and urine (by catheterization) were taken at various time intervals for 120 hours following administration of the drug. A suspension of the drug (finely ground) in 1% poly-

TABLE I.—DOG PLASMA LEVELS[a] OF C^{14} AFTER A SINGLE ORAL DOSE OF C^{14}-OXAZEPAM

	A	B	C	Av.
Dog Wt., Kg.	11.0	11.8	9.5	
Dose, mg.	23.0	25.0	20.0	
30 min.	0	0	0.20	0.07
1 hr.	0	0	0.67	0.22
2 hr.	0.28	0.07	2.34	0.90
4 hr.	1.62	0.79	2.2	1.50
6 hr.	1.53	1.43	1.15	1.37
24 hr.	0.12	0.54	0.14	0.27
48 hr.	0.06	0.06	0.01	0.04
120 hr.	0.01	0.04	0	0.02

[a] Expressed in mcg./ml. of equivalent oxazepam.

TABLE II.—DOG PLASMA LEVELS[a] OF C^{14} AFTER A SINGLE INTRAVENOUS DOSE[b] OF C^{14}-WY-4426

	A	B	C	Av.
Dog Wt., Kg.	8.8	11.7	10.0	
15 min.	21.4	10.5	16.1	16.0
30 min.	19.1	8.15	11.9	13.1
1 hr.	11.4	5.05	9.25	8.57
2 hr.	3.09	2.76	5.00	3.62
4 hr.	1.23	1.35	1.99	1.52
6 hr.	1.14	1.09	1.50	1.24
24 hr.	0.14	0.20	0.18	0.17
48 hr.	0.08	0.27	0.09	0.14
72 hr.	0	0.10	0.10	0.07

[a] Expressed in mcg./ml. of equivalent oxazepam. [b] Dose was equivalent to 1.92 mg./Kg. of oxazepam.

sorbate 80[2] was prepared containing 0.4 mg. oxazepam/ml. This suspension was administered by stomach tube at a dose of 2 mg./Kg. to fasted white male rats weighing between 250 and 370 Gm.

For parenteral administration Wy-4426 was made up in physiological saline so that 1 ml. of solution contained the equivalent of 3.84 mg. of oxazepam. The dose was such that animals received an amount equivalent to a dose of 1.92 mg./Kg. oxazepam. The solution of Wy-4426 was given i.v. to three fasted female dogs. Urine (by catheterization) and plasma samples were drawn at intervals up to 72 hours. The solution was also injected i.m. into fasted male white rats at a dose of 1.92 mg./Kg. For the collection of feces and urine, all rats were kept in metabolism cages until sacrificed in groups of three at intervals up to 48 hours.

A similar dose of oxazepam was given p.o. to an 8.7-Kg. pig and Wy-4426 was given i.m. to a 6.7-Kg. pig.

A 60-mg. dose of oxazepam was given p.o. to each of two humans—one a healthy male (V. M.) and the other a female with sickle cell anemia, rheumatic heart disease, and rheumatic fever (A. R.).

RESULTS

A preliminary study was carried out in which unlabeled oxazepam, at doses of 5 to 10 mg./Kg., was given orally and parenterally to female dogs. Only after administration by the i.v. route could appreciable levels of unchanged drug be demonstrated in plasma. Examination of urine bore out these results; only 1 to 2% of metabolically unaltered drug could be found. Accordingly the C^{14}-labeled drug was employed in further studies.

[2] Marketed as Tween 80 by the Atlas Powder Co., Wilmington, Del.

Plasma Levels in Dogs.—Table I gives plasma levels of C^{14} in three dogs after an oral dose of C^{14}-oxazepam. Dog C showed the most rapid uptake of drug and also peaked earlier than the other two dogs. Peak levels were reached at from 4 to 6 hours in all three dogs. Radioactivity persisted in the blood of all three dogs for 48 hours and in two of the dogs for 120 hours, although the levels present at these times were insignificant.

Table II gives C^{14} plasma levels in dogs following an intravenous dose of Wy-4426. Peak levels were reached within the first 15 minutes after injection; radioactivity, although not at appreciable levels, persisted in the plasma of all three dogs for 48 hours and in two of the dogs for 72 hours. It should be noted that from the fourth hour the data closely corresponded with that obtained with oxazepam.

