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

Received March 13, 1964, from the Laboratory of Nuclear Medicine and Radiation Biology, Department of Biophysics and Nuclear Medicine, School of Medicine, University of California at Los Angeles, Los Angeles.
Accepted for publication April 27, 1964.
Presented to the Scientific Section, A.Ph.A., New York City meeting, August 1964.
These studies were supported by contract AT(04-1)-GEN-12, U. S. Atomic Energy Commission, Washington, D. C.

8 v./10 seconds. Two hours were allowed to elapse prior to drug administration. Intravenous doses of the drug used were: lutetium, 1 to 40 mg./Kg.; epinephrine, 5 mcg./Kg.; acetylcholine, 5 mcg./Kg.; histamine, 2 mcg./Kg.; and atropine, 2 mg./Kg. All injections were made at a constant volume of 1 ml. Where appropriate, the results were analyzed statistically by the Litchfield-Wilcoxon method (14), or standard errors were calculated.

Acute Toxicity.—The symptoms of acute toxicity were writhing, ataxia, labored respiration, walking on toes with back arched, and sedation. The first deaths occurred within 24 hours, and the peak was reached at 48 hours. Throughout the 7-day period of observation, the animals were unthrifty. The intraperitoneal LD_{50}/7 days for lutetium chloride was 315 (267–372) mg./Kg. with a slope value of 1.54 (1.11–2.13). The oral LD_{50}/7 days was 7100 (6630–7590) mg./Kg. with a slope value of 1.19 (1.03–1.38).

Chronic Toxicity.—Male and female rats ingesting various levels of lutetium chloride in the diet showed no differences in growth rate from the controls (Fig. 1). Moreover, the chemical had no significant effect on the hematology of the animals. A normal growth pattern would account for the increases in both the cellular elements and the hemoglobin. In fact, the values given in Table I are in good agreement with those listed for the rat by Gardner (15). No data are given for the differential cell counts because there was no significant difference between the medicated and the control groups. At autopsy, the internal organs of all groups of rats appeared normal; there were no outward signs of damage from ingestion of lutetium chloride for 12 weeks. Histopathological examination of the heart, lung, liver, kidney, spleen, pancreas, adrenal, and small intestine revealed no changes that could be attributed to ingestion of lutetium chloride.

Ocular Irritation.—Instillation of 0.1 ml. of a 1:1 solution of lutetium chloride into the eyes of two albino rabbits produced no effect upon the cornea. However, within 1 hour the conjunctiva was a diffuse beefy red, chemosis had caused swelling of the lids closing the eyes, and a profuse discharge had moistened the lids and a considerable area surrounding the eyes. At 24 hours the maximum irritation index of 20 was still present, and minute ulcers appeared. The irritation index was reduced to 6 in 1 week, but complete healing required 2 weeks. There was no evidence of residual damage.

Skin Irritation.—No reaction was observed when 0.5 Gm. of crystalline lutetium chloride was applied to intact rabbit skin. The response of abraded skin was very severe, showing a maximum irritation index of 8 at 24 hours. No changes occurred in the irritation index up to 14 days. However, healing with the formation of scars 25 to 30 mm. in diameter occurred at 35 days. Intradermal injection of lutetium chloride at concentrations of 1:10, 1:100, and 1:1000 produced necrosis within 1 hour and an irritation index (erythema plus edema) of 8 to 3, respectively. These reactions persisted for 7 days; complete healing with epilation and scar formation did not occur for 5 weeks. The concentrations of $1:10^4$ to $1:10^6$ gave a 24-hour irritation index of 2 and healing with nodule formation at 46 days. Histopathological examination of these nodules revealed the presence of crystalline

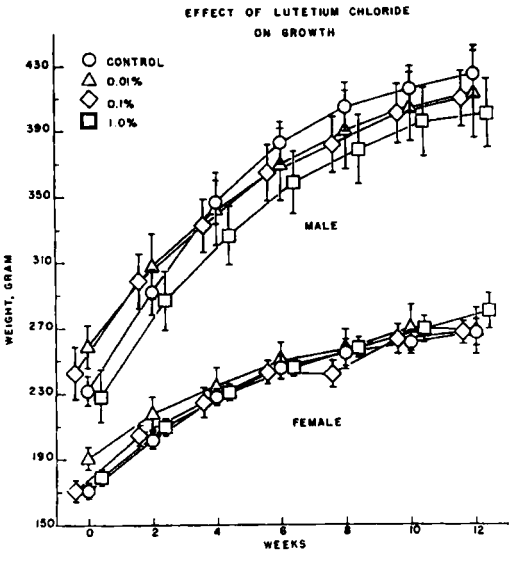

Fig. 1.—Growth of male and female CRW rats on feeding various levels of lutetium chloride in the diet for 90 days. Bars signify the standard errors. Each group contained six animals for each dosage level.

