cleavage, the duration of the stages becomes shortened by about half. The stage of 4 blastomeres is the shortest in its duration, which is 8-10 h.

A similar duration of the individual stages of cleavage has been found in the case of C57BL mouse embryos [2]. However, the duration of the zygote stage in C57BL embryos is 26-30 h, whereas in (CBA × C57BL)F_1 embryos it is 14-16 h. These differences may perhaps be attributed to the use of different methods for dating pregnancy. The duration of all stages of cleavage in the mouse embryos studied in the present investigation differs from that in mice of inbred lines BALB and 129 and their hybrids [3, 6]. For instance, the zygote stage in mouse embryos of line 129 takes 29 h, compared with 26 h for BALB mice. The stage of 2 blastomeres in mouse embryos of line 129 occupies 21 h, compared with 25 h for BALB mice. The stage of 4 blastomeres in embryos of both lines was fairly long, taking 14 h. The shortest stage was that of 4-8 blastomeres, which lasted 4 h in BALB embryos and 10 h in mouse embryos of line 129. The features distinguishing the duration of the stages of cleavage in (CBA × C57BL)F_1 hybrid mice from those of other strains of mice, observed in the present investigation, agree with the view that genetic differences between mice are reflected in the duration of the stages of cleavage [3, 6].

LITERATURE CITED

1. N. N. Rott, Ontogenez, No. 1, 3 (1980).
2. N. A. Samoshkina, "Histoautoradiographic analysis of nucleic acid synthesis and mitotic cycles in early mammalian embryogenesis," Author's Abstract of Doctoral Dissertation, Leningrad (1978).
3. A. N. Gates, L. L. Doyle, and R. W. Noyes, Am. Zool., 1, 449 (1961).
4. M. Krishna and W. M. Generoso, J. Exp. Zool., 202, 245 (1977).
5. A. K. Tarkowski, Cytogenetics, 5, 394 (1966).
6. W. K. Whitten and C. P. Dagg, J. Exp. Zool., 148, 173 (1961).

EFFECT OF DIHYDROTACHYSTEROL ON BONE TISSUE IN RATS WITH EXPERIMENTAL RENAL INSUFFICIENCY

V. S. Yagodovskii, V. M. Ermolenko, V. B. Spirichev, N. V. Blazheevich, and N. A. Migunova*

UDC 616.61-008.64-092.9-085.356:577.161.2]-07:616.71-091-07

KEY WORDS: experimental nephrogenic osteodystrophy; effect of dihydrotachysterol.

Recent data on the character of the effect of the vitamin D analog dihydrotachysterol (DTS) in renal osteodystrophy are contradictory. According to Kaye et al. [5], the compound stimulates calcium absorption in the intestine, raises its blood level, depresses alkaline phosphatase activity in the blood serum, and weakens the roentgenological and histological manifestations of renal osteodystrophy. However, Pogglitsch et al. [7] found that DTS aggravates the manifestations of this process in the bones and increases the "demineralization of the skeleton."

It was accordingly decided to analyze the effect of DTS on the state of the bones in experimental renal insuffiency (RI).

*Deceased.

Central Research Institute of Traumatology and Orthopedics. Problem Laboratory for Nephrology, I. M. Sechenov First Moscow Medical Institute. Institute of Nutrition, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 611-613, November, 1980. Original article submitted August 17, 1979.

