Pharmacology and Toxicology of Europium Chloride

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The pharmacological and toxicological properties of europium chloride have been studied. The intraperitoneal and oral LD_{50}'s were 550 mg./Kg. and 5.0 Gm./Kg., respectively. Feeding various levels of the chemical for 12 weeks produced no effect on growth, the hemogram, or any internal organ. Transient ocular irritation with no permanent ocular damage was observed, but abraded skin showed extensive scar formation. Permanent nodule formation resulted from intradermal injection. Progressive depression was observed on all biological systems studied, and death was the result of cardiovascular collapse coupled with respiratory paralysis. Neither atropinization nor epinephrine injections could counteract the lethal effects.

THERE IS an increasing awareness of the potential application of rare earths to many industrial processes (1–3). Anderson and Dunning (4) have suggested that europium oxide dispersion could be used as reactor control elements. Ransohoff (5) pointed out that the element could be used as a control rod poison if its cost could be reduced. Perhaps cost is also the reason for the general lack of information on the biological effects produced by europium chloride. Muroma (6) found europium chloride toxic to *Salmonella typhimurium*, *Escherichia coli*, *Micrococcus pyogenes*, and *Streptococcus pyogenes*. Bruce *et al.* (7) reported an intraperitoneal LD_{50} for europium nitrate in female mice of 320 mg./Kg. and for female rats 210 mg./Kg. The oral LD_{50} in the latter species was 75 Gm. Inhalation toxicity studies indicate that europium vapor causes heat sensitivity and itching of the skin but no real over-all effects on blood pressure, heart function, or respiration (8). Lung damage was seen in guinea pigs inhaling rare earth oxides or fluorides, but the causative agent could not be stated because mixtures were used (9–11). Van Cleave (12) reported that when Eu^{154} was administered intravenously, radioautographs showed the greatest concentrations in the reticuloendothelial system of the liver and spleen. Durbin *et al.* (13) studied uptake and distribution of $Eu^{152, 154}$ and found 30% in the liver and 40% in the skeleton. Excretion occurred *via* the feces and urine. Inasmuch as more knowledge was required concerning europium, an extensive pharmacological and toxicological investigation has been undertaken.

METHODS AND MATERIALS

The intraperitoneal LD_{50} was obtained with 55 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 12 weeks to three groups of CRW rats. Each group contained six males and six females. Observations of total erythrocytes, total leukocytes, differential cell count, platelets, hemoglobin, hematocrit and body weight were made every 2 weeks. Upon completion of the study, histopathological examination was made of the heart, lung, liver, kidney, spleen, pancreas, adrenal, and small intestine.

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The method of Draize *et al.* (14) was used to study ocular and skin irritation in rabbits and intradermal irritation in guinea pigs. In the ocular studies, three rabbits were used, and 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.* (14). Three guinea pigs were used for the compound in the intradermal studies; 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 (15). Studies also were 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 2.18 to 4.6 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 (16). Preganglionic stimulation of the cervical sympathetic fibers and the contralateral vagus fibers was accomplished with a Grass model S-4 stimulator at 8 v./10 sec. Two hours were allowed to elapse prior to beginning drug administration. Intravenous doses of the drug used were: europium, 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 (17), or standard errors were calculated.

Acute Toxicity.—The symptoms of acute toxicity from europium chloride were arched back, writhing, ataxia, lachrymation, stretching of the hind limbs on walking, and a labored and depressed respiration. The first deaths occurred within 24 hr., but the peak was not reached until 120 hr. The acute intraperitoneal LD_{50} and its range was 550 (515.5–586.9) mg./Kg., and the acute oral LD_{50} and its range was 5000 (4505–5500) mg./Kg. The slope values and their ranges were 1.18 (1.03–1.36) and 1.31 (1.05–1.64), respectively.

