Drug-Induced Resistance to Tissue Calcification

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New concepts of calcergy and calciphylaxis are defined concisely. In rats pretreated with a single intravenous injection of lead acetate, topical connective-tissue calcification was produced by minute doses of various mast-cell components (histamine, 5-HT) or mast-cell dischargers (48/80, polymyxin). This mast-cell-dependent form of calcergy or "mastocalcergy" was inhibited by large doses of any one of the mast-cell compounds just mentioned. Epinephrine, another biogenic amine, exerted a similar protective effect, although previously it has been shown not to produce a mastocalcergy at any dose level tested. A sympathomimetic compound (methoxamine) as well as two severe stressor techniques (restraint and spinal cord transection) were ineffective in preventing this form of mastocalcergy. Hence, the protective action of the biogenic amines appears to be largely specific. In a second experimental series, rats similarly pretreated with a single intravenous injection of lead acetate were given increasing doses of 5-HT or histamine. It was found that small doses regularly produce intense local calcification, while larger doses do not. These apparently paradoxical findings are explained tentatively by the "phenomenon of the intersecting dose-effect curves."

THE RECENTLY observed biologic reactions, calciphylaxis and calcergy, now make the study of the mechanism of soft-tissue calcification possible from a fresh approach.

Calciphylaxis is a phenomenon which can induce selective calcification in various organs. It is brought about by pretreatment with a systemic calcifying compound, *e.g.*, parathyroid hormone or vitamin D derivatives (the sensitizer), followed after a time interval (the critical period) by an eliciting agent (the challenger) (1).

Calcergy, an essentially different reaction, is produced without prior sensitization by the parenteral administration of so-called direct calcifiers or "calcergens" (2, 3).

Most calciphylactic challengers and calcergens have been metallic compounds, and all calcergens thus far evaluated also have been active as challengers, although the reverse has not always been true.

Through their mast-cell discharging effect, histamine liberators play an important role in the mechanism of certain calciphylactic syndromes (4, 5), and the same is true of some calcergic reactions (6). These mast-cell-dependent reaction types have been designated as "mastocalciphylaxis" (7) and "mastocalcergy" (6), respectively. Here, a mast-cell discharge presumably participates in the distribution of the challenging metal and of calcium phosphate to different tissues (6, 8). This laboratory became particularly interested in the last-mentioned reaction form upon observing that under certain conditions it can be directed to induce selective calcification of the autonomic nervous system. This "neurotropic calcergy" is obtained, for example, when immediately after an intravenous injection of a potent calcergen, such as lead acetate, rats are given a large subcutaneous injection of histamine (9). Calcification of the autonomic nerves also was obtained under similar conditions when, instead of histamine, mast-cell discharging histamine liberators (*e.g.*, polymyxin or compound 48/80) were given, while other mast-cell constituents, such as 5-HT (5-hydroxytryptamine) and heparin, proved to be devoid of this action.

Recently, it was noted that all the compounds that produce autonomic nerve calcification in the lead acetate pretreated rat also elicit topical calcium deposition in the subcutis at the site where they are injected (10). This paper reports experiments showing that rats can be made resistant to this topical calcification if they are treated with certain mast-cell dischargers or mast-cell constituents just before the intravenous injection of lead acetate.

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MATERIALS AND METHODS

Two experimental series were performed on rats prepared for mastocalcergy by the intravenous injection of lead acetate. The first series was designed to test the specificity of the protective effect by establishing what agents can block the topical skin calcification normally induced by various mast-cell dischargers or mast-cell constituents; the second series was supposed to show the dose dependence of this cutaneous mastocalcergy.

Eight hundred and twenty Sprague-Dawley rats of the Holtzman strain with a mean initial body weight of 100 Gm. (range 90–110 Gm.) were subdivided into 82 equal groups for the various experiments of the two series. All animals received a single intravenous injection of 5 mg. of neutral lead acetate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$, Fisher Scientific Co., Fair Lawn, N. J.] under light ether anesthesia on the first day of the experiment. The time of this lead acetate injection was considered zero hour; consequently, the time intervals at which pretreatments were given before this injection are preceded by a minus sign (−) in the text and tables.