0007-4888/80/9011-1601$07.50

© 1981 Plenum Publishing Corporation

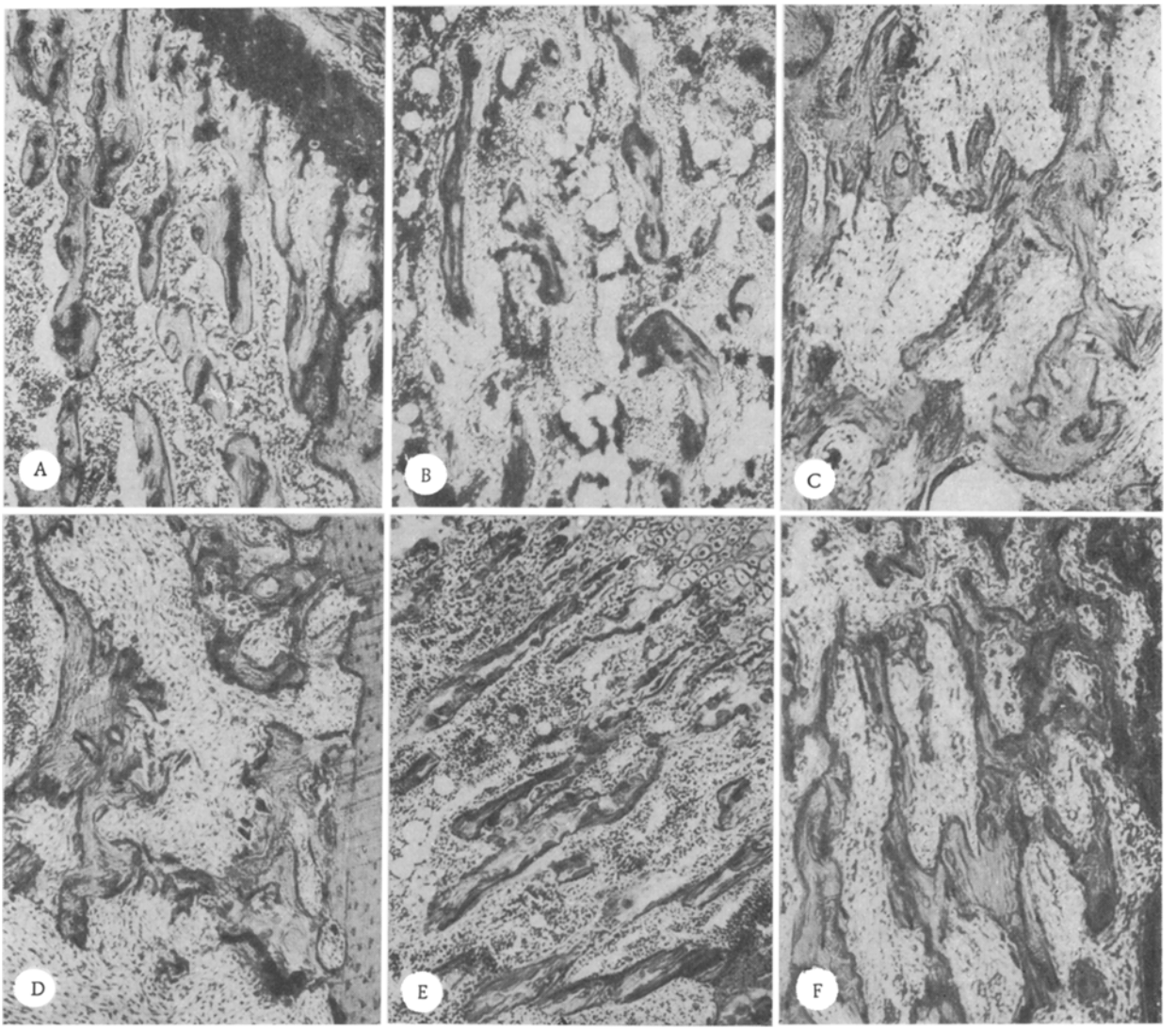

Fig. 1. Rat femur. A) Control: Spongiosa of distal femoral metaphysis. B) 1-1.5 months after removal of five-sixths of kidney tissue: moderate rarefaction of spongiosa of distal femoral metaphysis; some proliferation of fibrocellular tissue near bony trabeculae. C) 4-4.5 months after removal of five-sixths of kidney tissue: bony trabeculae of spongiosa of distal femoral metaphysis curiously shaped because of frequent occurrence of lacunar resorption of bone substance; interosseous spaces filled with fibrocellular tissue; picture similar to that observed in parathyroid osteodystrophy. D) 4-4.5 months after removal of five-sixths of kidney tissue; cortical lamina of femoral diaphysis; considerable rarefaction of lamina with proliferation of fibrocellular tissue to replace resorbed structures; picture similar to that observed in parathyroid osteodystrophy. E) 1-1.5 months after removal of five-sixths of kidney tissue of animals treated with DTS: structure of distal femoral metaphysis close to normal. F) 4-4.5 months after removal of five-sixths of kidney tissue of animals treated with DTS: distal femoral metaphysis; proliferation of fibrocellular tissue between bony trabeculae with signs of lacunar resorption. Hematoxylin-eosin, 75×.

