EXPERIMENTAL PRODUCTION OF CUTANEOUS CALCINOSIS AND SCLEROSIS WITH DIHYDROTACHYSTEROL (AT-10)*

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It was observed some twenty-five years ago that rats treated with parathyroid extract during the first days of life develop a cutaneous disease which, in many respects, closely resembles scleroderma. The condition begins with a hardening of the skin on the back, and extends bilaterally from the forehead to about the middle of the thorax. Then, the indurated plaques become whitish and eventually tend to exulcerate, leaving behind a hairless atrophic skin. Histologically, during the acute stage, there is edema and marked calcium deposition in the derma of the affected areas, and if the process is sufficiently severe, the entire calcified layer eventually becomes necrotic and is cast off in the form of dry scabs. If the process is not sufficiently intense to cause widespread necrosis, marked connective-tissue proliferation ensues and the derma becomes sclerotic (1). Often these skin lesions are accompanied by inflammatory infiltrations, even outside the derma, for example, in the connective tissue that surrounds the nerves and vessels of muscles.

On the basis of these observations, several investigators have focused their attention on possible calcium-metabolism disturbances in scleroderma, and it has been claimed that in this disease the calcium content of the skin is sometimes considerably increased (2, 3), and that partial parathyroidectomy may induce marked improvement (4).

In young rats acutely overdosed with parathyroid hormone, calcification in soft tissues other than the skin does not occur as readily as in older animals. However, the immature bones tend to respond with an extraordinarily severe osteitis fibrosa, that greatly predisposes them to the occurrence of multiple spontaneous fractures (5). On the other hand, more chronic overdosage with smaller doses of parathyroid hormone produces an excessive degree of bone formation, that tends to obliterate the marrow cavities of long bones, and presents an aspect not unlike that of the so-called "marble-bone disease" of Albers-Schönberg. Most of these changes produced by an excess of parathyroid hormone—the reactive changes in the skin, the diffuse perivascular and perineural infiltrations and, to some extent, even the osteitis fibrosa—exhibit morphologic characteristics of inflammation. Indeed, much before we became aware of the role of corticoids in the regulation of the "inflammatory potential," these observations on experimental hyperparathyroidism were considered to be "an example showing that a hormone normally produced by the body can cause inflammation and that the parathyroid hormone might well be regarded as a regulator of connective tissue in general;" it was felt that this humoral factor

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might participate in the pathogenesis of "diverse forms of systemic connective-tissue diseases" (6).

A certain relationship between parathyroid hormone and the steroids of the Vitamin-D group was also suspected long ago, on the basis of both experimental and clinical findings. It was shown, for example, that in young rats, heavy overdosage with irradiated ergosterol preparations (containing various members of the Vitamin-D group) can produce not only ectopic calcium deposition in various soft tissues, but also, excessive bone absorption with the consequent development of multiple spontaneous fractures (7–10). All these changes are reminiscent of those seen in hyperparathyroidism. This resemblance became particularly evident when it was found that even ectopic soft-tissue calcification can occur in man under the influence of a hyperfunctional parathyroid adenoma (11).

More recent progress in the chemical separation of the individual members of the Vitamin-D group has shown that, among these, it is dihydrotachysterol (AT-10) which most closely resembles the parathyroid hormone in its pharmacologic actions. This steroid can not only relieve clinical hypoparathyroid tetany in man, it can even produce osteitis-fibrosa-like changes in the rat (12).

In view of these findings it is hardly surprising that only few dermatologists have considered it justified to treat clinical scleroderma with AT-10. However, interestingly, all three publications of this kind that we were able to find in the literature uniformly reported favorable results (13–15). It is not unusual for humoral agents to produce diametrically opposed results, depending upon dosage, duration of treatment and other "conditioning factors." To mention but a few relevant examples: testosterone may cause involution or stimulation of the spermatogenic epithelium, and folliculoids can enhance or suppress the growth of corpora lutea, depending upon experimental conditions (16).

In view of all these considerations it was decided to determine whether a great excess of AT-10 could produce scleroderma-like lesions, under the experimental conditions that made it possible for us to induce such changes with parathyroid hormone.

