Neurotropic Calciphylaxis.* (28339)

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When calciphylactically sensitized rats are challenged with certain metallic dextrin complexes, the metal is fixed in the anaphylactoid shock organs, and secondarily attracts calcium. For example, in rats pretreated with dihydrotachysterol (DHT), the subsequent intravenous administration of thorium dextrin (Th-Din, Thorotrast®) or ferric dextrin (Fe-Din, Ferrigen®), produces a typical anaphylactoid reaction with acute swelling of the lips, ears and paws, followed by calcium deposition in these regions. Such a calcifying anaphylactoid reaction can also be obtained by separate intravenous administration of the anaphylactoidogenic agent and the calcium-attracting metal(1,2). In all these circumstances, the cutaneous lesions are often associated with calcification in certain internal organs in which metal deposition also occurs, particularly in the esophagus, mediastinum and the joints.

Using diverse calciphylactic systems, it has been possible to cause more or less selective calcium deposition in various organs such as the thyroid, parathyroid, carotid body, skeletal musculature or salivary glands(1). In many cases, not only the intensity but even the localization of the calciphylactic reactions depends upon the "critical period," that is, the time interval between sensitization and challenge. The experiments to be described here show that, by varying the critical period between sensitization with DHT and challenge with an aluminum dextran chelate (Al-Dex), it is possible to produce either a calcifying anaphylactoid reaction of the conventional type, or a neurotropic calciphylaxis in which the vagi are selectively affected.

Materials and methods. Seventy female rats of the Holtzman strain, with an initial body weight of 100 g (90-110 g), were subdivided into 7 equal groups and treated as indicated in Table I. Dihydrotachysterol or DHT (Calcamin,® Dr. A. Wander, S. A., Bern, Switzerland) was given as a single dose of 1 mg in 1 ml corn oil by stomach tube on the first day, for sensitization. Aluminum dextran or Al-Dex (Benger Ltd., Holmes Chapel, England) was administered intravenously at the dose of 0.5 ml to 5 rats, and 1 ml to the remaining 5 rats of each group that was to receive the challenger. This Al-Dex treatment was given 24 or 48 hours before (–2nd and –3rd day respectively), or at corresponding intervals after (+2nd and +3rd day respectively), or simultaneously with the DHT administration. Throughout the experiment, the animals were kept exclusively on Purina Fox Chow and tap water.

The experiment was terminated on the tenth day after DHT administration. At autopsy, the organs were examined with a dissecting loupe, and specimens of all regions which showed white calcium deposits were fixed in alcohol-formol (4 parts of absolute alcohol, 1 part of 10% formalin) for subsequent embedding in paraffin and histochemical examination for calcium phosphate deposits with the von Kóssa technique. The nerves were also stained with PAS and Bodian's myelin sheath technique. The intensity of the lesions was expressed in terms of an arbitrary scale in which 0 = no lesion, 1 = minimal, 2 = medium and 3 = maximal calcification, as described elsewhere(2). Since the lesions obtained with 0.5 ml and 1 ml Al-Dex respectively, were essentially similar in intensity and quality, the results are pooled in Table I.

Results. Neither DHT nor Al-Dex produced any calcification by itself, although the Al-Dex injection was immediately followed by an acute anaphylactoid reaction, which reached a maximum after about 1 hour and disappeared during the following 2 to 3

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TABLE I. Neurotropic Calciphylaxis.

Treatment	Calcification	
	Vagus	Anaphylactoid
DHT 1st day	0	0
Al-Dex +2nd day	0	0
" −3rd " + DHT 1st day	2.8	.1
" −2nd " + "	2.2	0
" 1st " + "	1.8	.4
" +2nd " + "	.7	2.8
" +3rd " + "	0	2.5

hours (Table I). Among the rats challenged with Al-Dex on the −3rd day, only 1 showed slight calcification around the lips. In the group challenged on the −2nd day, no calcification was noted in the anaphylactoid shock organs of any animal. Challenge on the first day (day of DHT treatment) resulted in minimal calcification of the anaphylactoid shock organs, while challenge on the +2nd and +3rd day produced intense calcification, not only of the forepaws and lips but of the entire head and neck region, tongue, palate, esophagus, mediastinum and, less commonly, the joints and the musculature of the fore limbs. In the anaphylactoid shock organs, calcium deposition was particularly pronounced within the cutis, but it spread also into the subcutis and often into the adjacent musculature. Curiously, the hind paws, which also participated in the acute anaphylactoid reaction, did not undergo calcification. In all these respects, the syndrome elicited by challenge with Al-Dex, strikingly resembles that induced by administration of thorium dextrin (Th-Din) or ferric dextrin (Fe-Din) after DHT sensitization(1).

