servations further strengthen the concept that the term "estrogen" can be rather vague and should be redefined in terms of chemical structure and physiological effect.

The inability of testosterone to affect the binding of estradiol is to be expected since competition studies carried out *in vivo* also gave negative results. The compound 2α-methyl-5α-dihydrotestosterone,[1] which is used clinically for suppression of human breast tumors, is also ineffective.

The concept of specificity of nuclear binding must be treated with proper caution. It has been demonstrated that *in vitro* binding is at least a two-step mechanism requiring a supernatant receptor probably followed by enzymatic transfer of the steroid to the nuclear receptor (13). Thus specificity may involve any or all of the receptors and transferring enzyme systems. Moreover, specificity may vary somewhat between these macromolecules.

Summary. The inhibitory effects of several compounds on the *in vitro* binding of estradiol to the rat uterine nuclear pellet were tested. The binding properties of the compounds tested were related to their known physiological effects. Chemical modifications of the estradiol molecule produced marked changes in binding properties, indicating a high degree of specificity for the transfer reactions.

[1] This compound was generously supplied by Dr. A. Segaloff.

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Rare Earth Metals and Soft-Tissue Calcification (33041)

B. TUCHWEBER AND L. SAVOIE (Introduced by H. Selye)

Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada

In the rat certain metallic salts can induce topical calcification at the site of injection (1); this phenomenon is called calcergy. Among these salts (calcergens) are the chlorides of lead, zinc, indium, cerium, and cobalt; numerous other metallic compounds ($CrCl_3$, $FeCl_3$, $SnCl_2$) have proved ineffective in this respect (2). The calcergic deposits show the X-ray diffraction pattern of hydroxylapatite (3). Many calcergens given

intravenously predispose the rat to calcification at sites of topical orthophosphate injection (4), whereas only lead salts sensitize for calcification those sites treated with a histamine liberator (5,6). Recently it has been demonstrated that rare earth metals also induce accumulation of calcium salts at the site of subcutaneous injection (7). Hence we wished to determine whether these metals also could sensitize the rat for calcification at the site of administration of a histamine liberator.

Materials and Methods. One hundred and twenty Sprague-Dawley rats of the Robidoux Farm (Montreal, Qué., Canada) with a mean initial body weight of 100 gm (range 94–110 gm) were divided into 12 equal groups and treated as shown in Tables I and II. The trichlorides of dysprosium, erbium, holmium, lutetium, scandium and thulium (K and K Laboratories Inc., Plainview, New York) were injected intravenously under light ether anesthesia in 1 ml of distilled water. In the first experiment (Table I) they were given at the dose of 3 mg, except scandium which was given at the dose of 4 mg. In the second experiment (Table II), various amounts of $HoCl_3$ (1–8 mg) were tested for their efficacy in predisposing the rat to calcification. In addition, immediately after the intravenous injection, 30 μg of polymyxin-B sulfate in 0.2 ml of distilled water (Pfizer Company of Canada Ltd., Montreal, Canada) were injected subcutaneously on the back.

During the experiments, the rats were fed Purina Laboratory Chow (Purina Co. of Canada) and given tap water. On the sixth day they were killed with chloroform. At autopsy, the diameter of the calcified wheal produced by the topical treatment was measured and splenic calcification was expressed in terms of an arbitrary scale of 0–3 in which 0 = no lesion, 1 = just detectable, 2 = moderate, and 3 = maximal lesion. Specimens of spleen and skin from the site of polymyxin injection were fixed in alcohol–formol (4 parts of absolute alcohol and 1 part of 10% neutral formalin) embedded in paraffin and stained with the von Kóssa technique for the histochemical demonstration of phosphate and carbonate, and with chloranilic acid for the demonstration of calcium. Skin specimens were incinerated at 600°C for 17 hours for the determination

TABLE I. Production of Soft-Tissue Calcification by Rare Earth Metals.

Group	Treatment[a]	Skin calcification at polymyxin site		
		Macroscopy (mm)	Calcium (%)	Phosphorus (%)
1	None	0	1.3 ± 0.05	10.7 ± 0.33
2	$DyCl_3$	24 ± 1.1	25.7 ± 2.59	19.0 ± 0.15
3	$ErCl_3$	25 ± 1.2	22.5 ± 1.50	18.6 ± 0.22
4	$HoCl_3$	26 ± 1.1	23.3 ± 0.97	18.9 ± 0.18
5	$LuCl_3$	0	1.8 ± 0.04	11.8 ± 0.36
6	$ScCl_3$	0	1.8 ± 0.04	11.7 ± 0.36
7	$TmCl_3$	24 ± 1.1	22.9 ± 1.18	19.1 ± 0.13

[a] In addition, all rats received 30 μg of polymyxin as described in the text.

FIG. 1. (Top) Normal aspect of the reversed skin: viewed from inside. (Bottom) A large calcified plaque at polymyxin injection site in a rat treated with $HoCl_3$ i.v.

