MORPHOLOGICAL EFFECTS OF THYROCALCITONIN IN EXPERIMENTAL HYPERVITAMINOSIS D_2

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Biochemical and morphological changes in bone tissue and in the thyroid and parathyroid glands were studied in 80 albino rats with experimental hypervitaminosis D_2. Administration of thyrocalcitonin to the animals not only stimulated osteogenesis, but also led to early calcification of the bone trabeculae. Thyrocalcitonin also reduced the hyperplasia of the thyroid C-cells.

The endocrine regulation of calcium metabolism in the body has recently contracted considerable attention. Interest in this subject grew sharply after the discovery of the new thyroid hormone thyrocalcitonin (TCT) [2, 4], which inhibits catabolism in bone tissue and, consequently, lowers the serum calcium level. The action of TCT has been studied on a reasonably wide scale in hyperparathyroidism and in other disturbances of calcium metabolism [6]. However, there are only individual reports in the literature on the effect of this hormone on hypervitaminosis D_2, in which there are frequently serious disturbances not only of the skeletal bones, but also of many internal organs [5].

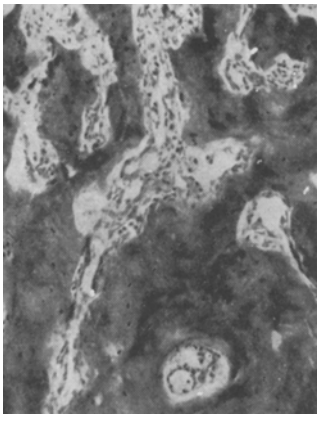

Fig. 1. Decrease in thickness of femoral bone trabeculae in a rat. Intertrabecular sclerosis. Picrofuchsin, 120 ×.

The object of the present investigation was to study the morphological changes in experimental hypervitaminosis D_2 and to ascertain whether TCT can diminish the toxic action of large doses of this vitamin.

EXPERIMENTAL METHOD

Experiments were carried out on 80 young male albino rats weighing 80-120 g. Vitamin D_2 was given by mouth daily to the animals in a dose of 16,000 units in 1 ml of vegetable oil. Some animals also received TCT (a slightly purified Soviet preparation obtained from freshly frozen bovine thyroid glands and containing 3000 MRC units/mg powder) three times a day by subcutaneous injection. Blood was taken and urine collected from the animals 3, 8, and 14 days after the beginning of the experiment for determination of the calcium and phosphorus concentrations, after which the animals were sacrificed. Pieces of the femur and tissue from the thyroid and parathyroid glands were fixed in Lillie's reagent and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, picrofuchsin-fuchselin, and by the PAS reaction. In the histological investigations of the

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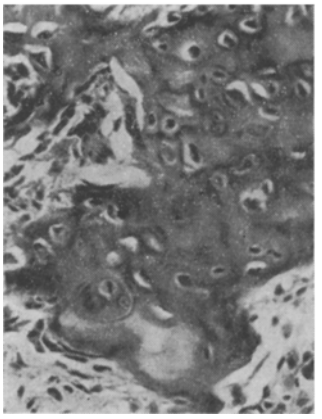

Fig. 2

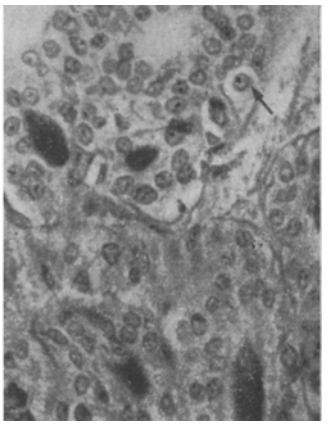

Fig.3

Fig. 2. Focus of cartilage tissue in a bone trabecula, irregular distribution of osteocytes. Picrofuchsin, 250 ×.