On the other hand, in the animals which were challenged with Al-Dex on the −2nd or −3rd day, that is, before DHT sensitization, and showed little or no calcification of the anaphylactoid shock organs, there was an extraordinary dilatation of the stomach. This called our attention to possible derangements in the function of the vagi. Inspection of these nerves revealed white deposits along their course, beginning near their emergence from the skull and continuing, with minor interruptions, along the whole course of both major vagus trunks throughout the neck, chest, and abdomen, up to the stomach. The smaller ramification of the vagi which enter the gastric wall, showed no calcium depositions.

Histologic examination confirmed the macroscopic observations, demonstrating von Kóssa-positive calcified material in the stroma (often also around the discharged mast-cell granules) in the anaphylactoid shock organs of the animals challenged after DHT administration. Here, the vagi were only rarely affected in the group given Al-Dex on the +2nd day and unaffected in those challenged on the +3rd day. Conversely, in the rats challenged on the −3rd or −2nd day, that is, prior to DHT treatment, massive calcium deposits were seen around the nerve fibers of the vagi. The exact position of these deposits cannot be established with certainty by the light microscope, but on cross sections the von Kóssa-positive material appeared to be arranged in the form of rings around each fiber, as well as more diffusely in the stroma of the nerves (Fig. 1). In the affected regions of the vagi there was also some inflammatory response, and degeneration of the myelin sheath could be demonstrated by means of the Bodian stain. Careful search for calcification in other nerves (sympathetic chain, femoral and brachial plexuses, cranial nerves other than the vagus, sciatic) showed no calcification. Sections through various regions of the brain and spinal cord likewise failed to reveal calcium deposition. In rats treated with DHT alone or Al-Dex alone, no histologic lesion could be demonstrated in the vagi, either by the von Kóssa or the Bodian procedure.

In an ancillary experiment, we tested the possible topical calciphylactic challenging effect of Al-Dex on 20 female rats, with an initial body weight of 90 g (range 86-94 g). Ten of these animals were sensitized with 1 mg of DHT in 0.5 ml of corn oil on the first day; the remaining 10 acted as controls. Both groups received 3 subcutaneous injections of Al-Dex at the concentration of 0.01%, 0.1% and 1%, always in 0.2 ml of water, subcutaneously, along the midline on the back, 24 hours after sensitization of the first group