First Experimental Series.—This consisted of four parts in which were tested the possible inhibition by various agents of the cutaneous calcinosis produced (after the standard pretreatment with lead acetate) by 48/80, polymyxin, histamine, and 5-HT, respectively. All four calcification-eliciting materials were given immediately after the lead acetate injection (0 hr.) under the shaved skin of the back at four points (as shown in Fig. 1), in 0.2 ml. of distilled water containing 0, 0.3, 3, and 30 mcg., respectively, as indicated in Tables I–IV.

The agents to be tested for their possible protective effect were similarly injected under the shaved skin of the belly at the doses listed in Tables I–IV, either simultaneously with the intravenous lead acetate injection (0 hr.) or 1 hr. previously (−1 hr.).

For this purpose, the following mast-cell constituents, mast-cell dischargers, and vasopressor compounds were employed at the doses indicated in Tables I–IV: histamine phosphate, serotonin creatinine sulfate,[1] compound 48/80, epinephrine bitartrate,[2] polymyxin-B sulfate, and methoxamine hydrochloride.

To verify if any protective effect was due merely to the stressor action of these materials, two additional groups have been added because it is well known that stress can produce "nonspecific resistance" against a variety of injurious agents (11). In one of these groups, the rats were restrained on a board according to a previously described technique (12) during the 24 hr. just preceding the lead acetate injection. In the second group, the spinal cord was severed with a thermocautery at the level of the first lumbar vertebra 1 hr. before the lead acetate injection.

Second Experimental Series.—Various dose levels of histamine (1 mcg.–60 mg.) and 5-HT (0.1 mcg.–6 mg.) were tested for their efficacy in producing local calcification when given immediately after the standard intravenous dose of lead acetate (Tables V and VI).

Throughout the experiment, the animals were maintained exclusively on laboratory chow (Purina Co., Canada) and tap water. The experiment was terminated on the sixth day by killing the rats with chloroform. At autopsy, the diameter of the calcified wheals which developed at the subcutaneous injection sites was measured in millimeters, and the means of these measurements (with their standard errors) are listed in the tables.

Specimens of the injection sites were then fixed in alcohol–formol (1 part of neutral formalin and 4 parts of absolute alcohol), embedded in paraffin, and stained with the von Kóssa technique for the histochemical demonstration of calcium salts.

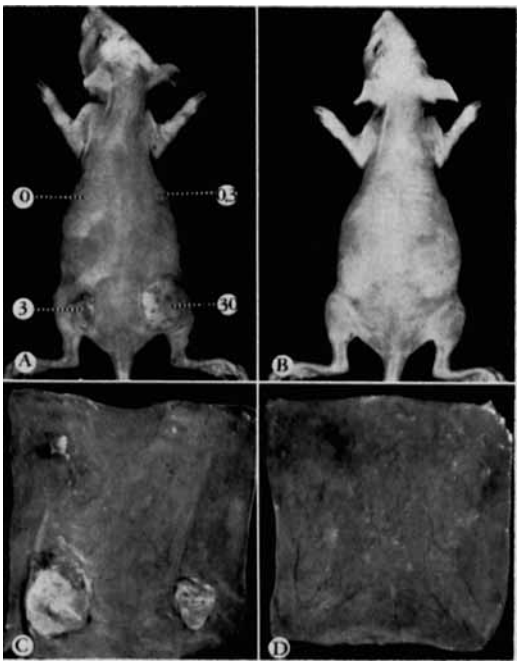

Fig. 1.—Inhibition by 5-HT of local mastocalcergy produced by compound 48/80. Key: *A*, shaved skin of a lead acetate pretreated rat which, at the points indicated, received 0, 0.3, 3, and 30 mcg., respectively, of compound 48/80 in 0.2 ml. of distilled water. No calcification occurs at the site where only water was injected, while at the other sites the size of the calcified wheals increases in proportion to the dose of 48/80 given; *B*, similarly pretreated rat completely protected against mastocalcergy by pretreatment with a single dose of 3 mg. of 5-HT (see text); *C* and *D*, skin specimens of the rats shown above, reflected to the left, to show subcutis. Here the dose dependence of the calcification is even more clearly visible in the unprotected animal (left), while the 5-HT protected rat shows no trace of subcutaneous mineralization.

