increase in the number of AFC in the spleen. Heating the mice for 30 days was accompanied by restoration of the number of AFC in the spleen, while the proliferative response of SC to the mitogens remained depressed.

During chronic exposure to interrupted hyperthermia of the animals the immune response was restored as the animals became adapted to this type of exposure to heat, as shown by the return of proliferative activity of the spleen cells to normal in response to stimulation by B-cell mitogens and preservation of the number of AFC at the control level 40 days after the beginning of hyperthermia.

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# VITAMIN D ENDOCRINE SYSTEM AND BONE TISSUE MINERAL METABOLISM IN RATS WITH ADJUVANT ARTHRITIS: EFFECT OF 1,25-DIHYDROXYVITAMIN $D_3$


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Adjuvant arthritis in rats is an autoimmune disease induced by subcutaneous injection of mycobacteria in mineral oil, and it reflects reasonably adequately pathological changes developing in rheumatoid arthritis in man, and is widely used as an experimental model of this disease in the quest for antiinflammatory and antiarthritic agents. In the chronic stage of adjuvant arthritis intensive destruction of cartilage takes place, the content of collagen and calcium in the bones is reduced, and osteoporosis develops [7]. A basic role in the regulation of mineral metabolism in bone tissue is played by the vitamin D endocrine system. The most active form of vitamin D, and responsible for realization of its function in the body, is 1,25-dihydroxyvitamin $D_3$ [1,25$(OH)_2D_3$]. It is now known that 1,25$(OH)_2D_3$ possesses marked immunomodulating activity [1]. In vivo 1,25$(OH)_2D_3$ functions through a hormonal mechanism, interacting in target tissues with specific receptors [5].

The aim of this investigation was to study the state of the vitamin D endocrine system of bone tissue and mineral metabolism in rats with adjuvant arthritis, and the effect of 1,25$(OH)_2D_3$ on these parameters.


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TABLE 1. Effect of $1,25(OH)_2D_3$ on Calcium and Phosphorus Metabolism, State of Bone Tissue, and Content of Vitamin D Metabolites in Blood Serum of Rats with Adjuvant Arthritis

| Parameter | Group of animals | | |
|---|---|---|---|
| | control | adjuvant arthritis | adjacent arthritis + $1.25(OH)_2D_3$ |
| Density of whole femur, $g/cm^3$ | 1,43±0,01 | 1,24±0,03[a] | 1,38±0,02 [b] |
| Ca in whole bone, $mg/cm^3$ | 145±17,7 | 107±13,0 | 119±8,7 |
| P in whole bone, $mg/cm^3$ | 113±15 | 65±4,7[a] | 72,9±7,3 |
| Serum Ca level, mg/dl | 9,5±0,20 | 9,1±0,14 | 9,0±0,20 |
| Serum $P_i$ level, mg/dl | 6,8±0,32 | 6,62±0,10 | 7,0±0,35 |
| Serum 25(OH)D, ng/ml | 7,2±2,0 | 5,4±1,5 | 10,1±3,8 |
| Serum 1, $25(OH)_2D$, pg/ml | 82,2±8,4 | 66,0±7,2 | 132±16,3[a] |

**Legend.** a) Difference from control significant, b) difference from group of rats with adjuvant arthritis significant ($p < 0.05$).

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing initially 160-180 g. Adjuvant arthritis was induced by subcutaneous injection of 0.1 ml of Freund's complete adjuvant, containing 10 mg of dry killed BCG in 1 ml of a mixture of anhydrous lanoline and mineral oil (1:7), into the footpad of the right hind limb. The rats were given $1,25(OH)_2D_3$ perorally daily in a dose of 0.15 $\mu$g/kg (close to the physiological dose) for 16 or 35 days after injection of the adjuvant. The $1,25(OH)_2D_3$ was dissolved in a mixture of: propylene-glycol–alcohol–water (8:1:1). Control rats received the mixture of solvents. The rats were decapitated on the 16th or 35th day after induction of arthritis. The serum concentration of the 25-hydroxyvitamin and of $1,25(OH)_2D_3$ was determined by radiocompetitive protein binding, using the following kits: "vitamin D screening kit" (Buhlman Lab., Switzerland) and "1,25-dihydroxyvitamin D [$^3$H] assay reagent system" (Amersham International, UK) [4]. The content of receptors for $1,25(OH)_2D_3$ in the lymphocytes was determined by measuring their specific uptake of [$^3$H]-$1,25(OH)_2D_3$ in a saturating concentration (0.5 nM) by intact lymphocytes at 37°C [3]. The concentrations of calcium and phosphorus in the blood serum were determined as described previously [2]. The state of the bone tissue was assessed from the calcium and phosphorus content in the diapheses and epiphyses of the femur and according to their density [2].

## EXPERIMENTAL RESULTS

Marked signs of polyarthritis were observed in the rats 2 weeks after injection of Freund's adjuvant: the joints of the upper and lower limbs and in some cases of the tail were swollen. By the 35th day of the experiment the intensity of swelling of the joints showed no significant change.