Urinary Excretion in Dogs.—Following an oral dose of C^{14}-oxazepam, amounts of C^{14} excreted at varying time intervals in urine corresponded roughly with its blood level (Table III). By the twenty-fourth hour each dog had excreted about three-fifths of the dose. All the dogs continued to excrete detectable amounts of C^{14} up to 96 hours, but virtually none beyond this time.

After i.v. administration of C^{14}-labeled-Wy-4426, a peak rate of urinary excretion of about 15%/hour occurred for the first 30 minutes (Table IV). Sixty per cent of the drug was excreted by the twenty-

TABLE III.—EXCRETION[a] OF C^{14} AFTER A SINGLE ORAL DOSE OF C^{14}-OXAZEPAM TO DOGS

Hr.	A	B	C	Av.
		Urine		
0.5	0	0	0	0
1	0.01	0	0.50	0.17
2	4.25	0.11	6.65	3.67
4	11.20	2.03	24.95	12.73
6	27.10	8.75	32.86	22.90
24	62.50	57.15	57.61	59.09
48	69.03	68.85	61.35	66.41
96	70.33	70.65	61.85	67.61
120	70.41	70.65	61.86	67.64
		Feces		
24		8.68	38.10	23.39
48	31.50[b]	19.93	41.66	31.03
96	34.58	24.81	42.90	34.90
144	35.54	27.21	44.08	35.61

[a] In cumulative per cent of dose. [b] 0–48 hours.

TABLE IV.—EXCRETION[a] OF C^{14} AFTER A SINGLE INTRAVENOUS DOSE OF C^{14}-WY-4426 TO DOGS

Hr.	A	B	C	Av.
		Urine		
0.25	...	4.36	1.71	3.02
0.5	5.36	7.48	8.91	7.25
1	9.21	12.23	13.15	11.53
2	14.01	20.98	18.50	17.83
4	24.71	32.08	33.36	30.05
6	31.30	42.98	42.16	32.15
24	44.01	65.41	72.90	60.77
48	45.06	78.08	77.03	66.72
72	45.06	79.03	78.12	67.40
		Feces		
24	39.0	0	0.1	13.0
48	44.5	11.2	18.8	24.5
72	44.5	23.5	22.4	30.4

[a] In cumulative per cent of dose.

fourth hour and an additional 6% by the forty-eighth hour. When the experiment was terminated at 72 hours, somewhat over two-thirds of the dose had been excreted in urine.

Fecal Excretion in Dogs.—That part of the dose not accounted for by urinary excretion appeared in feces (Tables III and IV). There appeared to be considerable individual variation in the amounts excreted in feces with both forms of the drug; animals receiving oxazepam ranged from about 20 to 42% of the dose in feces in 48 hours, and those on Wy-4426 ranged from 11 to 45% in the same period.

Rat Tissue Levels.—Table V summarizes the levels of C^{14} in tissues and excreta of rats dosed orally with C^{14}-oxazepam. A more rapid uptake can be noted than was apparent in the dogs (each evidenced C^{14} in all tissues in the first 30 minutes). Contributing to this more rapid uptake might be the effect of liquid volume on increasing the rate of gastric emptying, and of polysorbate 80 and fine particle size on absorption. Values for liver exceeded those for all other organs outside the gastrointestinal tract for up to 6 hours. Liver, however, showed no tendency to accumulate drug, as indicated by the rapid decline in radioactivity after the 30-minute peak and the virtual clearance of C^{14} from this organ by the twenty-fourth hour.

There was apparently good uptake by all organs examined and fairly rapid clearance from many by the sixth hour. Continued appearance of low levels of activity in kidney and several other organs may be accounted for by reabsorption from gut of drug or metabolites.

In contrast to the dog, the rat appeared to favor the fecal route for excretion of drug and metabolites, since about 1.5 to four times as much radioactivity appeared in the feces of the rat as in the urine by 48 hours. At the end of the same period the dog had excreted twice as much in urine as in feces (Table III).