Fig. 3. Pale C-cells in the thyroid gland. PAS reaction, 300 ×.

bone the width of the trabeculae was measured by means of an ocular micrometer and the number of osteocytes per unit area of trabecula was determined. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Under the influence of vitamin D_2 the serum calcium level of the animals rose progressively, and the increase was particularly marked (by 1.2-1.3 mg% compared with the control) two weeks after the beginning of the experiment. In the rats which received TCT simultaneously with vitamin D_2 the hypercalcemia was somewhat less marked. The changes were much greater in the blood phosphorus level, and summation of the hypophosphatemic effects of vitamin D_2 and TCT was observed (a decrease of 1.4-1.8 mg% compared with the control). Under the influence of TCT the calciuric action of vitamin D_2 was somewhat weakened, but its phosphatiuric action was strengthened. In the animals receiving vitamin D_2 delay in growth and adynamia were observed, and calcinosis of the kidneys was found postmortem.

Histological examination of the bones showed that in animals receiving vitamin D_2 new bone trabeculae were formed but existing trabeculae were reduced in thickness and replaced by fibrosis (Fig. 1).

By contrast with the control animals, in rats receiving vitamin D_2 a ground substance rich in mucopolysaccharides could be seen in the young bone structures. The cartilage tissue has the appearance of irregular islands undergoing resorption and replacement by bone structures. The Haversian canals were narrowed. Many of the bone structures were calcified. In certain areas the osteoid tissue was reorganized into compact lamellar bone, while elsewhere there was delay in the reorganization of the bone structures, with the result that layers of osteoid material were piled one above the other. Signs of osteoclastic resorption of uncalcified bone structures were almost completely absent. The number of osteocytes in the various bone trabeculae varied within wide limits. The mean width of the bone trabeculae was $23.55 \pm 1.37\ \mu$ (compared with $30.9 \pm 0.1\ \mu$ in the control animals). The number of cells in 100 fields of vision was 132 ± 1.5 (145.6 ± 0.6 in the control). Proliferation of fibrous connective tissue was observed between the individual bone trabeculae.

According to the literature [1] hypervitaminosis D_2 stimulates the development of fibrosis in the intertrabecular spaces. However, as the investigations of Weinmann and Sicher [8] showed, in hypervitaminosis D_2 the morphological changes in bone tissue vary depending on the degree of damage to the renal filter. If the damage is slight osteosclerosis of the bones develops, but if the damage is considerable, osteoporosis develops.

In the present experiments, during the first 3-8 days of vitamin D_2 administration to the rats signs of stimulation of metabolism were actually observed in the tissue structures of the bone. In the later stages,

marked changes evidently due to stimulation of osteoclastic resorption were observed [3, 5]. The intertrabecular spaces were enlarged. A slight reaction of the osteoblastic layer could be seen in the islands of osteoid tissue. In these areas of osteoid tissue vacuolated cells with indistinctly outlined nuclei could be seen. Mineralization of the structures of the bone trabeculae was slight. Extensive areas with masses of cartilage were present. Many bone trabeculae contained large foci of cartilage cells (Fig. 2). The bone tissue of osteocytes in 100 fields of vision was 143.6 ± 4.0. The width of the bone trabeculae was 25.6 ± 1.8 μ; however, the bone structures contained large numbers of cells and few intercellular structures.

It is an interesting fact that in the animals receiving vitamin D_2 for long periods (two weeks) hyperplasia of the pale cells of the parathyroid glands was observed (Fig. 3); this developed against the background of marked congestion of their blood vessels, probable evidence of increased production of parathyroid hormone. These changes can be regarded as secondary and unconnected with the action of vitamin D_2 on the parathyroid glands, for the characteristic effect of vitamin D_2 on bone is also manifested on parathyroidectomized animals [7].

Simultaneous administration of vitamin D_2 and TCT led to the development of complex morphological changes in the bones. First, excessive administration of vitamin D_2 and TCT induced the formation of new osteoid material followed by the formation of bone trabeculae, and second, the deposition of calcium salts in the trabeculae was increased. Following the combined administration of vitamin D_2 and TCT to the animals the number of C-cells in the thyroid gland tissue was not increased but was normal or significantly below it, namely 10.2 ± 0.1 in 100 fields of vision. The changes in the parathyroid glands were distinctive. They showed a focal enlargement of the pale, chief cells. However, no evidence of marked hyperemia or diffuseness of hyperplasia of their component structural elements could be found.

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