The effect of adjuvant arthritis on the state of the bone tissue was assessed 35 days after its induction. As the data in Table 1 show, in adjuvant arthritis the density of the femur and its content of calcium and phosphorus were significantly reduced, and osteoporosis developed. Injection of $1,25(OH)_2D_3$ into the rats largely prevented the development of osteoporosis: the density and mineral saturation of the bone in these rats did not differ from those in the controls. A similar improvement of the mineral content of the bone was observed previously after injection of synthetic 1$\alpha$-hydroxyvitamin $D_3$, a vitamin D analog which is metabolized in vivo to the natural active form – $1,25(OH)_2D_3$ into rats with adjuvant arthritis [6]. It has to be pointed out that neither the development of arthritis nor injection of $1,25(OH)_2D_3$ into rats had any effect on the serum calcium and phosphorus levels (Table 1).

The state of the vitamin D endocrine system in the rats was assessed on the basis of serum level of two of its metabolites, namely 25-hydroxyvitamin D [25(OH)D] and $1,25(OH)_2D$, transport and hormonal forms respectively, and also on the basis of the number of $1,25(OH)_2D_3$ receptors in peripheral blood lymphocytes. The serum concentration of 25(OH)D was unchanged compared with the control after both 16 and 35 days of the disease, whereas the $1,25(OH)_2D$ level had a clear tendency to fall 35 days after the beginning of development of arthritis (Table 1).

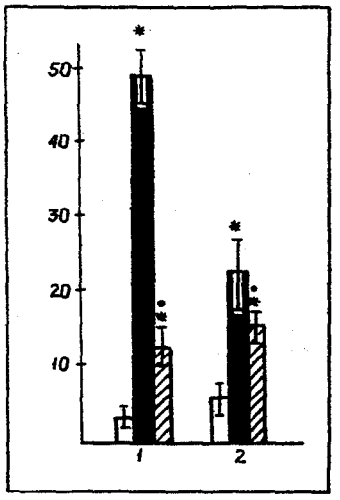


Fig. 1. Effect of $1,25(OH)_2D_3$ on concentration of $1,25(OH)_2D_3$ receptors in lymphocytes of rats with adjuvant arthritis. Vertical axis – concentration of $1,25(OH)_2D_3$ receptors (in fmoles/mg protein). 1) 16th day after induction of arthritis, 2) 35th day after induction of arthritis. Unshaded column – control rats, black shading – rats with adjuvant arthritis, obliquely shaded – rats with adjuvant arthritis receiving $1,25(OH)_2D_3$. Asterisk indicates significant differences from control ($p < 0.05$); dots indicate significant differences from group of rats with adjuvant arthritis, not receiving preparation ($p < 0.05$).

Injection of $1,25(OH)_2D_3$ did not affect the 25(OH)D concentration but led to a sharp rise in the serum $1,25(OH)_2D$ level; this was true, moreover, not only compared with the affected rats not receiving the preparation, but also by comparison with the control rats. The fall in the blood level of the hormonal form of vitamin D, namely $1,25(OH)_2D$, observed in adjuvant arthritis, may evidently be one cause of the development of the disturbances of mineral metabolism of bone tissue. For instance, it was recently shown that the calcium balance in rats with adjuvant arthritis correlates directly with the blood level of $1,25(OH)_2D$ [8]. We also know that $1,25(OH)_2D_3$ is the main hydroxylated metabolism of vitamin D in bone, accumulates in the nuclei of osteoblasts, which possess $1,25(OH)_2D$ receptors, and plays an active role in regulation of the function of these cells [10].

The development of adjuvant arthritis induced expression of $1,25(OH)_2D_3$ receptors in lymphocytes, for no such receptors could be found in the control animals (Fig. 1). After 16 days of the disease the number of receptors was twice that found in the rats after 35 days. This expression of receptors evidently reflects activation of lymphocytes in adjuvant arthritis, for we know that no such receptor is present in lymphocytes at rest, but appears when they are activated, by widely different factors (mitogens, antigens, cytokines, infectious agents) [1]. The decrease in the number of receptors toward the 35th day was evidently connected with changes in the course of the disease and with its transition from the acute to the chronic stage. The presence of $1,25(OH)_2D_3$ receptors is direct evidence that the lymphocytes of rats with adjuvant arthritis are sensitive to $1,25(OH)_2D_3$. In the affected rats receiving $1,25(OH)_2D_3$ the concentration of receptors in the lymphocytes was significantly lower than in rats not receiving the compound, after both 16 and 35 days of the disease (Fig. 1). This fall in the level of receptors may indicate normalization of the functional state of the lymphocytes relative to the hormonal signal (vitamin D). However, it has to be pointed out that in the present investigation we determined the concentration predominantly of unoccupied receptors of the

vitamin, and for that reason this decrease may reflect the higher level of occupation of receptors of the hormone in lymphocytes after administration of exogenous $1,25(OH)_2D_3$ compared with the control.

The vitamin D system is currently regarded as an immunoregulatory system, the function of which is aimed at correcting deviations of immune homeostasis with the aid of $1,25(OH)_2D_3$ [1]. The decrease in concentration of the hormonal form of vitamin D, namely $1,25(OH)_2D$, in the blood serum, found in the present investigation, and the marked expression of vitamin D receptors in the lymphocytes in adjuvant arthritis are clear evidence of an increase in the demand of the body for the hormonal form of vitamin D in this autoimmune process. It was recently shown that lymphocytes of most patients with rheumatoid arthritis, unlike lymphocytes from healthy individuals, also possess a $1,25(OH)_2D_3$ receptor [9]. It follows from the facts described above that the use of $1,25(OH)_2D_3$ in the combination treatment of rheumatoid arthritis, in order to correct its blood level, the mineral metabolism of bone tissue, and functional activity of lymphocytes, is indicated.

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