Within 30 minutes of injection of C^{14}-labeled-Wy-4426, half the dose was absorbed from the intramuscular injection site (Table VI). By the twenty-fourth hour, no drug remained at the injection site in the three animals of that group.

Peak levels occurred in plasma within 30 minutes of injection. Except for the gastrointestinal tract, only liver and kidney exhibited higher values than the general average for all organs. These higher levels did not persist beyond the first hour, and at 24 and 48 hours, liver and kidney contained amounts of C^{14} comparable to over-all organ averages. Small intestine remained high for the first 6 hours, but by 48 hours, small intestine was free of drug and metabolites.

Excretion of Wy-4426 and metabolites was accomplished largely *via* the fecal route, which accounted for over two-thirds of the dose in 48 hours. During this period, less than one-fifth of the dose was excreted in urine. This urine to feces ratio, similar to that in the oxazepam rats, was the reverse of the ratio obtained in dogs.

Metabolism.—Virtually all of the radioactivity in the urine of dogs dosed with C^{14}-oxazepam was found in a single metabolite (Fig. 1). Treatment of this urine with propionic anhydride in pyridine resulted in the quantitative conversion of this metabolite to a new substance. On the chromatograms this detoxi-

TABLE V.—TISSUE LEVELS IN MCG./GM. IN RATS AFTER A SINGLE ORAL DOSE[a] OF C^{14}-OXAZEPAM

	30 Min.		1 Hr.		2 Hr.		4 Hr.		6 Hr.		24 Hr.		48 Hr.	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Urine[b]	0.09	0.38	2.23	0.74	0.66	3.18	1.63	40.6	1.45	0.54	12.55	10.42	10.70	9.80
Plasma[c]	0.18	0.32	0.24	0.18	0.15	0.30	0.11	0.10	0.15	0.08	0	0	0	0
Lung	0.88	1.00	0.48	0.31	0.12	0.59	0.14	0.19	0.06	0	0	0	0	0
Liver	3.58	5.55	4.86	1.85	2.89	4.55	0.53	0.83	0.82	0.60	0.08	0	0.08	0.05
Brain	0.28	0.76	1.15	0.25	0.65	0.39	0.09	0.14	0.05	0.34	0	0	0.19	0
Heart	0.54	0.95	0.61	0.32	0.98	0.44	0.26	0.17	0	0	0	0	0	0
Spleen	0.50	0.34	0.27	0.12	0.12	0.23	0.08	0.12	0.26	0	0	0	0	0
Kidney	1.23	2.19	1.79	0.89	0.71	1.97	0.34	0.61	0.80	0.60	0.15	0	0.21	0.21
Fat	0.21	0.74	0.21	0.17	0.47	0.12	0.07	0.11	0.10	0	0	0	0	0.18
Muscle	0.24	0.43	0.23	0.14	0.14	0.26	0.13	0.13	0.19	0.08	0.09	0	0	0
Skin	0.17	0.51	0.13	0.05	0.53	0.29	0.12	0.14	0.35	0	0	0	0	0
Bladder	0.20	0.84	0.31	0.09	0.89	0.53	0.10	0.17	0.06	0.06	0.07	0	0	0
Stomach and contents	131	0.39	0.15	0.16	0.89	0.13	0.49	0.18	0.06	0.06	2.3	0	0	0
Small intestine	17.2	49	220	72	18.5	39	5.16	21.9	3.5	2.4	0	0	0	3.35
Caecum	0.97	71.5	176	17.0	19.1	0.25	29.8	15.1	24.8	16.0	9.45	0	0.24	0.33
Feces[b]	...	28.7	32.1	14.1	0.35	6.50	0.42	3.90	37.0	22.6	49	3.25	4.85	27.2
		9.60	0.69	0.55	0.65	...	...	7.65	6.90	0.03	0.02	3.96	16.0	12.2
		0	...	...	...	...	...	...	1.57	0.50	30.0	24.5	18.2	40.6
		0.32	...	...	...	...	...	...	...	...	...	...	...	...
		...	...	...	...	...	...	...	...	...	...	...	...	...