MATERIALS AND METHODS

Sixteen lactating Wistar rats, with a mean initial body-weight of 214 gm. (range: 163–228 gm.), were used for our experiments. Each mother, with her litter, was placed in a separate cage. All animals were fed exclusively on "Purina Fox Chow" and "Pablum," throughout the experiment.

Treatment with AT-10 ("Hytakerol," Winthrop Laboratories of Canada, Ltd.) was initiated on the third day after delivery. Daily, six of the mothers received 1.25 mg. (in 1 ml. of sesame oil), and the remaining ten, 500 μg. (in 0.4 ml. of sesame oil) of this steroid, through a thin plastic catheter, by gavage.

Both the mothers and the young, of the group given 1.25 mg. of AT-10 per day, became manifestly ill within four days and, since several of the young died during the subsequent 48 hours, we decided to kill both the mothers and the surviving young after six days of treatment. In this series histologic studies were not made.

The group receiving 500 μg. of AT-10 per day were in a much better condition, but even here the mothers lost much weight and several of the young died during the first week, so that, on the seventh day, AT-10 treatment was discontinued. To help the nutrition of the

young, the litters (originally comprising between 5 and 13 offspring) were uniformly reduced to 5, so as to provide more milk for each of the young. All the mothers in this second series were killed on the 20th day after initiation of AT-10 treatment. Autopsy revealed extensive calcification of the heart, aorta, peripheral vessels, kidney and gastrointestinal tract, in each of the lactating animals of both series. Among the suckling rats of the second series, eight were killed for histologic study at various intervals, between the 7th and the 14th day of treatment; the others were kept under observation during four weeks, in order to follow the clinical course of their recovery.

Histologic specimens of the skin, bones, heart, kidneys, blood-vessels and skeletal muscles of both the mothers and their young were fixed, either in Susa solution (for subsequent staining with the PAS technic) or in neutral formalin (for the demonstration of calcium deposits with the v. Kóssa technic).

RESULTS

In the litters of both experimental series, macroscopically visible skin lesions began to appear between the fourth and the sixth day of treatment. Their incidence within a litter varied from 20% to 80%. Only one litter, in the group receiving the lower dosage of AT-10, remained totally unaffected.

Usually, the disease began by the development of a few whitish, swollen patches on the skin that covers the calvarium; these spots gradually extended

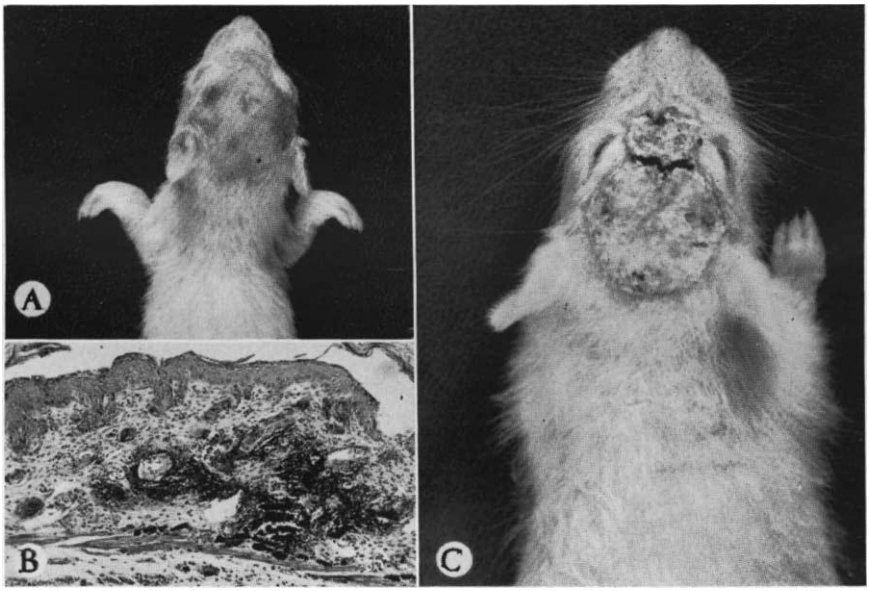

FIG. 1, A. Ten-day-old suckling rat on the seventh day after initiation of AT-10 treatment in its mother. Note the hairless, indurated and edematous patch of scleroderma-like skin on the head. The front paws are severely deformed due to multiple spontaneous fractures. *B.* Section through the skin of the scalp of the above rat. The numerous dust-like calcium granules between the epithelium and the cutaneous muscle are clearly visible (v. Kóssa × 75). *C.* Seventeen-day-old suckling rat on the 14th day after the initiation of AT-10 treatment in its mother. Here the indurated skin over the scalp has become hard and brittle. One transverse crack is clearly visible between the eyes; the frontal part of the patch is beginning to be detached.