deposits of unknown composition. Foreign body giant cells and histocytes surrounded the crystals, while fibroblasts and granulation tissue extended into the deposits. Such cellular changes were not found in control skin sections from the same animals. Inasmuch as necrosis was not observed at these concentrations, the response does not appear to be related to the hydrogen ion concentration of the injected solutions. The pH values were 5.61 to 6.04. It seems more likely that there was a reaction between the lutetium and tissue phosphate to produce insoluble lutetium phosphate; however, the small number and size of the crystals prevented a more quantitative analysis.

Effects on Isolated Ileum.—In the dosage range of 10 to 40 mg., lutetium chloride produced an increasing depression of intestinal tonus and contractility of the rabbit ileum ending in a nonreversible paralysis. This depressant effect counteracted the spasmogenic effect of both acetylcholine and nicotine. The antispasmodic ED_{50}'s were 0.6(0.5–0.7) mg./ml. and 0.5(0.3–0.8) mg./ml., respectively, with slope values of 1.33(0.91–1.94) and 2.22 (0.96–5.13). A similar depression also occurred with the Trendelenburg guinea pig enteric ganglia preparation, where the ED_{50} figures for blocking the circular and longitudinal muscular contractions were 0.5(0.4–0.7) mg./ml. and 0.3(0.2–0.4) mg./ml., respectively. Although this would seem to indicate that lutetium chloride had ganglionic blocking properties, such activity appears unlikely because the compound failed to block the cat superior cervical ganglion preparation.

Pharmacological Effects.—No pharmacological effects were observed on the cat in the dosage range of 1 to 10 mg./Kg. When death did not occur at 20 mg./Kg., there was a transient hypotension of 10 to 15 mm. Hg with a concomitant decrease in peripheral blood flow. Complete cardiovascular collapse, coupled with respiratory paralysis, was