	48 Hr.	
	A	C
Urine[b]	10.70	24.55
Plasma[c]	0	0
Lung	0	0.19
Liver	0.08	0
Brain	0.19	0
Heart	0	0
Spleen	0	0.31
Kidney	0.21	0.14
Fat	0.18	0.08
Muscle	0	0
Skin	0	0
Bladder	0	0.75
Stomach and contents	0.33	3.87
Small intestine	1.90	17.5
Caecum	12.2	45.6
Feces[b]	40.6	

[a] 2 mg./Kg. [b] Per cent of dose. [c] mcg./ml.

TABLE VI.—C^{14} TISSUE LEVELS[a] IN RATS FOLLOWING A SINGLE INTRAMUSCULAR DOSE[b] OF C^{14}-LABELED-WY-4426

	30 Min.		1 Hr.		2 Hr.		4 Hr.		6 Hr.		24 Hr.		48 Hr.	
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Injection site (% of dose)	44.6	55.5	51.5	45.2	34.9	35.7	3.04	7.24	27.0	5.15	1.91	2.41	3.52	10.2	7.2	0	0	0.19	0.2	0	0.22
Plasma[c]	1.65	1.51	1.20	0.92	1.19	1.29	0.76	0.69	0.52	0.34	0.24	0.34	0.20	0.25	0.24	0.22	0.22	0.81	0.32	0.2	0.78
Lung	1.32	1.57	2.20	0.85	1.67	1.34	1.21	1.87	1.06	0.98	0.56	0.56	0.45	0.38	0.54	0.17	0.55	0.29	0.41	0.79	0.38
Liver	7.32	6.95	4.75	4.39	7.61	8.7	3.73	2.92	3.84	1.5	1.21	1.77	1.15	1.37	0.81	0.25	0.26	0.20	0.20	0.28	0.24
Brain	0.48	0.78	0.82	0.36	0.71	0.64	0.75	0.71	0.59	0.31	0.33	0.29	0.28	0.29	0.18	0.17	0.20	0.22	0.23	0.22	0.41
Heart	1.25	1.91	1.78	0.85	1.91	1.50	1.17	1.16	1.05	0.42	0.35	0.47	0.33	0.36	0.36	0.29	0.44	0.40	0.31	0.25	0.52
Spleen	0.44	2.56	1.28	0.54	1.00	1.0	0.82	1.32	0.68	0.39	0.24	0.48	0.24	0.21	0.21	0.16	0.49	0.31	0.31	0.29	0.46
Kidney	5.60	5.9	5.3	3.40	4.39	5.57	2.47	3.14	2.36	1.35	1.1	1.62	1.39	1.08	1.23	0.38	0.50	0.46	0.29	0.46	0.46
Fat	0.30	0.93	0.46	0.17	0.44	0.33	0.74	0.89	0.47	0.07	0.16	0.41	0.11	0.09	0.13	0	0.12	0.04	0.05	0.15	0.19
Muscle	0.20	1.17	0.79	0.35	0.50	0.42	0.54	0.61	0.53	0.32	0.14	0.26	0.14	0.15	0.16	0.12	0.09	0.12	0.09	0.21	0.15
Skin	0.44	0.87	0.8	0.18	0.28	0.42	0.56	0.61	0.63	0.40	0.14	0.26	0.26	0.12	0.29	0.15	0.34	0.28	0.08	0.30	0.11
Bladder	0.65	1.21	1.93	1.41	1.98	1.28	3.28	9.33	2.95	0.60	0.4	1.52	0.36	0.29	2.41	0.38	0.44	0.42	0.20	0.25	0.23
Stomach and contents	0.60	0	2.8	2.0	0	0.5	1.2	8.0	1.8	1	0	1.1	0	0	0	0	0.9	0.7	0	0	0
Small intestine	13.0	14.4	11.0	17	27.4	20.4	57.5	33	37.4	46.1	56	24.2	8.42	33.2	7.64	0.2	1.2	12.7	0	0.45	0
Caecum	0.38	0.3	0.73	0.15	0.47	0.2	0.56	0.42	0.57	1.74	0	17.2	53.0	12.8	35.6	8	20	16.7	0.28	20.9	18.75
Urine[d]	0.16	...	...	...	0.91	1.23	5.2	...	2.35	12.1	8.14	11.9	30.0	18.9	10.2	21.6	14.0	16.7	17.2	68.7	76.5
Feces[d]	...	...	...	...	...	...	...	...	...	...	...	...	...	0	...	65.9	46.9	72.5	72.9		

[a] Values are given in mcg./Gm. [b] Equivalent to 1.92 mg./Kg. oxazepam. [c] mcg./ml. [d] Per cent of dose.