RESULTS

First Experimental Series.—*Specificity of the Protection Against Mastocalcergy.*—The results of the first series are summarized in Tables I–IV, dealing with the protective effect of various agents against the mastocalcergy elicited by lead acetate combined with 48/80, polymyxin, histamine, and 5-HT, respectively.

Table I shows that, in the lead acetate pretreated rat, 48/80 is highly efficacious in producing topical mastocalcergy in the subcutis at doses of

[1] Nutritional Biochemicals Ltd., Cleveland, Ohio.
[2] Brickman & Co., Montreal, Quebec, Canada.

TABLE I.—MASTOCALCERGY PRODUCED BY LEAD ACETATE AND 48/80 AS INFLUENCED BY VARIOUS AGENTS

Group	Treatment[a]	Mean Diam. (mm.) of Calcified Wheal at Sites where 48/80 was Injected at the Dose of 0	0.3 mcg.	3 mcg.	30 mcg.	Mortality, %
1	None	1 ± 0.6	7 ± 0.6	17 ± 1.3	29 ± 0.8	0
2	Histamine (20 mg.) 0 hr.	3 ± 1.4	2 ± 0.8	5 ± 1.7	9 ± 2.4	0
3	Histamine (20 mg.) −1 hr.	3 ± 0.7	1 ± 0.6	2 ± 1.0	4 ± 1.3	0
4	5-HT (3 mg.) 0 hr.	0.5 ± 0.3	1 ± 0.7	4 ± 1.8	8 ± 3.7	40
5	5-HT (3 mg.) −1 hr.	1 ± 0.3	1 ± 0.5	1 ± 0.5	0	30
6	48/80 (400 mcg.) 0 hr.	1 ± 0.5	2 ± 0.8	8 ± 2.2	21 ± 2.2	10
7	48/80 (400 mcg.) −1 hr.	0	0	0	0	20
8	Polymyxin (1 mg.) 0 hr.	0	0	2 ± 0.9	10 ± 4.0	30
9	Polymyxin (1 mg.) −1 hr.	0	0	0	0	50
10	Epinephrine (500 mcg.) 0 hr.	1 ± 1.7	1 ± 0.4	1 ± 0.4	12 ± 3.0	30
11	Epinephrine (500 mcg.) −1 hr.	0	0.5 ± 0.4	0	2 ± 0.7	0
12	Methoxamine (3 mg.) 0 hr.	1 ± 0.4	3 ± 0.9	9 ± 1.7	18 ± 3.7	70
13	Methoxamine (3 mg.) −1 hr.	3 ± 1.0	5 ± 1.7	12 ± 1.4	29 ± 1.4	50
14	Restraint −24 to 0 hr.	6 ± 1.0	7 ± 0.9	12 ± 0.9	27 ± 1.8	0
15	Spinal cord lesion −1 hr.	3 ± 0.9	7 ± 1.7	13 ± 0.8	27 ± 1.4	80

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously.

