OSTEITIS FIBROSA INDUCED BY CALCIPHYLAXIS IN THE ABSENCE OF THE PARATHYROIDS

By

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ABSTRACT

Following a brief description of calciphylaxis, its influence upon the skeleton of the rat is described. After sensitization with dihydrotachysterol (DHT) challenge by subcutaneously administered ferric dextran (Fe-Dex) or egg white produces extensive topical calcium deposits and severe osteitis fibrosa. At a later stage this is followed by an excessive osteogenesis. All these reactions can proceed in the absence of the parathyroids.

An experimental simile of osteitis fibrosa is most readily produced by overdosage with exogenous parathyroid hormone. Similar skeletal lesions can also be induced by bilateral nephrectomy, certain nephrotoxic sulfa drugs or deviation of the gastric juice to the outside through a gastric fistula; however, in all these latter instances parathyroidectomy prevents the development of osteitis fibrosa presumably because such interventions act upon the bones merely by inducing metabolic changes that stimulate parathyroid hormone secretion. The relevant literature has been discussed at length elsewhere (Selye 1961). More recently we noted that typical osteitis fibrosa can likewise be produced in rats by various calciphylactic techniques (Selye 1961 a); the question arose whether these also act merely by stimulating parathyroid hormone secretion.

Calciphylaxis is a condition of hypersensitivity in which, during a «critical period» after sensitization by a systemic calcifying factor (e.g., vitamin-D compounds, parathyroid hormone, sodium sulfathiazole) treatment with certain

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challengers (e. g., metallic salts, ferric dextran, egg white) causes an acute local tissue calcification followed by inflammation and sclerosis. A topical calciphylaxis thus induced by subcutaneous injection of challengers results in a cutaneous calcinosis reminiscent of calcareous scleroderma. However, in suitably sensitized (e. g., dihydrotachysterol- or »DHT«-treated) rats, calciphyllactic reactions can also be elicited rather selectively at predetermined sites (e. g., in the pancreas, bile ducts, uterus, spleen, Kupffer cells, lungs, salivary glands, lacrimal glands or the carotid body) by the intravenous administration of challengers that have a particular affinity for one or the other organ (Selye et al. 1960; Selye & Nielsen 1961; Selye 1961 b, 1962; Selye & Dieudonné 1961).

If in suitably sensitized rats large areas of the subcutaneous tissue are infiltrated by a challenger during the »critical period«, severe osteitis fibrosa results as a consequence of topical calciphylaxis. Presumably, here, the attraction of very large amounts of calcium into the challenged area secondarily mobilizes calcium from the bones (Selye 1961 a). We shall attempt to show that the osteitis fibrosa thus produced by calciphylaxis does not depend upon the presence of parathyroid tissue.

**MATERIAL AND METHODS**

Thirty-six female Holtzman rats with a mean initial body-weight of 98 g (range 95–100 g) were parathyroidectomized and then subdivided into three equal groups treated as follows: *Group 1*, DHT; *Group 2*, DHT plus ferric dextran (»Fe-Dex«); *Group 3*, DHT plus egg white.

**Parathyroidectomy** was performed on the first day of the experiment under ether anaesthesia by destroying the parathyroids with a pointed thermocautery. Completeness of the extirpation can readily be verified by direct inspection if the operation is performed under the control of a stereoscopic loupe. However, to ascertain the absence of functional accessory parathyroids that might have escaped attention we administered 2 mmol of NaH₂PO₄ in 2 ml of water through a stomach tube just prior to autopsy. This treatment causes no obvious change in the behaviour of normal rats but invariably elicited severe tetanic convulsions in our parathyroidectomized animals.

**DHT** (»Calcamin«, Dr. A. Wander, S. A.) was given at the dose of 1 mg in 0.5 ml corn oil by stomach tube once on the sixth day after parathyroidectomy to the animals of all three groups. *Fe-Dex* (ferric dextran, »Imferon«, Benger Laboratories, London) is a well tolerated iron preparation which contains 50 mg of metallic iron per ml. For the purpose of these experiments, it was diluted with distilled water to one tenth of the original strength. To obtain a particularly extensive soft-tissue calcification, the entire subcutaneous tissue was infiltrated with 10 ml of this diluted solution under light ether anaesthesia. Only the head, the extremities and the ano-genital region remained untreated in order to make certain that the expected calcification does not prevent locomotion, the ingestion of food and the excretion of metabolic products. This procedure was carried out on the day following DHT administration which happens to be the »critical period« for this type of calciphyllactic challenging.

Also 24 hours after the DHT, the rats of the third group received egg white in the form of a 50 % aqueous solution, using the same subcutaneous infiltration technique.
that was employed for the administration of Fe-Dex. Egg-white solutions tend to form precipitates which are difficult to remove by routine filtration, hence the solution was filtered through tissue paper (»Kleenex«) 15 minutes prior to injection.