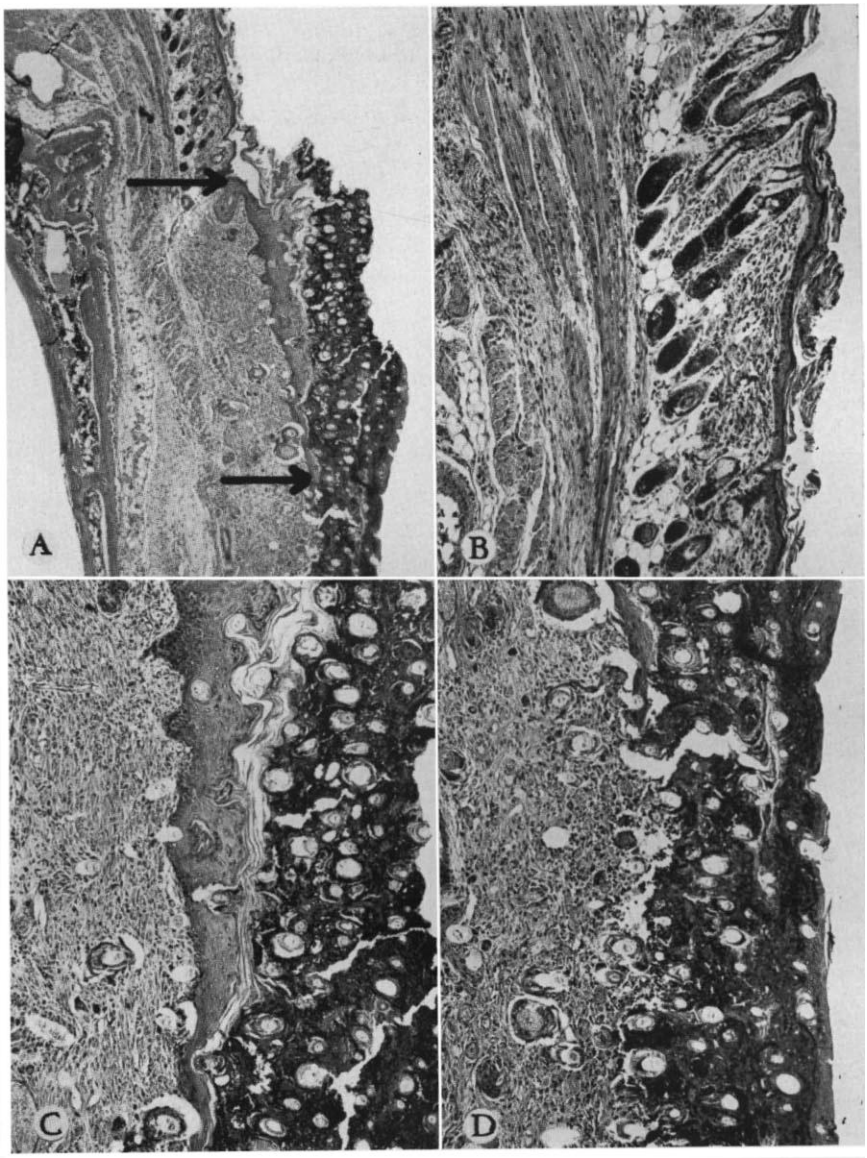

FIG. 2. Low magnification of a transverse section through the scleroderma-like patch on the head of the rat shown in Fig. 1, *C*. The temporal bone is on the left and the skin surface on the right. From the top of the figure to the upper arrow, the skin is of normal appearance and contains numerous hair follicles and sebaceous glands. Below that level, the derma is thickened and sclerotic; it consists of newly-formed tissue. The original (dark) calcified skin—which contains hair follicles and necrotic sebaceous glands—is in the process of being separated from the healthy tissue by a thick layer of epithelial ingrowth that penetrated the area from the upper to the lower arrow. Below the lower arrow there is no epithelial separation between the old calcified and the new sclerotic skin (PAS × 30). *B*. Higher magnification of the healthy skin that corresponds to the region above the upper arrow in Fig. A (PAS × 75). *C*. Higher magnification of a region situated between the arrows in Fig. *A*. The ingrowth of epithelium between the dark, calcified, necrotic skin on the right and the dense, sclerotic, newly-formed derma on the left is clearly visible here (PAS × 75). *D*. Higher magnification of the region below the lower arrow in Fig. *A*. Here, only the beginning of an epithelial ingrowth is visible, near the upper margin of the field; elsewhere, the calcified, necrotic, old skin is separated from the underlying healthy tissue only by a sclerotic and an inflamed open wound surface (PAS × 75).

symmetrically on both sides, towards the neck and upper thorax. In the first experiment, which was terminated on the sixth day of treatment, the condition did not progress beyond this point, but in the second group, the originally soft, swollen spots eventually became confluent and formed a large, hard, yellowish plaque on the head and neck. Still later, these plaques became dry, necrotic and brittle, so that they tended to break up into small scabs and then to be cast off (Fig. 1, *A* and *C*).

While these skin lesions developed, many of the suckling rats became seriously deformed as a result of multiple spontaneous bone fractures (Fig. 1, *A*), similar to those observed in our earlier work with irradiated ergosterol (7) and parathyroid hormone (5).

Histologic study of the skin specimens, taken at various stages in the development of the scleroderma-like lesions, reveals that, in the indurative stage, the derma is heavily infiltrated with calcium. On sections stained with v. Kóssa's technic, this manifests itself by the appearance of numerous black, dust-like granules between the epithelium and the cutaneous muscle layer (Fig. 1, *B*).