TABLE II.—MASTOCALCERGY PRODUCED BY LEAD ACETATE AND POLYMYXIN AS INFLUENCED BY VARIOUS AGENTS

Group	Treatment[a]	Mean Diam. (mm.) of Calcified Wheal at Sites where Polymyxin was Injected at the Dose of 0	0.3 mcg.	3 mcg.	30 mcg.	Mortality, %
1	None	1 ± 0.1	6 ± 0.1	24 ± 1.4	33 ± 1.0	0
2	Histamine (20 mg.) 0 hr.	1 ± 0.5	3 ± 0.8	4 ± 1.0	9 ± 2.6	10
3	Histamine (20 mg.) −1 hr.	0.1 ± 0.2	0.5 ± 0.5	1 ± 0.6	6 ± 1.4	10
4	5-HT (3 mg.) 0 hr.	0.4 ± 0.2	2 ± 0.6	5 ± 1.7	18 ± 1.7	10
5	5-HT (3 mg.) −1 hr.	0	0	0	0	40
6	48/80 (400 mcg.) 0 hr.	0 ± 6.03	1 ± 0.7	13 ± 2.5	25 ± 1.7	20
7	48/80 (400 mcg.) −1 hr.	0	0	0	0	10
8	Polymyxin (1 mg.) 0 hr.	0.9 ± 0.3	1 ± 1.0	2 ± 1.0	9 ± 3.3	20
9	Polymyxin (1 mg.) −1 hr.	0	0	0	0	0
10	Epinephrine (500 mcg.) 0 hr.	1 ± 0.5	2 ± 2.0	2 ± 0.7	8 ± 1.0	10
11	Epinephrine (500 mcg.) −1 hr.	0	0	0	4 ± 0.5	10
12	Methoxamine (3 mg.) 0 hr.	5 ± 0.1	10 ± 0.6	14 ± 1.3	23 ± 1.7	50
13	Methoxamine (3 mg.) −1 hr.	4 ± 0.4	8 ± 0.9	15 ± 2.2	29 ± 0.8	70
14	Restraint −24 to 0 hr.	4 ± 1.2	8 ± 0.3	13 ± 2.2	26 ± 3.1	0
15	Spinal cord lesion −1 hr.	5 ± 1.0	9 ± 0.7	15 ± 0.3	26 ± 2.2	50

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously

TABLE III.—MASTOCALCERGY PRODUCED BY LEAD ACETATE AND HISTAMINE AS INFLUENCED BY VARIOUS AGENTS

Group	Treatment[a]	Mean Diam. (mm.) of Calcified Wheal at Sites where Histamine was Injected at the Dose of 0	0.3 mcg.	3 mcg.	30 mcg.	Mortality, %
1	None	1 ± 0.6	4 ± 1	4 ± 1.4	15 ± 2.0	0
2	Histamine (20 mg.) 0 hr.	0	0	0	1 ± 1.0	20
3	Histamine (20 mg.) −1 hr.	6 ± 1.7	5 ± 1.2	3 ± 1.0	1 ± 1.0	0
4	5-HT (3 mg.) 0 hr.	0	0	0	6 ± 3.1	40
5	5-HT (3 mg.) −1 hr.	4 ± 2.1	0	0	0	0
6	48/80 (400 mcg.) 0 hr.	3 ± 1.4	0	1 ± 1.4	3 ± 1.4	10
7	48/80 (400 mcg.) −1 hr.	0	0	0	0	40
8	Polymyxin (1 mg.) 0 hr.	1 ± 1	1 ± 0.4	0	3 ± 1.3	10
9	Polymyxin (1 mg.) −1 hr.	0	0	0	0	30
10	Epinephrine (500 mcg.) 0 hr.	0	1 ± 1.0	0	0.4 ± 0.4	40
11	Epinephrine (500 mcg.) −1 hr.	0.5 ± 0.4	0.5 ± 0.3	0	0	40
12	Methoxamine (3 mg.) 0 hr.	2 ± 1.0	7 ± 3.4	7 ± 3.4	16 ± 8.7	70
13	Methoxamine (3 mg.) −1 hr.	0	0	0	0	90
14	Restraint −24 to 0 hr.	1 ± 1.2	2 ± 2	1 ± 2	5 ± 2.1	40
15	Spinal cord lesion −1 hr.	4 ± 0.8	5 ± 1.7	4 ± 1.7	8 ± 2.2	50

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously.

