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ACTION OF VARIOUS METALLIC CHLORIDES ON CALCAEMIA AND PHOSPHATAEMIA

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Compounds which produce tissue calcification at the site of injection in otherwise untreated rats are known as calcergens or direct calcifiers. These include lead acetate, potassium permanganate, indium trichloride, the chlorides of rare earth metals and zinc chloride. On the other hand, numerous other metallic compounds—for example, aluminium trichloride, chromium chloride, ferric chloride, scandium trichloride—are inactive in this respect (Selye, 1962 ; Padmanabhan, Tuchweber & Selye, 1963 ; Gabbiani, Jacqmin & Richard, 1966). Calcergy must be distinguished from calciphylaxis, in which tissue calcification can be produced by certain challengers (for example, aluminium trichloride, ferric chloride, chromium chloride, etc.) only after suitable sensitization with systemic factors such as vitamin D compounds or parathyroid hormone (Selye, 1962). While all calcergens are calciphylactic challengers, the reverse is not true.

Among the calcergens, lead acetate given intravenously prepares the rat for the production of topical calcinosis at the site of administration of various histamine liberators (Selye, Tuchweber & Gabbiani, 1962 ; Selye, Gabbiani & Tuchweber, 1963). The other calcergens given intravenously cause calcium deposition in the skin if orthophosphate is added to the extracellular fluid. Moreover, the oral administration of phosphate increases the sensitivity of the animals for the induction of calcification, while pretreatment with calcium acetate or parathyroid extract completely prevents this phenomenon (Gabbiani & Tuchweber, 1965a, b). These experiments indicate that phosphate may play a leading part in calcification induced experimentally.

More recently it was shown that in the rat, lead acetate given intravenously increases serum calcium and phosphorus concentrations (Kumagai & Sakai, 1966). Here we report experiments designed to test the effect on serum calcium and phosphorus concentrations of various metallic chlorides (calcergens and non-calcergens) given intravenously.

METHODS

Three hundred and eighty female Sprague-Dawley rats from the Robidoux Farm (Montreal, Quebec, Canada) with a mean initial body weight of 102 g (range 95–106 g) were divided into seventy-six equal groups to perform three experiments.

In the first experiment (Table 1) we tested the following chlorides: cadmium ($CdCl_2$) 0.7 mg ; cerium ($CeCl_3.7H_2O$) 6 mg ; cobalt ($CoCl_2$) 0.5 mg ; indium ($InCl_3$) 2.5 mg ; lead ($PbCl_2$) 5 mg ; zinc

($ZnCl_2$) 2.5 mg; aluminium ($AlCl_3.6H_2O$) 2.5 mg; chromium ($CrCl_3.6H_2O$) 5 mg; iron ($FeCl_3.6H_2O$) 4 mg; strontium ($SrCl_2$) 5 mg; thorium ($ThCl_4$) 5 mg; mercury ($HgCl_2$) 0.8 mg; magnesium ($MgCl_2$) 3.3 mg and nickel ($NiCl_2$) 2 mg (all compounds were supplied by Fisher Scientific Co. Fair Lawn, N.Y., U.S.A.). The trichlorides (anhydrous salts) (K and K Laboratories, Plainview, N.Y., U.S.A.) of rare earth metals were given at the following doses: gadolinium, holmium and neodymium, 10 mg; yttrium, 8 mg; erbium, 12 mg; scandium, 6 mg. Groups of animals were killed 1 hr, 3 hr and 24 hr after injection, by drawing blood from the abdominal aorta, under ether anaesthesia, for determination of serum calcium and phosphorus.

In the second experiment (Tables 2 and 3) we tested various doses of cerium and holmium chlorides. Blood samples were taken 3 hr after treatment and analysed for calcium and phosphorus.

TABLE 1

ACTION OF VARIOUS METALLIC CHLORIDES ON SERUM CALCIUM AND PHOSPHORUS CONCENTRATIONS.

Bold type indicates calcergens; italic type indicates calciphylactic challengers; roman type indicates compounds which induce no calcification. Figures indicate mean ± standard error. Control animals: serum calcium (mg%), 10·0±0·09; serum phosphate (mg%), 8·4±0·12. * $P<0.001$. † $P<0.005$.