TABLE I.—HEMATOLOGIC EFFECTS OF LUTETIUM

Dosage	Sex	Erythrocytes, mm.³ × 10⁶ 0 Wks.	Erythrocytes, mm.³ × 10⁶ 12 Wks.	Leukocytes, mm.³ × 10³ 0 Wks.	Leukocytes, mm.³ × 10³ 12 Wks.	Hematocrit, Vol. % 0 Wks.	Hematocrit, Vol. % 12 Wks.	Hemoglobin, Gm. % 0 Wks.	Hemoglobin, Gm. % 12 Wks.	Platelets, mm.³ × 10³ 0 Wks.	Platelets, mm.³ × 10³ 12 Wks.
Control	♂	7.19 ± 0.22[a]	8.37 ± 0.28	25.43 ± 0.22	20.89 ± 0.24	41 ± 0.88	46 ± 0.88	10.9 ± 0.77	13.7 ± 0.50	4.11 ± 0.009	4.35 ± 0.004
	♂♂	5.98 – 8.14[b]	7.45 – 9.80	16.8 – 36.4	12.8 – 32.2	37 – 45	42 – 50	10.0 – 11.9	11.1 – 15.4	3.04 – 5.20	3.80 – 4.86
	♀	6.88 ± 0.35	8.02 ± 0.30	25.71 ± 0.37	19.93 ± 0.21	43 ± 0.21	46 ± 1.07	11.9 ± 1.20	13.5 ± 0.39	4.66 ± 0.012	4.18 ± 0.008
	♀	5.62 – 8.32	6.30 – 9.55	13.6 – 45.4	13.2 – 30.8	39 – 46	38 – 48	9.7 – 13.7	10.7 – 14.4	3.40 – 7.00	3.04 – 5.44
Lutetium 0.01%	♂	7.54 ± 0.45	9.39 ± 0.48	24.33 ± 0.32	19.57 ± 0.23	43 ± 1.28	50 ± 0.92	12.0 ± 0.69	15.2 ± 0.25	5.09 ± 0.023	5.27 ± 0.016
	♂♂	6.18 – 8.82	7.10 – 10.35	12.0 – 36.6	13.8 – 29.8	39 – 48	47 – 52	10.0 – 14.3	14.4 – 16.1	3.56 – 7.80	4.00 – 6.80
	♀	6.95 ± 0.36	8.86 ± 0.48	18.20 ± 0.20	13.12 ± 0.11	42 ± 1.00	46 ± 0.49	12.1 ± 0.40	14.5 ± 0.22	3.93 ± 0.009	5.18 ± 0.006
	♀	6.18 – 8.48	7.45 – 10.10	10.0 – 24.4	10.0 – 16.6	38 – 45	45 – 48	11.0 – 13.6	13.9 – 15.1	3.05 – 4.92	4.68 – 5.80
Lutetium 0.1%	♂	6.87 ± 0.40	9.36 ± 0.33	23.43 ± 0.42	22.33 ± 0.30	43 ± 0.99	47 ± 0.88	12.2 ± 0.27	14.4 ± 0.27	3.70 ± 0.006	4.37 ± 0.008
	♂♂	5.08 – 7.74	8.40 – 10.40	12.6 – 42.2	15.8 – 36.2	38 – 45	44 – 50	11.0 – 12.8	13.2 – 14.9	3.16 – 4.48	3.44 – 5.00
	♀	6.97 ± 0.39	8.81 ± 0.37	23.20 ± 0.23	23.68 ± 0.66	43 ± 1.09	47 ± 1.81	12.9 ± 0.60	14.8 ± 0.51	3.96 ± 0.004	4.87 ± 0.015
	♀	5.16 – 7.80	8.20 – 10.25	18.8 – 34.4	9.8 – 48.6	40 – 48	43 – 54	10.8 – 15.0	13.7 – 16.4	3.44 – 4.44	3.42 – 5.88
Lutetium 1.0%	♂	6.43 ± 0.10	8.46 ± 0.25	30.40 ± 0.50	25.00 ± 0.28	41 ± 1.14	44 ± 0.60	11.5 ± 0.25	12.8 ± 0.45	4.43 ± 0.014	4.56 ± 0.014
	♂♂	6.18 – 6.84	7.80 – 9.30	14.2 – 43.8	16.4 – 33.4	37 – 45	42 – 46	10.4 – 12.0	11.3 – 14.5	3.40 – 6.44	3.40 – 6.36
	♀	6.99 ± 0.46	9.07 ± 0.36	19.67 ± 0.16	13.33 ± 0.16	42 ± 1.18	45 ± 2.55	11.7 ± 0.50	13.5 ± 0.92	5.30 ± 0.022	5.37 ± 0.017
	♀	5.44 – 8.44	7.40 – 9.70	10.7 – 34.4	9.8 – 19.8	38 – 46	33 – 50	9.9 – 13.3	9.0 – 14.8	3.64 – 8.48	4.04 – 7.04

[a] Mean ± standard error. [b] Range.

produced by 20 mg./Kg. in five animals and by 40 mg./Kg. in the other five. The respiration was not affected prior to exitus. Terminal electrocardiographic changes included inversion of the QRS complex, transient ventricular fibrillation, 2:1 and 3:1 heart block, diphasic T-waves, inverted T-waves, increase in height of T-wave, and high takeoff of T-waves. Injection of epinephrine caused QRS complexes of low amplitude and an absence of the T-wave. In the presence of atropine, epinephrine induced a fatal ventricular fibrillation. Within the dosage range employed, lutetium chloride had no effect on the pharmacological responses to acetylcholine, histamine, or vagal stimulation. Moreover, it had no effect on transmission in the superior cervical ganglion or on contraction of the nictitating membrane. None of the above effects of lutetium chloride could be modified by atropinization, and the cardiovascular collapse was not counteracted by epinephrine.

DISCUSSION

Lutetium chloride is less toxic—both acutely and chronically—than gadolinium and samarium (16) or niobium (17) or hafnium (18) and about equal in toxicity to scandium (19), terbium, thulium, and ytterbium (20), and praseodymium and neodymium (21). The ocular irritation produced by lutetium was similar to and of the same duration as that produced by the other rare earths studied (16, 19–21). The same was true for its ability to produce skin irritation and nodule formation in the dermal layers (22). The depressant effects on other biological systems was similar to the observations of Mines (23) concerning the frog heart. The mode of death—respiratory paralysis coupled with cardiovascular collapse—was identical to that observed with the other rare elements (16–21). Precautionary measures to be instituted in handling lutetium chloride should include respirators and protective clothing.

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