the various groups again may be attributed to depth of anesthesia or perhaps an inherent difference between hypothermic responses of cats and dogs.

Summary. Evidence is presented which indicates that pressor response to injections (2 μg/kg) of epinephrine and nor-ephinephrine is potentiated in the anesthetized dog at blood temperatures of 27-28°C. The augmented response to nor-epinephrine was greater than that for epinephrine. Evaluation of respiratory and circulatory responses to bilateral carotid occlusion and hypoxic hypoxia indicates that the hypothermic dog retains reflex activity but magnitude of the responses is less than that at normal body temperature.

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Plasma Free Fatty Acids and the Rare-Earth Fatty Liver.* (26286)

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Some of the lanthanide elements when injected intravenously into rats and other species can produce a fatty liver cycle that is affected by certain endocrines(1,2). The influence of endocrines on release of free fatty acids (FFA) from adipose tissue and the implied significance of FFA in lipid transport (3-6) suggested to us that mobilized extrahepatic lipids might be the source of at least part of the increased liver glyceride level caused by cerium. The experiments described here have tested this possibility; the data demonstrate that cerium does cause an increase in free fatty acids of the plasma and that substances which bind cerium or aggregate it prevent the FFA response and the fatty liver.

Method. Female Carworth-Farms-Nelson (CFN) albino rats weighing 150-200 g were used in all of the experiments except for one group of 7-male CFN rats (136-160 g) and another group of Charles River hypophysectomized female rats (139-170 g). Cerous chloride† (2 mg Ce/kg) was injected intravenously into the tail vein. All animals were maintained on a Dietrich and Gambrill‡ laboratory chow before the cerium injection; some animals were fasted 24 hr before death and others were fed *ad libitum.* Water was available at all times. At 24, 30, 48, and 72 hr after cerium injection, the animals were anesthetized with ether and blood was drawn in a heparinized syringe from the abdominal aorta. The plasma free fatty acids were extracted and determined in duplicate by the procedure of Dole(3). An Ultra Micro-Buret, model 200, obtained from Scientific Industries, Inc., was used for all titrations. In a few rats, glucose was measured(7) in serum obtained from blood after decapitation.

Cerium in combination with versene, albumin, adenosine triphosphate, plasma, phosphate, hydroxide, adenine, adenosine, cytidine, citric acid, and heparin was injected into rats to see whether these compounds protected the animal from the fatty liver degeneration. The method for extraction and measurement of total liver lipids has been described(1).

Results and discussion. A significant in-

* Under contract with U. S. Atomic Energy Commission.

† Cerous chloride, Fisher, C-252, dissolved in saline.

‡ Dietrich & Gambrill, Inc., Laboratory Animal Food Division, Frederick, Md.

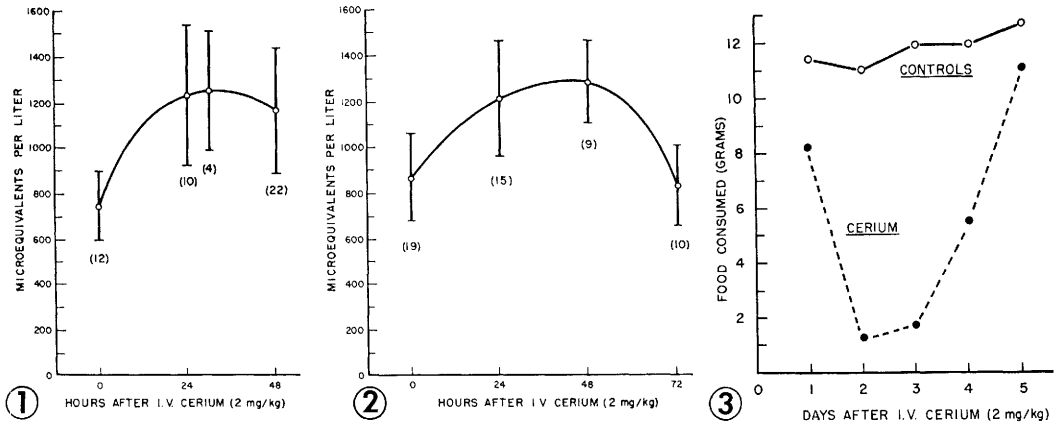

FIG. 1. Effect of cerium on plasma free fatty acids in female rats fed *ad libitum*. Vertical bars represent stand. dev. No. of animals are shown in parentheses. The probability for chance occurrence was less than 0.001 when the test of significance was applied to the difference between the mean of control group and means of 24, 30, and 48 hr post-cerium groups.

FIG. 2. Effect of cerium on plasma free fatty acids in female rats fasted 24 hr before sacrifice. Vertical bars represent stand. dev. No. of animals shown in parentheses. The probability for chance occurrence was less than 0.001 when the test of significance was applied to the difference between means of control group and means of 24 and 48 hr post-cerium groups.

FIG. 3. Effect of cerium on food consumption. Each group consisted of 5 rats.

crease in plasma free fatty acids precedes the development of fatty livers in rats injected intravenously with cerium (Fig. 1). The high levels of titratable acid at 24 hr after cerium administration cannot be explained by the reduced food intake which occurs during this period (8 g, as seen in Fig. 3), since the effect of cerium on FFA is also observed in fasted rats (Fig. 2); furthermore, plasma FFA levels are not significantly altered in control rats fed a restricted diet (6 g, as seen in Table I).