Chronic Toxicity.—Ingestion of various dietary levels of europium chloride by male and female rats indicate that this chemical had no effect on growth (Fig. 1). Hematological studies indicated

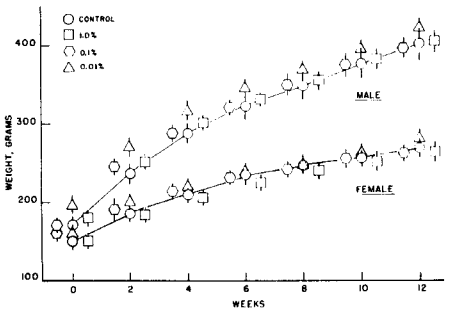

Fig. 1.—Growth of male and female CRW rats on feeding various levels of europium chloride in the diet for 12 weeks. Bars signify the standard errors calculated with the formula

$$\text{S.E.} = \sqrt{\frac{\Sigma X^2 - (\Sigma X)^2/N}{N(N-1)}}$$

Each group contained six animals for each dosage level.

that this chemical had no effect on the total erythrocyte count, total leukocyte count, differential cell count, thrombocyte count, hemoglobin, or hematocrit values. The data obtained were in agreement with the values listed by Gardner (18) for the rat. At autopsy, all organs appeared normal and histopathological examination of heart, lung, liver, kidney, spleen, pancreas, adrenal, and small intestine revealed no changes which could be attributed to ingestion of europium chloride for 12 weeks.

Ocular Irritation.—No detectable damage to the cornea or the iris was observed after application of 0.1 ml. of a 1:1 concentration of europium chloride to the eye. One hour after the application of the chemical, the rabbit's conjunctival membranes were a diffuse beefy red, the eyelids were almost completely closed, and a discharge moistened the lids and hairs about the eye. This high degree of irritation, irritation index of 20, persisted for 7 days; and 1 mm. conjunctival ulcers were observed also. Healing was complete at 36 days. Such high irritation did not appear to be related to pH because the pH of the solution, 2.45, produced a greater effect than pH 1 aqueous HCl.

Skin Irritation.—The application of 0.5 Gm. of europium chloride to abraded and unabraded rabbit skin resulted in an average irritation index of 7.5 on the former and no effect on the latter. Such irritation resulted in punched out ulcers which required 28 days for complete healing with scars of 30 to 35 mm. in diameter.

Intradermal injection of europium chloride at concentrations of 1:10 to 1:1000 produced local necrosis within 1 hr. and an irritation index of 8 which persisted for 1 wk. When healing occurred at 74 days, epilation and scar formation were observed at the injection site. Such reactions were not seen with the concentrations of 10^4 to 10^6, although the irritation indexes were 2 to 5 during the first 24 hr. Nodules were formed, and histopathological examination of these areas in the 10^6 concentration revealed the presence of crystalline deposits of unknown composition. Histocytes and foreign body giant cells 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. These effects do not appear to be related to pH because the pH of the injected solutions was 6.39 to 6.78. The deposits may have been the result of a combination of the europium with tissue proteins.

Effects on Isolated Ileum.—An increasing dose dependent depression of tonus and contractility of the rabbit ileum was produced by europium chloride in the dosage range of 10 to 40 mg. Repeated washing would not restore the europium-induced intestinal paralysis. This depression counteracted the spasmogenic effects of both acetylcholine and nicotine. The antispasmodic ED_{50} figures and their ranges for europium chloride were 0.5 (0.38–0.6) mg./ml. and 0.5 (0.27–0.91) mg./ml., respectively, with slope values and ranges of 1.3 (1.0–1.72) and 2.2 (0.85–5.72). A similar depressant action was observed on the Trendelenburg guinea pig enteric ganglia preparation, where the ED_{50} figures and their ranges for blocking the circular and longitudinal muscular contractions were, respectively, 0.14 (0.07–0.3) mg./ml. and 0.09 (0.04–0.2) mg./ml. with slope values and ranges of 2.77 (0.99–7.73) and 3.0 (0.91–9.9). Such effects appear to indicate ganglionic blocking properties, but this appears to be unlikely because europium chloride was ineffective on the cat superior cervical ganglion preparation.

Pharmacodynamic Effects.—No detectable pharmacodynamic effects were observed in the cat with doses of 1 to 10 mg./Kg. of europium chloride. At the 20 mg./Kg. dose, there was a transient hypotension in eight of 10 cats and no effect on heart rate or femoral blood flow. This dose was fatal to two animals, whereas it required 40 mg./Kg. for the other cats. Death resulted from cardiovascular collapse coupled with respiratory paralysis. Prior to this, the respiratory function was not affected. The terminal electrocardiographic changes included a decrease in the size of the entire complex, low voltage QRS, absence of the QRS, notched QRS, high takeoff of the T-wave, increased T-wave, inverted T-wave, 2:1 to 3:1 heart block, and transient ventricular fibrillation. Within the dosage range studied, europium chloride had no effect on the pharmacological responses to acetylcholine, epinephrine, histamine, vagal stimulation, transmission in the superior cervical ganglion, or contraction of the nictitating membrane. Furthermore, neither epinephrine nor atropine was able to modify the responses to europium chloride or counteract the cardiovascular collapse.