Group	Treatment	Serum calcium (mg%) at following times			Serum phosphate (mg%) at following times		
		1 hr	3 hr	24 hr	1 hr	3 hr	24 hr
1	**$CdCl_2$**	9·3±0·09	9·7±0·14	10·9±0·16	8·8±0·17	7·7±0·29	7·6±0·17
2	**$CeCl_3$**	20·8±0·63*	19·8±0·71*	9·7±0·28	15·8±0·71*	17·4±0·50*	9·3±0·27
3	**$CoCl_2$**	9·8±0·06	10·3±0·10	10·2±0·12	8·6±0·53	9·8±0·30	10·1±0·25
4	**$ErCl_3$**	8·8±0·29*	8·9±0·21*	8·7±0·32*	6·8±0·46*	7·8±0·42*	7·1±0·15
5	**$GdCl_3$**	9·2±0·12†	9·4±0·22	8·7±0·32*	8·0±0·18	9·0±0·35	7·5±0·32
6	**$HoCl_3$**	8·7±0·25*	8·9±0·21*	9·8±0·31	7·0±0·27*	7·0±0·19*	8·0±0·37
7	**$InCl_3$**	15·4±0·46*	15·1±0·62*	11·1±0·33*	14·5±0·74*	13·5±0·70*	6·8±0·37*
8	**$NdCl_3$**	10·8±0·71	13·6±0·79*	11·4±0·18	7·6±0·11	10·0±0·66*	8·5±0·32
9	**$PbCl_2$**	25·2±1·89*	23·1±0·85*	12·6±1·22*	18·4±1·09*	17·1±0·96*	9·5±0·21
10	**$ScCl_3$**	23·9±2·25*	25·7±1·28*	12·8±0·59*	25·2±1·13*	23·8±1·69*	9·2±0·66
11	**YCl_3**	9·7±0·12	9·0±0·22†	10·0±0·23	7·3±0·15	8·5±0·48	8·0±0·63
12	**$ZnCl_2$**	11·9±0·55*	10·3±0·44	11·1±0·41	8·2±0·35	8·4±0·42	8·1±0·80
13	*$AlCl_3$*	14·6±0·65*	13·2±0·54*	10·4±0·19	12·5±0·63*	11·5±0·09*	9·6±0·22
14	*$CrCl_3$*	11·8±0·32*	11·6±0·33*	10·7±0·24	10·1±0·63*	10·4±0·38*	9·6±0·47
15	*$FeCl_3$*	16·8±0·59*	14·2±0·60*	10·8±0·17	14·6±0·59*	12·4±0·60*	9·6±0·33
16	*$SrCl_2$*	10·2±0·20	10·2±0 04	9·9±0·13	9·4±0·36	9·0±0·49	9·5±0·20
17	*$ThCl_4$*	15·3±1·18*	12·4±1.21*	10·3±0·05	11·6±0·77*	10·1±0·79*	9·0±0·50
18	$HgCl_2$	9·7±0·10	10·3±0·10	9·5±0·48	9·1±0·45	9·9±0·54	17·8±0·7*
19	$MgCl_2$	9·5±0·10	9·5±0·14	9·7±0·10	8·6±0·22	8·8±0·22	9·1±0·45
20	$NiCl_2$	9·2±0·14	10·1±0·29	10·2±0·11	9·2±0·21	7·9±0·60	9·1±0·33

TABLE 2

EFFECT OF VARIOUS DOSES OF $CeCl_3$ ON SERUM CALCIUM AND PHOSPHATE.

Blood samples were taken 3 hours after $CeCl_3$ injection. * $P<0.01$. † $P<0.001$.

Group	Dose (mg) of $CeCl_3$	Serum calcium (mg%)	Serum phosphate (mg%)
1	0·5	11·3±0·6	9·9±0·2*
2	1	12·2±0·3†	11·9±0·3†
3	3	16·1±0·4†	14·8±0·6†
4	6	18·1±0·6†	16·9±0·9†
5	11	11·6±0·9*	10·8±0·6*
6	15	9·7±0·20	9·8±1·4

TABLE 3

EFFECT OF VARIOUS DOSES OF $HoCl_3$ ON SERUM CALCIUM AND PHOSPHATE.
Blood samples were taken 3 hr after $HoCl_3$ injection. *$P<0{\cdot}001$. †$P<0{\cdot}01$.