During the entire experimental period, all animals were kept exclusively on »Purina Fox Chow« (Purina Co. of Canada) and tap water. Six animals of each group were sacrificed on the fifth and the remaining six on the tenth day after DHT treatment to follow the progress of the skeletal lesions.

At autopsy, specimens of the skin were fixed in alcohol-formol (8 parts of absolute alcohol, 2 parts of 10 % formalin) and, after embedding in paraffin, stained for calcium with the von Kossa- and celestine-blue-methods (Selye & Nielsen 1961). The development of the skeletal lesion was followed histologically on specimens of the femora, ribs and lumbar vertebrae of all animals. For this purpose, the bones were fixed and simultaneously decalcified in Susa solution saturated with picric acid embedded in paraffin and stained with haematoxylin-phloxine or the PAS-method.

**RESULTS**

In itself the single dose of DHT used in these experiments produced no cutaneous lesions and only negligible skeletal changes (Group 1). On the other hand, intense cutaneous calcinosis and bone lesions developed when DHT treatment was followed by the administration of either Fe-Dex (Group 2) or egg white (Group 3), but these changes were essentially identical and, hence, they can be discussed conjointly here.

The skin became intensely oedematous and obviously painful within two days after infiltration of Fe-Dex or egg white and it was subsequently transformed into a rigid carapace-like calcified shield. Histologically the challenged area exhibited the manifestations of intense cutaneous calcinosis that is characteristic of calciphylaxis (Selye & Nielsen 1961). Both the derma and the subcutaneous tissue were heavily incrusted with calcium salts and, especially in animals killed on the tenth day after DHT administration, this was followed by an inflammatory infiltration containing many eosinophil and pseudo-eosinophil leucocytes.

The bones of the entire skeleton became extremely fragile so that quite frequently spontaneous fractures developed, especially in the ribs. Histologically all the examined bone specimens of the animals killed on the fifth day after DHT administration showed the changes characteristic of osteitis fibrosa. Most of the trabeculae in the spongiosa disappeared or were in the process of absorption by osteoclasts and the normal haemopoietic bone marrow was replaced by hyperaemic fibrous tissue.

The picture was entirely different in the animals killed on the tenth day after DHT administration. By this time a secondary (apparently reactive) osteosclerosis appeared with an excessive development of dense, newly formed bone trabeculae; this was especially marked in the regions that had been most
Fig. 1.
Effect of calciphylaxis upon epiphyseal zone of femur in parathyroidectomized rats. 

Fig. 2.
Effect of calciphylaxis upon ribs of parathyroidectomized rats. A: DHT alone: essentially normal rib. B: Fifth day of DHT plus Fe-Dex treatment: intense osteitis fibrosa conducive to fracture of rib (arrow). Insert on bottom (higher magnification of same fracture region) shows fibrosis, necrosis and osteoclast proliferation. C: Similarly treated rat, killed on tenth day. Excessive bone formation with »club-shaped« thickening of costochondral region (haematoxylin-phloxine × 24, only insert in »B« × 280).

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severely affected by osteitis fibrosa in rats killed five days earlier (Figs 1 and 2).

It may be added that, in numerous control experiments, we found that in itself (i.e., without previous sensitization by DHT) similar subcutaneous infiltration with Fe-Dex or egg white causes no cutaneous calcification or severe skeletal changes.

DISCUSSION

Under ordinary circumstances osteitis fibrosa with secondary osteosclerosis is the result of a transient parathyroid hormone overdosage. This may be induced by brief treatment with parathyroid hormone or stimulation of endogenous parathyroid hormone secretion. The present experiments show, however, that the same sequence of bone changes can also be elicited by calciphylaxis in the absence of the parathyroids. Apparently, overdosage with exogenous or endogenous parathyroid hormone primarily affects the skeleton (presumably through stimulation of osteoclastic activity) and the resulting hypercalcaemia secondarily produces soft-tissue calcification, thereby removing excess calcium from the circulation. Here, the initial bone absorption itself is probably the stimulus for compensatory osteogenesis; the latter may result in severe osteosclerosis – comparable to clinical marble-bone disease – when the parathyroid hormone level of the blood returns to normal (Selye 1942).

Calciphylaxis, on the other hand, appears to influence the skeleton in an inverse manner: it first attracts so much calcium into the challenged area that a secondary bone absorption must ensue to compensate for the loss of calcium from the circulation. Yet, even here, the initial osteoclastic response is followed by excessive compensatory osteogenesis and both the initial bone absorption and the subsequent bone deposition can proceed in absence of the parathyroids. It is true that DHT possesses effects quite similar to those of parathyroid hormone but the amount given here was insufficient to produce any noteworthy skeletal change; hence it could have exerted only a »conditioning« influence, comparable to that of corticoids in many stress-induced homeostatic reactions.

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REFERENCES


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