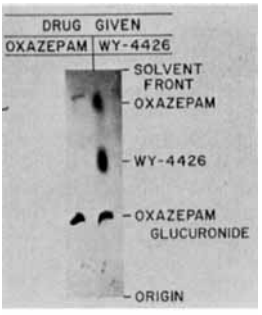

Fig. 1.—Metabolites of oxazepam and Wy-4426 found in dog urine. Developing solvent: butanol 17, ethanol 3, water 20.

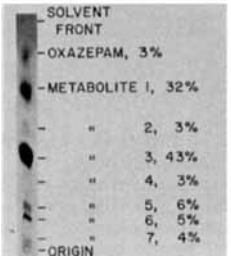

FIG. 2.—Metabolites of oxazepam found in rat urine. Developing solvent: butanol 17, ethanol 3, water 20.

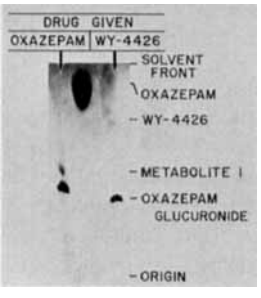

Fig. 3.—Metabolites of oxazepam and Wy-4426 found in pig urine. Developing solvent: butanol 17, ethanol 3, water 20.

TABLE VII.—DISPOSITION OF OXAZEPAM IN HUMANS

Time, Hr.	Plasma Oxazepam, mcg./ml.		Urine Oxazepam Glucuronide[b]		Feces Oxazepam[c]
	A. R.	V. M.	A. R.	V. M.	V. M.
1	...	0	...	0	...
1.5	0.39	...	...	...	...
2	1.11	0	0.18	0.12	...
4	2.08	0.68	...	2.02	...
8	1.49	0.62	...	7.30	...
10	...	...	1.56	...	...
11	...	...	...	23.06	...
24	1.19	0.47	9.51	24.81	...
28	0.88	...	...	26.50	...
31	...	...	...	...	10.9
32	0.63	...	...	29.20	...
35	...	...	...	30.93	...
48	0.36[a]	0.23	17.86	35.44	...
60	...	...	...	...	20.9

[a] This sample also contained oxazepam glucuronide equivalent to 0.75 mcg./ml. [b] Values are equivalent amounts of oxazepam expressed as cumulative per cent of dose. [c] Values are cumulative per cent of dose.

cation product ran near the solvent front, lending support to the view that it is a glucuronide. Treatment of the urine with β-glucuronidase liberated oxazepam, thus establishing the structure of the metabolite as a β-glucuronide. That this glucuronide is of the "ether" type was indicated by its failure to form a hydroxamate, its relative stability to mild acid hydrolysis, and its failure to reduce alkaline copper reagent.

Intramuscular injection of C^{14}-labeled-Wy-4426 in dogs gave rise to three radioactive substances in

urine (Fig. 1). Two of these were identified as unchanged Wy-4426 and oxazepam. When this urine was subjected to mild acid hydrolysis, Wy-4426 was converted to oxazepam, but the glucuronide was relatively unaffected. When the urine was treated with glucuronidase instead of acid, only Wy-4426 and oxazepam remained. Chromatograms of rat urine (Fig. 2) indicated more complex biotransformation of both drugs in this species; at least seven distinct metabolites were evident. Pigs appeared to metabolize oxazepam and Wy-4426 like dogs (Fig. 3); almost all the radioactivity appearing in oxazepam glucuronide. Traces of radioactivity were distributed among four or five additional metabolites.

Disposition of Oxazepam in Humans.—As seen in Table VII, plasma levels peaked at 4 hours and persisted for at least 48 hours in both subjects following a single oral 60-mg. dose. Only one plasma sample, 48 hour of A. R., contained glucuronide in addition to unchanged drug. As in dog and pig, the urine of both subjects contained only glucuronide, and the feces (V. M.) only unchanged drug.