TABLE I. Effect of Various Treatments on Plasma Free Fatty Acids in CFN Rats Injected with Cerium.

Treatment	No. of rats	Free fatty acids	
		μeq/l	Range
Controls	12*	754	(512-1025)
24 hr cerium	10*	1237	(867-1900)
Versene-cerium	4*	613	(537- 713)
Cerium, followed by versene	4*	1004	(900-1160)
Reduced food intake (6 g per day)	5	681	(549- 750)
Controls (24 hr fast)	3	976	(855-1217)
" (male)	4*	641	(523- 834)
Cerium—24 hr (male)	3*	666	(525- 750)
Cerium hydroxide	4*	515	(435- 563)
Hypophysectomized control (24 hr fast)	4	709	(665- 771)
Hypophysectomized, 24 hr cerium (24 hr fast)	5	782	(650- 938)

* Fed *ad libitum*. All animals were female, unless otherwise indicated in Table.

TABLE II. Effect of Various Compounds on Total Liver Lipids 24 Hr after an Intravenous Injection of Cerium.

Compound admin. with cerium, I.V.	Molar ratio: Compound/Ce	Liver lipids, % wet tissue
None	—	13.92, 14.87
Versene	5.65	4.60, 6.87
Albumin	.03	10.01, 11.66
ATP	.60	4.39, 5.53
Phosphate	2.26	5.88, 5.98
Hydroxide	excess	5.29, 6.14
Adenine	1.04	5.51, 7.19
Adenosine	1.00	10.67, 11.95
Cytidine	1.00	9.90, 10.71
Citric acid	5.00	10.19, 12.85
Plasma	—	6.40, 7.26
Heparin*	—	14.94, 16.59

* 1 mg at 15 min. before and 6, 24, 30 and 47 hr after cerium inj. All other compounds were administered as a single intrav. inj. in combination with cerium.

Conditions that prevent the induction of the cerium fatty liver also prevent the free fatty acid response; *e.g.*, use of male or hypophysectomized rats, versene binding, and particle formation *via* hydroxide. Table II shows the protective action of certain compounds mixed with cerium before injection in development of this fatty liver infiltration. All the substances that either bound with

TABLE III. Effect of Cerium (2 mg/kg, I.V.) on Serum Glucose.

Treatment	No. of rats	Glucose (range), mg/100 ml	Liver fat
Control (*ad libitum*)	3	172 (161-178)	—
Control (24 hr fast)	6	144 (137-164)	—
4 hr cerium (*ad libitum*)	4	141 (134-145)	—
24 hr cerium (24 hr fast)	4	91 (76-126)	+
48 hr cerium (24 hr fast)	4	88 (74- 95)	++++
Idem, alloxan diabetic rats)	3	308 (205-468)	—

cerium (versene, plasma proteins) or produced particles (phosphate, hydroxide, ATP) also prevented the usual increase in liver lipids of animals killed 48 hr after an intravenous cerium dose (2 mg/kg). The results obtained with adenine or adenosine are difficult to explain. It can be seen from Table I that the cerium must be reacted with the substance in question before injection, since the fatty liver and the FFA response will not be prevented if versene, for example, is administered immediately before or after cerium injection. Preliminary experiments in our laboratories, in which versene has been infused continuously in dogs attached to an artificial kidney with hopes of speeding up excretion of cerium, have also demonstrated that the biological binding of cerium is quite tenacious.

The inverse relationship of blood glucose and FFA levels that occurs after certain hormonal stimuli(3,6) also exists with the cerium-induced free fatty acid response, *i.e.*, a decrease in serum glucose occurs as early as 4 hr after administration of cerium (Table III). A similar effect of rare earths on blood sugar is described in a review by Trapmann (8). Because of the involvement of carbohydrate, the effect of insulin deprivation was tested, and the alloxan diabetic rat, like the hypophysectomized animal, will not develop the cerium-induced fatty infiltration (Table III). The fact that serum glucose decreases at a time when plasma FFA levels from cerium-treated rats (0.5 h, 728 μEq/L and 4 hr, 759 μEq/L) are in the same range as controls, indicates that carbohydrate metabolism is interrupted before the lipid disorder occurs.

The consistent occurrence of elevated free fatty acids during accumulation of total liver lipids suggests that mobilization from other sites is an important factor in the mechanism of fatty infiltration due to cerium. Other experiments, including measurements of distribution of total body lipids and changes in their composition by gas chromatographic analyses, have been initiated to evaluate this concept. A depressed mitochondrial oxidation of lipids (octanoic acid) is also a contributing factor in their abnormal accumulation in liver after cerium administration(9).

Summary. 1. Cerium (2 mg/kg) causes an early decrease in serum glucose and a later significant increase in plasma free fatty acids followed by fatty degeneration of the liver. 2. Conditions that prevent the free fatty acid and fatty liver responses are (a) administration of the cerium as a bound complex or particle, and (b) use of hypophysectomized, diabetic, or male rats.

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