TABLE IV.—MASTOCALCERGY PRODUCED BY LEAD ACETATE AND 5-HT AS INFLUENCED BY VARIOUS AGENTS

Group	Treatment[a]	Mean Diam. (mm.) of Calcified Wheal at Sites where 5-HT was Injected at the Dose of 0	0.3 mcg.	3 mcg.	30 mcg.	Mortality, %
1	None	0	16 ± 1.4	23 ± 0.8	28 ± 0.9	10
2	Histamine (20 mg.) 0 hr.	0	19 ± 1.4	18 ± 1.8	21 ± 2.2	10
3	Histamine (20 mg.) −1 hr.	2 ± 1.4	6 ± 2.1	10 ± 3.4	18 ± 3.6	10
4	5-HT (3 mg.) 0 hr.	0	0	0	0	20
5	5-HT (3 mg.) −1 hr.	0	0	0	0	50
6	48/80 (400 mcg.) 0 hr.	1.4 ± 0.5	8 ± 2.2	20 ± 1.0	29 ± 1.4	0
7	48/80 (400 mcg.) −1 hr.	0	0	0	0	30
8	Polymyxin (1 mg.) 0 hr.	0.8 ± 0.5	4 ± 1.4	7 ± 2.4	15 ± 2.4	0
9	Polymyxin (1 mg.) −1 hr.	0	0	0	0	50
10	Epinephrine (500 mcg.) 0 hr.	2 ± 2.4	2 ± 2.4	0	2 ± 2.4	80
11	Epinephrine (500 mcg.) −1 hr.	0	0	0	0.5 ± 0.5	40
12	Methoxamine (3 mg.) 0 hr.	3 ± 0.7	9 ± 1.0	15 ± 1.6	23 ± 1.4	30
13	Methoxamine (3 mg.) −1 hr.	0	2 ± 1.4	2 ± 1.0	4 ± 2.6	100
14	Restraint −24 to 0 hr.	5 ± 0.7	11 ± 0.2	19 ± 0.2	23 ± 0.8	60
15	Spinal cord lesion −1 hr.	1 ± 0.7	8 ± 0.7	13 ± 0.7	21 ± 1.5	10

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously.

0.3–30 mcg. (group *1*). This effect is only slightly diminished when histamine is given at 0 hr. (group *2*), while the same dose of histamine given *1* hr. earlier induced almost complete protection (group *3*). The slight calcification at the injection site, where 30 mcg. of histamine was administered in this group, is not significantly above the control site treated only with distilled water; hence, the inhibition may be regarded as virtually complete. It must be kept in mind that even distilled water (13), physiological saline (14), and mechanical trauma (15) can produce some degree of mast-cell degranulation. Furthermore, in rats pretreated for calcergic reactivity by an intravenous injection of lead acetate, mechanical trauma to the skin also has been shown to produce topical calcification (3). That the control injection site was so rarely calcified in the present experimental series is probably due to the fact that only 0.2 ml. of distilled water was administered through a fine injection needle, thereby minimizing the effect of trauma due to puncture of the skin and infiltration with hypotonic fluid.

It is not necessary to comment upon each of the other groups separately since it is quite evident from the tables that histamine, 5-HT, 48/80, polymyxin, and epinephrine were all capable of suppressing mastocalcergy under the experimental conditions. This protective effect was most consistently evident when the protective agents were administered 1 hr. prior to the intravenous injection of lead and the topical treatment with 48/80. However, in some instances, the inhibition was complete, even when the protective agent was administered at 0 hr., *i.e.*, simultaneously with the evocative agents (Fig. 1).

On the other hand, methoxamine and the two severe stressors, 24 hr. of restraint and complete transection of the spinal cord, offered no statistically significant protection against mastocalcergy elicited by 48/80.

Essentially similar results were obtained when the potential protective effect of these agents was tested against mastocalcergy elicited by polymyxin (Table II), histamine (Table III), and 5-HT (Table IV).