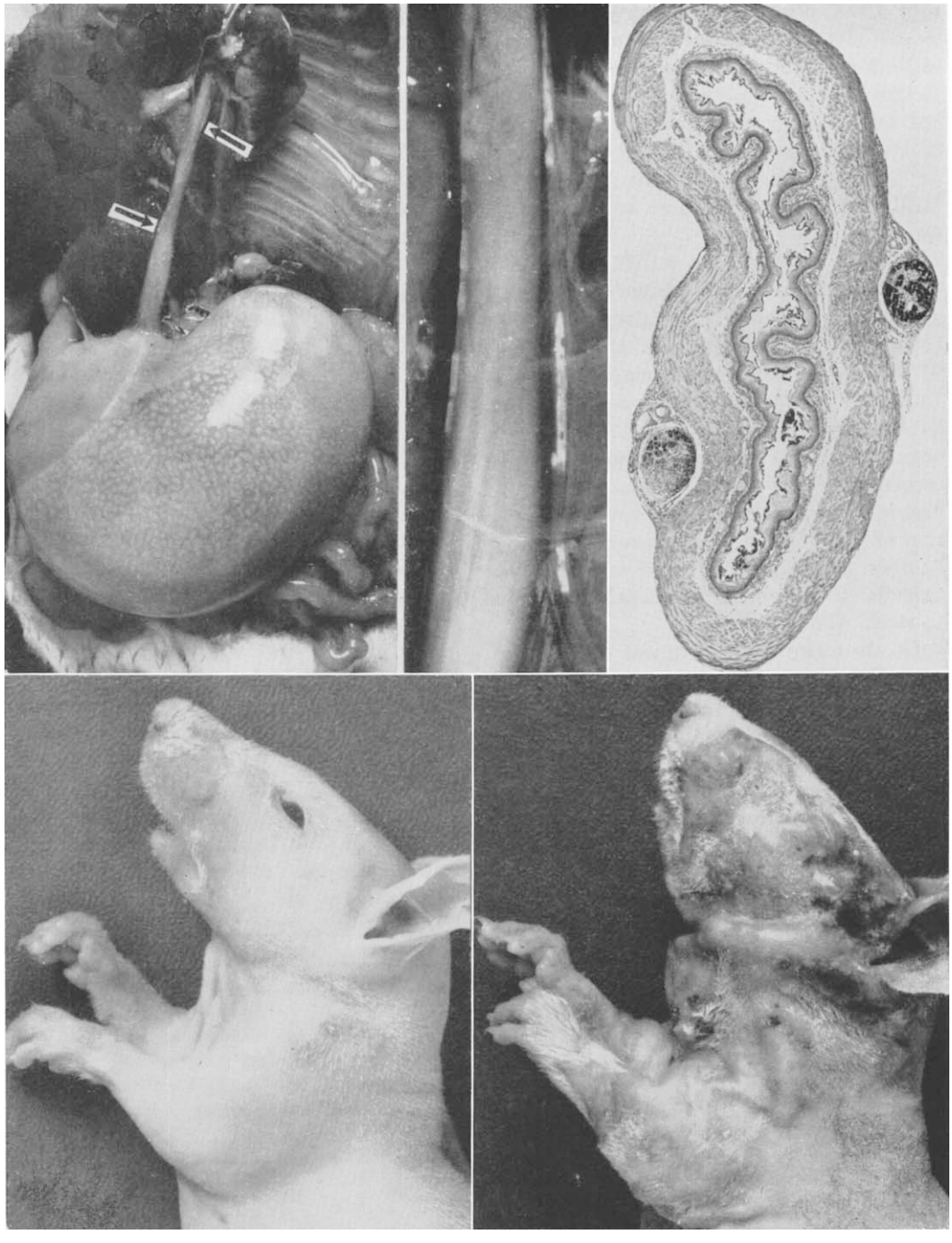

FIG. 1. Neurotropic and anaphylactoid calciphylaxis, produced by varying the critical period. TOP: Al-Dex given 3 days prior to DHT results in selective calcification of the vagus nerves. *Left:* Bead-like calcific deposits in the left vagus as it courses along the esophagus (arrows). *Middle:* Closer view of both calcified vagi, as they descend along the esophagus in the thorax. *Right:* Cross section through the esophagus, showing the 2 calcified vagi (von Kóssa × 35). BOTTOM: *Left:* Rat treated with DHT alone, shows no calcification of the shaved skin surface. *Right:* Rat treated with Al-Dex 3 days after DHT sensitization, shows intense calcification of the skin covering head and forepaws.

with DHT. In the sensitized rats, autopsy on the sixth day revealed pronounced local calcium deposition at the site of the 1% injection. The 0.1% solution produced definite, but less pronounced, calcification while the 0.01% solution caused no local calcification. In the rats which were not sensitized by DHT, the Al-Dex caused no topical calcinosis.

Discussion. The most striking feature of these experiments is the demonstration that a selectively neurotropic calciphylaxis can be induced, by intravenous administration of Al-Dex, prior to sensitization with DHT. Under these conditions, pronounced and selective calcium deposition occurs along the vagus nerves. This leads to functional disturbances, as manifested by paralysis of the gastric musculature, with pronounced dilatation of the stomach. Certain respiratory difficulties, which we noticed in several of the animals so treated, are presumably also due to calcification of the vagi.

On the other hand, treatment with Al-Dex, after DHT sensitization, caused calcification in the anaphylactoid shock organs, but little (+2nd day) or no (+3rd day) calcification of the vagus nerves. Challenge with Al-Dex on the day of DHT sensitization (1st day), resulted in an intermediate, mixed syndrome, with moderate degrees of calcification both in the vagi and in the anaphylactoid shock organs. Evidently here, the critical period elapsing between sensitization and challenge plays a decisive role as regards the localization of the lesions. Only a mild, patchy, generalized cutaneous calcification (followed by scaling and not limited to the classical anaphylactoid shock organs) occurred in all groups treated with Al-Dex plus DHT, irrespective of the time interval between the two treatments.

A search through the literature failed to reveal any instance of selective vagus calcification in man, and there is no evidence to suggest that any among the metal-induced clinical neuropathies are related to the process of calciphylaxis. However, amorphous basophilic and "hyaline" masses have been noted in connection with diverse forms of neuritis in man(3) and the calcified regions in the vagi of our rats, are basophilic and give a positive PAS reaction similar to that of hyalin. Perhaps the most intriguing question raised by these observations concerns the chemical process that can specifically attract calcium into the vagi, leaving other peripheral nerves intact.

Summary. Experiments on rats, calciphylactically sensitized with dihydrotachysterol (DHT), indicate that challenge with an aluminum dextran chelate (Al-Dex) prior to sensitization causes selective calcium deposition in the vagi, with gastric dilatation and respiratory difficulties. If, under otherwise comparable circumstances, the rats are challenged with Al-Dex after DHT sensitization, there is intense calcification of the analphylactoid shock organs (lips, paws, esophagus, mediastinum and joints), but not of the vagi. The observations show that, by varying the "critical period" between sensitization and challenge, a neurotropic calciphylaxis limited to the vagi, or a calcifying anaphylactoid reaction can be produced with considerable selectivity, at will.

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