Group	Dose (mg) of $HoCl_3$	Serum calcium (mg %)	Serum phosphate (mg %)
1	0·1	9·5±0·1	8·9±0·6
2	0·5	11·3±0·2*	11·0±0·2*
3	1	13·5±0·5*	12·2±0·5*
4	4	9·7±0·7	7·8±0·5
5	7	8·4±0·1†	7·1±0·4†
6	10	8·4±0·1†	7·2±0·2*

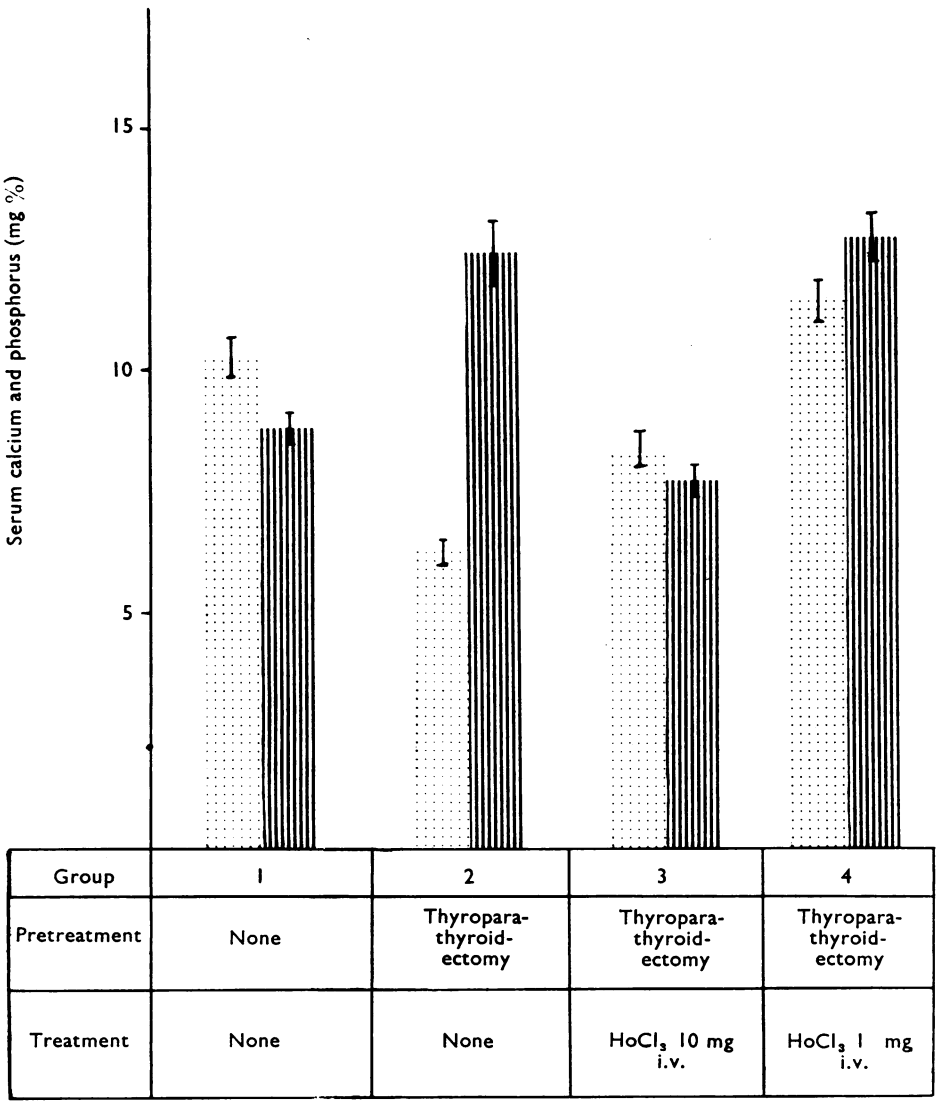

Fig. 1. Effect of holmium chloride on serum calcium and phosphorus levels in thyroparathyroidectomized rats. Blood samples were obtained 3 hr after injection of $HoCl_3$.