Second Experimental Series.—*Dose Dependence of Cutaneous Mastocalcergy.*—The experiments summarized in Table V indicate that there is a definite optimum dose for the production of local calcergy by histamine. Only small calcified foci were produced in a few animals treated with 3 mg., while large calcified wheals appeared at the injection site in all animals given either smaller or larger amounts. Doses below 3 mg. always produced solid calcified wheals, while more than 3 mg. of histamine elicited the formation of calcified rings surrounding an uncalcified or at least less calcified and usually necrotic central area (Figs. 2 and 3).

Table VI likewise indicates that the response is dose dependent, for 30 mcg. produced maximum calcification, while both smaller and larger doses were less effective or even totally inert in this respect. Because of its systemic toxicity (manifested principally by the production of fatal renal cortical necrosis), even 6 mg. of serotonin caused a high mortality; hence, larger doses could not be tested.

TABLE V.—EFFICACY OF VARIOUS DOSES OF HISTAMINE IN ELICITING MASTOCALCERGY

Group	Histamine Dose[a]	Mean Diam. (mm.) of Calcified Wheal at Site where Histamine was Injected	Mortality, %
1	0.1 mcg.	3 ± 1.2	0
2	10 mcg.	9 ± 2.2	10
3	300 mcg.	28 ± 2.4	0
4	3 mg.	6 ± 3.1	10
5	30 mg.	37 ± 0.7	0
6	60 mg.	44 ± 1.4	50

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously.

TABLE VI.—EFFICACY OF VARIOUS DOSES OF 5-HT IN ELICITING MASTOCALCERGY

Group	5-HT Dose[a]	Mean Diam. (mm.) of Calcified Wheal at Site where 5-HT was Injected	Mortality, %
1	0.1 mcg.	3 ± 1.2	0
2	1 mcg.	28 ± 2.2	0
3	30 mcg.	37 ± 0.5	0
4	300 mcg.	30 ± 2.8	0
5	3 mg.	6 ± 7.8	0
6	6 mg.	0.6 ± 0.3	70

[a] In addition to the treatments mentioned in this column, the rats of all groups received 5 mg. of lead acetate intravenously.

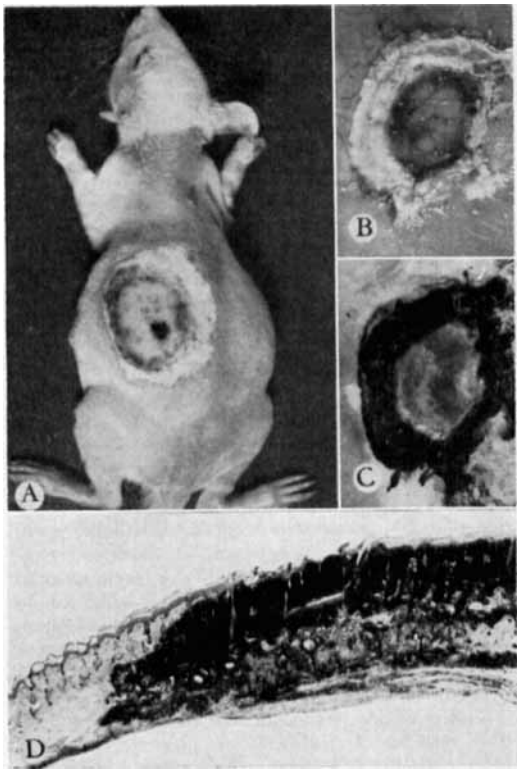

Fig. 2.—Annular calcification induced by a large dose of histamine in the lead acetate pretreated rat. Key: *A*, ring-shaped calcium deposit surrounds a necrotic uncalcified central area, where 60 mg. of histamine was injected subcutaneously; *B*, after reflecting the skin to the left, the calcified ring of the same rat is seen from the subcutis. (The central necrotic area is dark.) *C*, specimen shown above, after defatting and staining with the von Kóssa procedure. (The calcified areas are black.) *D*, general view of a histologic section taken from the transitional area between the necrotic central portion (left) and the calcified peripheral ring (black) of the specimen shown in the pictures above (von Kóssa, × 70).