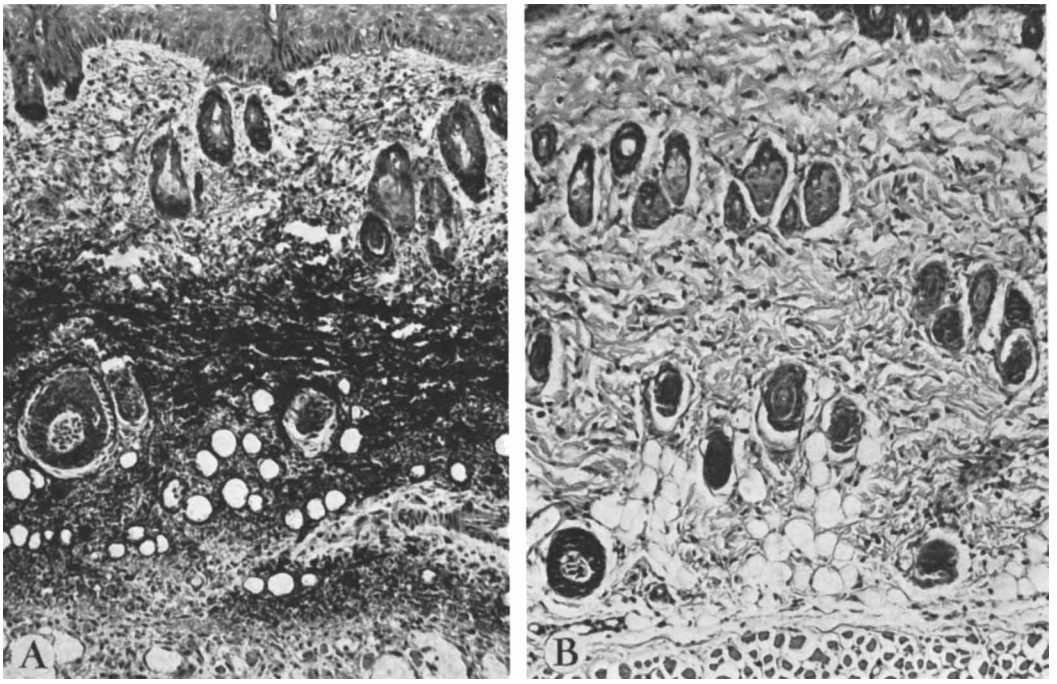

FIG. 2. A: Histologic appearance of the polymyxin injection site in a rat given $HoCl_3$ i.v. Calcium is evenly deposited along the collagen fibers. B: Normal skin (Von Kóssa × 120).

of calcium and phosphorus, respectively, by atomic absorption spectroscopy (8) and the Fiske and Subbarow technique (9).

Results. As shown in Table I, of the 6 rare earth metals tested, only the chlorides of Dy, Er, Ho, and Tm induced calcification at the site of polymyxin injection (Fig. 1). The macroscopic readings are in agreement with chemical analysis of the corresponding calcified plaques, in which the values of calcium and phosphorus are expressed as a percentage of the ashed weight. The calcium and phosphorus contents of these plaques (groups 2, 3, 4 and 7) were significantly increased ($p < 0.001$) when compared with those of the skin of control rats (group 1). Histologic examination revealed that calcified material was evenly deposited in close connection with collagen fibers of subcutaneous tissue and dermis (Fig. 2). In additional experiments, we observed that many intravenously administered calcergens (chlorides of Cd, Co, In, Zn) and noncalcergens (chlorides of Al, Bi, Cr, Fe) failed to sensitize for precipitation of calcium salts at sites of polymyxin injection.

The experiment summarized in Table II indicates that there is a definite optimum dose for the production of skin calcification by $HoCl_3$. The 3-mg dose produced maximal cutaneous calcification while both smaller (1 mg) and larger doses (5 and 8 mg) were totally inactive in this respect. As expected from earlier work (7), high amounts of $HoCl_3$ induced splenic calcification.

Discussion. Previous studies performed with large amounts of calcergens adminis-

TABLE II. Efficacy of Various Doses of $HoCl_3$ in Eliciting Cutaneous Calcification.

Group	$HoCl_3$ dose[a] (mg)	Mean diameter (mm) of calcified wheal at site of polymyxin	Splenic calcification (scale 0–3)
1	1	0	0
2	2	14 ± 0.85	0
3	3	23 ± 0.97	0
4	5	0	1.0 ± 0.2
5	8	0	3.0

[a] In addition, all rats received 30 μg of polymyxin as indicated in the text.

tered intravenously suggested that only lead acetate could induce connective tissue calcification at sites of increased capillary permeability (5,6). The present experiments demonstrate that this effect also is caused by several rare earth metals (Ho, Er, Dy, Tm) when given in small amounts. We have recently observed that small doses of rare earth metals increase, while large amounts decrease the serum concentrations of calcium and phosphorus (10,11). Large amounts of these metals are absorbed by the reticuloendothelial system and induce accumulation of calcium salts in the spleen (7). It is noteworthy that splenic calcification is induced when large amounts of metal are administered, while cutaneous calcification, at the site of polymyxin injection, is obtained only with low levels. The ability of rare earths to induce or to inhibit cutaneous calcification is reminiscent of the dual effect exhibited by different amounts of thrombohemorrhagic "sensitizers" (12).

Summary. In the rat, the intravenous administration of several rare earth metals (Ho, Tm, Dy, Er) induces an accumulation of calcium salts at sites treated with a histamine liberator, polymyxin. This reaction is obtained only with medium doses (3 mg) of metals; smaller (1 mg) and larger doses (5 and 8 mg) were inactive in this respect. The intensity of splenic calcification is directly proportional to the amount of rare earth administered.

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Blood Lactic Acid in Rats and Men: Comparison of Normo- and Hypertensive Individuals*† (33042)

KNUD D. KNUDSEN, LEWIS K. DAHL, AND LORRAINE M. TASSINARI

Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

Demartini *et al.* (1) reported that in patients with either renal or essential hypertension the concentration of lactic acid (LA) in both venous and arterial blood was significantly elevated. Somewhat similar results had been obtained by Gupta and Chakravarty (2). The reason for this finding was not clear.

We have studied the LA concentration in the blood of two strains of rats with an inborn susceptibility (S) or resistance (R), respectively, to experimental hypertension induced by NaCl as well as other techniques

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