In the third experiment (Fig. 1), animals of group 1 served as untreated controls and the remaining groups were surgically thyroparathyroidectomized 5 days before treatment. Group 2 received no further treatment. Groups 3 and 4 were given $HoCl_3$ at doses of 10 mg and 1 mg respectively. Blood samples were taken 3 hr after treatment and analysed for calcium and phosphorus.

All metallic chlorides were given intravenously under light ether anaesthesia in 1 ml. of distilled water. During the course of the experiments, the rats were maintained exclusively on Purina laboratory chow (Purina Co., Canada) and tap water.

Total serum calcium was determined by atomic absorption spectroscopy (Willis, 1960) using a Jarrell-Ash apparatus. Measurements were made in an air acetylene flame after removal of the proteins by coagulation and centrifugation. Strontium chloride was added to prevent interference from phosphate. Serum samples from animals receiving ferric chloride injection were analysed for calcium after direct dilution with ethylenediamine tetraacetic acid (Ramakrishna, Robinson & West, 1966). Serum inorganic phosphorus was determined using the method of Fiske & Subbarow (1925). Normal concentrations of serum calcium were obtained from fifty untreated rats. The mean values are indicated in the tables; the significance of the results was determined by Student's *t* test.

RESULTS

Effect of various metallic salts on serum calcium and phosphorus concentrations

Blood calcium. Data in Table 1 show that 1 hr after treatment with $CeCl_3$, $InCl_3$, $PbCl_2$, $ScCl_3$, $AlCl_3$, $CrCl_3$, $FeCl_3$, $ThCl_4$ and $ZnCl_2$, the serum calcium was significantly increased ($P<0.001$) when compared with the mean of 10.4 mg/100 ml. found in control rats of the same age. Similar results were obtained 3 hr after intravenous treatment, except from the group receiving $ZnCl_2$ in which the concentration of calcium had returned to normal.

After 24 hr, the calcium concentrations were still significantly increased ($P<0.001$) in the rats treated with $CeCl_3$, $PbCl_2$ and $ScCl_3$, while in the remaining animals the concentrations had returned to the normal range. The chlorides of erbium, gadolinium, yttrium and holmium caused a significant decrease ($P<0.001$) of serum calcium 1 and 3 hr after treatment. The hypocalcaemic action of erbium and gadolinium could still be observed 24 hr after injection. The remaining elements (Cd, Co, Hg, Mg and Ni) had no effect on the calcaemia.

Blood phosphorus. The phosphorus concentrations were influenced in a fashion similar to those of calcium; that is, both calcium and phosphorus increased or decreased simultaneously. The hyperphosphataemia induced by $HgCl_2$ 24 hr after treatment was caused by the development of nephrocalcinosis.

Effect of various doses of $CeCl_3$ and $HoCl_3$ on serum calcium and phosphate

Cerium chloride had no effect on the serum calcium and phosphate levels when given at a dose of 0.5 mg. From 1 to 6 mg, the increase of both electrolytes was dose-dependent. At the dose of 11 mg, the calcium and phosphate levels increased significantly ($P<0.01$) in comparison with normal levels, but decreased in comparison with rats injected with 6 mg. Calcium and phosphorus concentrations returned to normal after the injection of $CeCl_3$ 15 mg. Similar results were obtained with $HoCl_3$: 0.1 mg had no effect on calcium and phosphate levels; 0.5 mg and 1 mg caused a significant increase ($P<0.001$) of calcium and phosphorus concentrations. The injection of 4 mg of $HoCl_3$ caused no change in

the level of either electrolyte, whereas 7 and 10 mg produced a significant decrease ($P<0.01$).

Effect of holmium chloride on serum calcium and phosphorus levels in thyroparathyroidectomized rats

Five days after thyroparathyroidectomy (Group 2) the animals showed a significant decrease in serum calcium ($P<0.01$) and a corresponding increase in phosphorus ($P<0.05$) when compared with control rats (Group 1) (Fig. 1). The injection of 10 mg of $HoCl_3$ (Group 3) induced a significant decrease of both serum calcium and phosphorus ($P<0.01$) in comparison with intact animals. In thyroparathyroidectomized rats, only the phosphate was significantly decreased ($P<0.01$).