Therefore, it was impossible to verify whether here, as in the histamine series, amounts in excess of those causing no calcification would result in the formation of peripheral calcified rings surrounding an uncalcified center.

DISCUSSION

Perhaps the most interesting outcome of these experiments is the demonstration that a cutaneous mastocalcergy elicited by small doses of 48/80, polymyxin, histamine, or 5-HT can be inhibited by large doses of any one of these agents. This inhibition is most pronounced if the large doses are given as a pretreatment 1 hr. before the small doses that normally induce calcification.

This "cross-resistance" may suggest some close relationship in the mechanisms through which mast-cell dischargers and mast-cell constituents produce mastocalcergy. An initial uptake of intravenously injected lead by subcutaneous mast cells at sites treated with mast-cell dischargers has been demonstrated histochemically by the rhodizonate technique. It was concluded that this hematogenous lead subsequently attracts calcium salts, just as lead acetate does when it is injected directly into the subcutaneous tissue (16). In this connection, it is of interest that zinc can be demonstrated with the dithizonate reagent in the mast cells of the mesentery in rats and guinea pigs. This zinc was thought to be part of a histamine–zinc complex (17).

However, previous studies have shown that an essential difference exists between the structural characteristics of the calcifications obtained in lead acetate pretreated rats by mast-cell dischargers on the one hand and by mast-cell constituents on the other. Polymyxin and compound 48/80 first produce mast-cell discharge, followed by calcium incrustation of the metachromatic granules; on the other hand, 5-HT and histamine cause no mast-cell discharge at comparable dose levels, but they produce a fine precipitate of calcium salts in and around the connective-tissue fibers at the injection sites. Hence, it was suggested that when elicited by mast-cell dischargers, this form of calcergy depends upon the liberation of endogenous 5-HT and histamine or related compounds from mast-cell granules, while if 5-HT or histamine are administered as such, they cause calcification directly without the intermediary of a mast-cell discharge (10).

In view of these considerations, it may be assumed that in the present experiments calcification

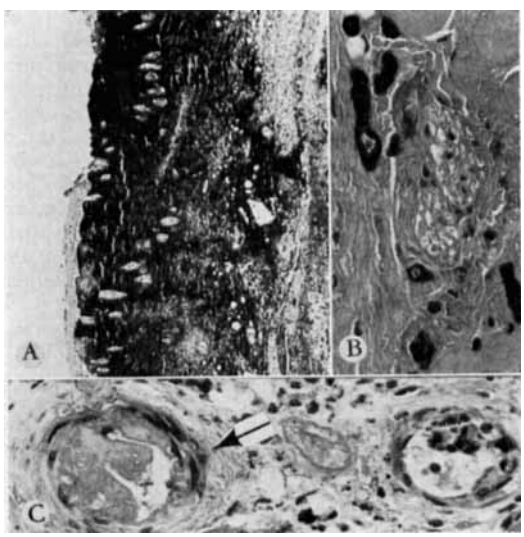

Fig. 3.—Annular calcification induced by a large dose of histamine in the lead acetate pretreated rat. (Higher magnification of the specimen shown in Fig. 2.) Key: *A*, part of the peripheral ring with heavy calcification throughout the dermis. [Near the upper edge of the picture, the epithelium is lost, and the calcified connective tissue is laid bare (von Kóssa, × 70).] *B*, in the central necrotic portion, only some small blood vessels show calcification (von Kóssa, × 460); *C*, two larger vessels from the necrotic central region, one of which (arrow) shows an almost occlusive hyaline thrombosis, while in the other, the endothelium has just begun to degenerate and to be infiltrated with inflammatory cells (von Kóssa, × 460).