DISCUSSION

Comparison of our acute intraperitoneal LD_{50} with the data of Bruce *et al.* (7) indicates that the female mouse is more susceptible to the chemical than the male. This is consistent with the observations of other members of the rare earth group (19–23). The inability to demonstrate chronic toxic effects upon prolonged ingestion of europium may be due to a low degree of absorption from the intestinal tract, resulting from a rapid conversion from the chloride to the oxide in an alkaline media. Durbin *et al.* (13) pointed out that only 0.1% of the administered dose of $Eu^{152, 154}$ was absorbed from the gastrointestinal tract. This would account for the low acute oral LD_{50} found. Both the ocular and

skin irritation produced by europium chloride were of the same magnitude and duration reported for the other members of the rare earth series (19–23). Moreover, the skin lesion from intradermal administration was the same (24). Europium chloride produced the same type and degree of depression as the other rare earths (19–23) did on the various biological systems studied. Also, the mechanism of death, cardiovascular collapse coupled with respiratory paralysis, was identical to that of the other elements of the series. Although this element has a relatively low toxicity, care should be exercised to prevent skin lesions with nodule formation by using appropriate clothing.

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Dissolution Rate Studies III. Penetration Model for Describing Dissolution of a Multiparticulate System

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Danckwerts' penetration model is used to derive equations for the dissolution of solids in a multiparticulate system. The equations obtained are capable of explaining the deviations from linearity of cube-root plots. The loss of sensitivity in distinguishing differences between polymorph dissolution rates at high agitation intensities also may be rationalized from these equations.

IT HAS BEEN shown in a previous publication (1) that the Hixon-Crowell cube-root law (2) does not appear to hold for rapidly stirred systems. The data showed that a square-root law described the system more accurately.

The cube-root law is derived using the diffusion layer model proposed by Nernst (3). It is of interest to derive an equation using the penetration theory described by Danckwerts (4). This model (derived for dissolution of a gas in a liquid) assumes that a turbulent liquid is a mass of eddies which are being exposed continuously to fresh surfaces of a gas and then returned to the bulk of the liquid. It is proposed further that free diffusion takes place into each of the packets during the short period of time in which the packet is in contact with the surface. The rationality of such a model is supported strongly by the work of Fage and Townend (5) who found evidence of turbulent flow in a tube as close as 6 μ from the interface. The validity of the model has been discussed by Danckwerts (4) and Hanratty (6). This model should be equally valid for the dissolution of a solid into a liquid and has been demonstrated for the case of dissolution from a flat surface (7).

Danckwerts defines the surface having ages between t and $t + dt$ as $\phi(t)dt$. Hence the flux per unit area, R, (Gm. sec.$^{-1}$ cm.$^{-2}$) from a spherical surface would be

$$R = \int_0^\infty \psi(t)\phi(t)\,dt \qquad \text{(Eq. 1)}$$

where $\psi(t)$ is the rate of diffusion into a stagnant liquid of infinite depth defined by

$$\psi(t) = -D \left.\frac{dC}{dr}\right|_{r=a} \qquad \text{(Eq. 2)}$$

Here C is the concentration, r the radial distance from the center, and a the radius of the sphere. The usual formulation for Fick's second law in spherical coordinates is (8)

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r} \text{ or } \frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial r^2} \qquad \text{(Eq. 3)}$$

where $u = rC$, and t is the time in seconds. The following boundary conditions are assumed:

$$r = a \qquad C = C_A \text{ at all } t > 0$$
$$r = \infty \qquad C = 0$$
$$t = 0 \qquad C = 0 \text{ for all } r$$

The Laplace transform of Eq. 3 is

$$s\bar{u} = D\frac{d^2\bar{u}}{dr^2} \qquad \text{(Eq. 4)}$$

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