Holmium chloride at the dose of 1 mg (Group 4) caused an increase in serum calcium and phosphorus in comparison wth both intact and thyroparathyroidectomized rats.

DISCUSSION

Kumagai & Sakai (1966) have shown that lead acetate causes hypercalcaemia and hyperphosphataemia; the present studies indicate that a similar effect is exerted by several other calcergic and non-calcergic metals. Thus there is no parallelism between the changes in calcaemia and phosphataemia caused by various metallic chlorides and their calcifying activity.

Kennedy (1966) reported that cadmium chloride given intravenously causes a marked hypocalcaemia in the rabbit 6 hr after injection, with a return to normal 24 hr later. In our conditions, cadmium chloride did not significantly change the level of blood calcium. The differences in the dosage and the species used may account for the apparent discrepancy in the results.

The second experiment clearly shows that the changes in serum calcium and phosphate caused by $CeCl_3$ and $HoCl_3$ are not merely dependent on the dose injected. Two different phenomena are observed, an increase of serum calcium and phosphate after the administration of low doses and a decrease after higher doses.

It is difficult to explain the mechanism by which the metallic compounds increase or decrease concentrations of calcium and phosphorus. They might act through renal damage; but a uraemia does not explain why, in the acute experiments, the serum calcium falls within 3 hr. As for the increase of both elements, even bilateral nephrectomy fails to duplicate the sharp rise in serum calcium and phosphate observed after treatment with various metals—for example, $ScCl_3$ and $PbCl_2$. In additional experiments (not reported here), we found no difference between the calciuria and phosphaturia of untreated control rats and those given metallic salts known to cause both hypo and hyper-calcaemia and phosphataemia.

The hypocalcaemic and hypophosphataemic action of some elements (for example, $HoCl_3$, $CeCl_3$) cannot be attributed to an increased secretion of thyrocalcitonin, since the results of our third experiment show that this effect occurs even in the absence of the thyroid and parathyroid glands.

It is known that many of the cations studied here can concentrate selectively in the skeleton and they have been termed " bone seekers " (MacDonald, Nusbaum, Alexander, Ezmirlian, Spain & Rounds, 1952; MacDonald, Ezmirlian, Spain & McArthur, 1951; Ondreička, Ginter & Kortus, 1966; Past, 1964; Neuman & Neuman, 1953; Jowsey, Rowland & Marshall, 1958). It therefore seems likely that the hypercalcaemic and hyperphosphataemic effect results from a displacement of calcium and phosphorus from the bone, but this is still a matter of conjecture.

MacDonald *et al.* (1952) reported that metal deposition in bone was marked after parenteral administration of small but not of large amounts of yttrium. This observation may be related to our findings showing that high doses of $CeCl_3$ and $HoCl_3$ cause either no change or a decrease of serum calcium and phosphate, while small amounts induce an increase of both electrolytes. In high doses, the rare earth metals are absorbed in the reticulo-endothelial system (Haley, 1965) and induce accumulation of calcium salts in the spleen (Gabbiani *et al.*, 1966). It seems probable that the hypocalcaemia and hypophosphataemia which follow intravenous administration of high amounts of rare earth metals result from the accumulation of calcium salts in the spleen, because the two phenomena occur almost simultaneously.

Although these results shed little light on the mechanism of this reaction, we may conclude that several metallic salts influence the calcium and phosphorus levels in blood. There is no direct correlation between the hypercalcaemic and hyperphosphataemic effect of these compounds on the one hand, and their topical calcifying action on the other. Furthermore, these effects are not mediated through the thyro-parathyroid apparatus.

SUMMARY

1. Previous experiments have shown that lead acetate, a so-called " calcergen " which produces soft-tissue calcification at the site of injection, can induce hypercalcaemia and hyperphosphataemia.

2. We have examined the effect of various metallic chlorides (calcergens and non-calcergens) given intravenously and have found no parallelism between their effect on serum calcium or phosphorus and their direct calcifying activity.