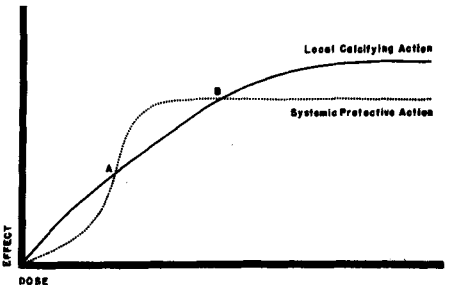

Fig. 4.—Illustration of the "phenomenon of the intersecting dose-effect curves." Key: —, local calcifying action of histamine; ---, systemic protective action. If the dose-effect curves of these two actions are correctly represented in this hypothetical graph, it is clear that calcification can occur only at dose levels lower than point *A*, or higher than point *B* because in the intermediate region, the systemic protective action exceeds the local calcifying effect.

depends upon the attraction of lead, and subsequently of calcium, by directly injected or endogenously liberated mast-cell constituents, such as histamine and 5-HT. On the other hand, large doses of these same compounds, be they of endogenous or exogenous origin, elicit a systemic defense reaction against this type of calcification.

However, it is difficult to understand why epinephrine, a biologic amine unrelated to those produced by mast cells, is also highly effective as a protector against mastocalcergy. Unlike the mast-cell dischargers and mast-cell constituents just mentioned, epinephrine is unable to produce mastocalcergy under comparable conditions (10). Having thus established that a compound in itself inactive can protect against mastocalcergy, it was interesting to test methoxamine, another sympathomimetic amine, but it was found to have little or no protective effect. The same may be said about the two severe stressor agents employed—restraint and spinal cord transection.

Evidently, the systemic protection against mastocalcergy offered by mast-cell constituents, mast-cell dischargers, and epinephrine cannot be traced easily to any one common action of these compounds. It is perhaps significant, however, that despite the considerable differences in the chemical structure and the pharmacologic actions of histamine, 5-HT, and epinephrine, all these compounds are biogenic amines.

The dose dependence of the reaction to 5-HT, as seen in the second experimental series, may be due to the fact that at high dose levels, the protective systemic actions of the compound counteract its local mastocalcergic effect. In other words, inhibition would be due merely to the same phenomenon seen in the first experimental series, where a large dose of 5-HT administered in one location inhibited the mastocalcergic effect of small doses of 5-HT administered elsewhere (Table IV, group *4*).

It is more difficult to understand why histamine did not show the same kind of dose dependence. It will be recalled that in the first experimental series, a large dose of histamine given in one region also prevented the mastocalcergic effect of small doses of the same compound administered elsewhere (Table III, group *2*). In the second experiment, it was found that both small and large doses of histamine produced extensive calcification, while an intermediate dose of 3 mg. was almost inactive. This curious dose-effect relationship might depend upon the "phenomenon of the intersecting dose-effect curves," first described in 1947, for the explanation of similar apparently paradoxical hormone effects (18). As applied to the present problem, the situation could be illustrated by the theoretical curves shown in Fig. 4. Assuming that with increasing doses the local calcifying action of histamine is expressed by the solid line and the systemic protective action by the dotted line, it is clear that calcification can occur only at dose levels lower than point A or higher than point B because in the intermediate region (between points A and B) the systemic protective action exceeds the local calcifying effect.

Possibly, in addition to its local calcifying action, histamine activates a systemic protective mechanism, and the dose-effect curves of these actions intersect at two points, indicated in Fig. 4. In the case of 5-HT (which could not be administered at high dose levels because of its systemic toxicity), apparently levels beyond point B have not been reached; hence, even the highest tested dose of this compound produced only protection against calcification.

This working hypothesis still leaves unexplained why high doses of histamine produce a ring-shaped calcified area around a noncalcified and usually necrotic center. The occurrence of such a nonreactive central zone in the midst of a calcified plaque is somewhat reminiscent of the phenomenon of "overchallenge" seen in calciphylaxis where an excessive amount of the challenger is injected subcutaneously (1).

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