A few days later the entire calcified skin becomes necrotic and is demarcated from the underlying derma by an inflammatory granuloma. Subsequently, a thick covering of squamous epithelium grows over the granuloma and separates it from the necrotic calcified skin layer, so that eventually the latter is cast off. This leaves a broad, hard layer of sclerosed derma, covered by a thick epithelium, but virtually deprived of hair follicles and sebaceous glands, since these cannot regenerate after the loss of the original skin structures (Fig. 2). Only much later does this hardened sclerotic skin eventually become thin and atrophic. Curiously, mast-cells are extraordinarily numerous, not only in the affected skin regions but virtually everywhere in the connective tissue of AT-10-treated rats.

The skeletal changes, that culminate in multiple spontaneous fractures, are characterized by a considerable deficiency in the development of both cancellous and solid bone. At the same time, there is proliferation of osteoclasts and, to a lesser degree, of fibrous tissue in the marrow cavity and underneath the periosteum. Soft-tissue calcification was much less common in the suckling young than in their mothers.

DISCUSSION

These experiments show that manifestations of AT-10 intoxication can be transmitted from the mother to her young through the milk. This may be due to the excretion, by the mammary glands, either of AT-10 itself, or of some metabolic product which mediates the activity of this steroid.

It is of special interest that AT-10, a synthetic steroid of known chemical structure, can imitate the parathyroid hormone, not only as regards the relief of tetany and the production of osteitis fibrosa, but also with respect to the singular effect which this hormone exerts upon the skin. It remains to be seen whether this experimental cutaneous disease is a true replica of clinical scleroderma, but it resembles the latter in many respects.

Highly purified, accurately standardized and stable preparations of parathyroid hormone are still difficult to obtain. This has greatly handicapped the

progress of research concerning the effect of the parathyroids upon connective tissue in general and the skin in particular. The observations reported here will facilitate animal experimentation concerning the pathogenesis of the peculiar scleroderma-like skin lesions that are caused by certain regulators of calcium metabolism. It is hoped that such studies will also further our understanding of the mechanism conducive to the development of scleroderma in man.

SUMMARY

Experiments on lactating rats indicate that oral administration of dihydrotachysterol (AT-10) to the mother can result in the development of a scleroderma-like skin disease in her suckling young. These cutaneous lesions are often associated with multiple spontaneous bone fractures.

It remains to be seen whether this experimental disease is fundamentally related to clinical scleroderma. However, we now have a simple, reliable means of producing such lesions in the common laboratory rat, so that the study of such possible relationships has become amenable to experimental analysis.

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