Pharmacology and Toxicology of Lutetium Chloride

By THOMAS J. HALEY, N. KOMESU, M. EFROS, L. KOSTE, and H. C. UPHAM

The pharmacological and toxicological properties of lutetium chloride have been investigated. The intraperitoneal and oral LD_{50}'s were 315 and 7.1 mg./Kg., respectively. Studies of chronic toxicity showed no effect on growth and the hemogram or any internal organ changes at autopsy. Transient ocular irritation was observed, and the chemical produced extensive scar formation when applied to abraded skin. Nodules were formed at the site of intradermal injection. Pharmacological studies showed the chemical to be a depressant on all systems studied. Death resulted from cardiovascular collapse coupled with respiratory paralysis. Such effects could not be counteracted by atropinization or epinephrine injection.

RECENTLY, there has been considerable interest in the chemistry of the rare earths and their employment as alloying agents (1–3). Studies of their biological effects have not been comparable, particularly concerning the heaviest member of the series, lutetium. Spode (4) employed Lu^{177} for interstitial radiotherapy in guinea pigs. Snyder *et al.* (5) showed that intravenous injection of lutetium chloride did not increase liver lipids like cerium did. Durbin *et al.* (6) reported that 50 to 65% of an injected dose of lutetium deposited in the skeleton and the extraskeletal burden was excreted in the urine within 2 weeks. Schepers *et al.* (7–9) studied the effects of mixtures of rare earth oxides or fluorides on the lungs; although damage did occur, the exact element involved could not be pinpointed because a mixture was used. Bruce *et al.* (10) reported that the intraperitoneal LD_{50}'s for lutetium nitrate in female mice and rats were 290(259–325) and 335(294–382) mg./Kg., respectively. Inasmuch as the above reports constitute the bulk of the known effects of lutetium, a more extensive investigation of the pharmacology and toxicology of this element has been undertaken.

METHODS AND MATERIALS

The intraperitoneal LD_{50} was obtained with 60 male CFl mice and the oral LD_{50} with 50 male CFl mice. Chronic toxic effects of the element were studied by including 0.01, 0.1, and 1.0% of the compound in the diet and feeding it over a period of 90 days to three groups of CRW rats. Each group contained six males and six females. Observations were made every 2 weeks of total erythrocytes, total leukocytes, differential cell count, platelets, hemoglobin, hematocrit, and body weight. Upon completion of the study, histopathological examination was made of the heart, lung, liver, kidney, spleen, pancreas, adrenal, and small intestine. The method of Draize *et al.* (11) was used to study ocular and skin irritation in rabbits and intradermal irritation in guinea pigs. In the ocular studies two rabbits were used; each animal had one eye exposed to 0.1 ml. of a 1:1 aqueous solution of compound, while the other eye served as a control. Rabbit skin irritation studies used six animals according to the design of Draize *et al.* (11). Three guinea pigs were used for the compound in the intradermal series; the concentrations were 1:10, 1:100, $1:10^3$, $1:10^4$, $1:10^5$, and $1:10^6$. Histopathological examination was made of the skin areas injected with the $1:10^6$ concentration. Effects of the chemical on guinea pig ileal strips bathed in Locke-Ringers solution were studied in a thermostatically regulated 25-ml. bath using the Trendelenburg method (12). Studies were also made on the isolated rabbit ileum in the presence of either 2.5 mcg. of acetylcholine or 0.5 mcg. of nicotine. Ten cats of both sexes, weighing 2.3–4.18 Kg., were anesthetized with 0.5 ml./Kg. of Dial-urethane intraperitoneally. A six-channel Offner Dynagraph with Statham transducers was used to record carotid arterial pressure, respiration, nictitating membrane contraction, ECG lead II, femoral arterial pressure, and femoral arterial flow. The latter was obtained with a 25-ml. Shipley-Wilson flowmeter (13). Preganglionic stimulation of the cervical sympathetic fibers and the contralateral vagus fibers was accomplished with a Grass model S-4 stimulator at

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