3. Most calcergically active metals increase serum calcium and phosphate. Decrease of both electrolytes was noted only after injection of high amounts of some rare earth elements (for example, Ho, Er). The changes were observed as soon as 1 hr after treatment; similar values were observed after 3 hr, and 24 hours later the calcium and phosphorus concentrations had reverted to the normal range in almost all groups.

4. When rare earth metals were administered (for example, $CeCl_3$ and $HoCl_3$), we observed two reactions: first, an increase of serum calcium and phosphorus induced by small doses of metallic chlorides, and second, a decrease of both elements after injection of high amounts.

5. Involvement of the thyro-parathyroid apparatus in the mechanism of the above reactions seems to be completely ruled out.

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REFERENCES

FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem.*, **66**, 375–400.

GABBIANI, G., JACQMIN, M. L. & RICHARD, R. M. (1966). Soft-tissue calcification induced by rare earth metals and its prevention by sodium pyrophosphate. *Br. J. Pharmac. Chemother.*, **27**, 1–9.

GABBIANI, G. & TUCHWEBER, B. (1965a). Studies on the mechanism of experimental soft-tissue calcification. *Can. J. Physiol. Pharmac.*, **43**, 177–183.

GABBIANI, G. & TUCHWEBER, B. (1965b). Inhibition of soft-tissue calcification by parathyroid extract and calcium. *Acta endocr. (Copenh.)*, **49**, 603–609.

HALEY, T. J. (1965). Pharmacology and toxicology of the rare earth elements. *J. Pharm. Sci.*, **54**, 663–669.

JOWSEY, J., ROWLAND, R. E. & MARSHALL, J. H. (1958). The deposition of the rare earths in bone. *Radiat. Res.*, **8**, 490–501.

KENNEDY, A. (1966). Hypocalcemia in experimental cadmium poisoning. *Br. J. ind. Med.*, **23**, 313–317.

KUMAGAI, A. & SAKAI, T. (1966). Effect of lead acetate on serum calcium and phosphorus in rats. *Archs int. Pharmacodyn. Thér.*, **160**, 248–252.

MACDONALD, N. S., EZMIRLIAM, F., SPAIN, P. & MCARTHUR, C. (1951). The ultimate site of skeletal deposition of strontium and lead. *J. biol. Chem.*, **189**, 387–399.

MACDONALD, N. S., NUSBAUM, R. E., ALEXANDER, G. V., EZMIRLIAN, F., SPAIN, P. & ROUNDS, D. (1952). The skeletal deposition of yttrium. *J. biol. Chem.*, **195**, 837–841.

NEUMAN, W. F. & NEUMAN, M. W. (1953). The nature of the mineral phase of bone. *Chem. Rev.*, **53**, 1–37.

ONDREIČKA, R., GINTER, E. & KORTUS, J. (1966). Chronic toxicity of aluminium in rats and mice and its effects on phosphorus metabolism. *Br. J. ind. Med.*, **23**, 305–312.

PADMANABHAN, N., TUCHWEBER, B. & SELYE, H. (1963). Über direkt wirkende Verkalkungstoffe. *Arzneimittel-Forsch.*, **13**, 429–432.

PAST, W. L. (1964). The uptake of Fe^{59} by osseous tissue arising in vitro. *Am. J. Path.*, **45**, 873–887.

RAMAKRISHNA, T. V., ROBINSON, J. W, & WEST, P. W. (1966). The determination of calcium and magnesium by atomic absorption spectroscopy. *Analytica chim. Acta*, **36**, 57–64.

SELYE, H. (1962). *Calciphylaxis.* Chicago: University of Chicago Press.

SELYE, H., GABBIANI, G. & TUCHWEBER, B. (1963). Factors influencing topical calcinosis induced by trauma following intravenous injection of lead acetate. *Archs int. Pharmacodyn Thér.*, **145**, 254–263.

SELYE, H., TUCHWEBER, B. & G. GABBIANI (1962). Calcinosis induced by lead acetate. *J. Pharmac. exp. Ther.*, **138**, 131–138.

WILLIS, J. B. (1960). The determination of metals in blood serum by atomic absorption spectroscopy. I. Calcium. *Spectrochim. Acta*, **16**, 259–272.