EXPERIMENTAL METHOD

RI was produced in 85 noninbred male rats weighing 70-100 g by removal of five-sixths of the weight of kidney tissue by Morrison's method [6]. One group of rats (43) received no medication, but rats of the outer group (42) were given DTS (from the Vitamin Institute, Ministry of Health of the USSR) perorally, starting from 3-5 days after the operation and continuing until the end of the experiment, in a dose of 2 µg/100 g body weight daily. Intact rats (35) served as the control. The animals were killed after 1-1.5, 2-2.5, and 4-4.5 months.

TABLE 1. Dependence of Frequency of Visible Changes in Bones of Rats with Experimental Renal Insufficiency (RI) on Administration of DTS to Animals

Duration of RI, months	RI	RI+DTS	P
1—1½	12/16	7/18	about 0,05
2—2½	8/19	4/13	>0,05
4—4½	7/8	2/11	<0,02
Total	27/43	13/42	<0,01

Legend. Numerator gives number of rats with visible changes in bones, denominator total number of rats in group.

Histological sections were cut along the longitudinal axis of the lower end of the femur (after decalcification and embedding in celloidin) through the middle of the epiphysis, metaphysis, and diaphysis, and also transversely to the axis of the bone in the middle of the diaphysis. The sections were stained with hematoxylin-eosin and picrofuchsin by Van Gieson's method. For quantitative estimation of the degree of rarefaction of the bone, the method of gravimetric projection morphometry (see the paper by Avtandilov [1]) was used: transverse sections through the diaphysis of the bone were placed in a diascope and their images were thrown on paper with a magnification of 25 times. The outlines of the periosteal and endosteal surfaces of the bone, and its vascular and haversian canals were traced. The figures thus obtained (the canals and the whole of the cortical layer of the bone separately) were cut out with scissors and weighed on torsion scales. The ratio (in per cent) of the total area (weight) of the channels to the total area (weight) of the cortical lamina (the total area of the channels plus the area of the remaining part of the cortical lamina of bone) was calculated. The chi-square test and the Wilcoxon—Mann—Whitney U criterion were used for statistical analysis of the data [2]. Nonprotein nitrogen in the blood was determined by Assali's method [3].

EXPERIMENTAL RESULTS

During the first days after the operation the rats developed uremia (nonprotein nitrogen 68.0 ± 3.1 mg %). The uremia subsequently increased.

Histological investigation of the bones 1-1.5 months after the operation revealed proliferation of a thin or moderately thick layer of osteogenic fibrocellular tissue in the metaphysis of 12 of the 16 rats, in places along the trabeculae of the spongiosa (Fig. 1B), by contrast with the control rats (Fig. 1A); a picture of lacunar resorption of bone substance was frequently observed. Many lacunae contained osteoclasts. The vascular canals in certain areas of the cortical lamina of the metaphysis (less frequently at the boundary of the metaphysis and diaphysis) were moderately widened and filled with fibrocellular tissue. These changes were evidence of resorption of the bony walls of the vascular canals. Only in isolated cases were individual vascular canals widened a little in the cortical lamina of the diaphysis.

In rats with RI for 2-2.5 months, the changes in the bones were more marked and were observed in eight of 19 rats. In the most manifest cases considerable proliferation of fibrocellular tissue was observed in the metaphysis. Its bony structures revealed an abundance of patterns of lacunar resorption. Osteoclasts were frequently found in the lacunae. Widening of the vascular canals was observed in the cortical lamina in all parts of the bone. In some rats, with the highest blood nonprotein nitrogen level, the cortical lamina was considerably rarefied because of widening of the vascular canals. The edges of the bony walls of these canals were indented, i.e., they showed signs of lacunar resorption, and the lumen of the canals was filled with fibrocellular tissue, often containing osteoclasts.

After RI lasting 4-4.5 months, bone lesions were found in seven of the eight rats; in four of them the structural changes in the bone tissue were on a considerable scale or were very clearly defined. The pattern of the structural changes was similar to that observed

in primary hyperparathyroidism [4] (Fig. 1C, D). The bony trabeculae of the metaphysis were curiously shaped because of the frequent presence of lacunar resorption. The cortical lamina in all parts of the bone was perforated by numerous greatly widened vascular canals or confluent areas of resorption of bony structures, and it consequently acquired the appearance of lace. Nearly all the interosseous spaces in the metaphysis and areas of resorption of structures of the cortical lamina were filled with osteogenic fibrocellular tissue with many osteoclasts. In many places an increased number of cementation lines was observed, evidence of frequent alternation of resorption and new formation of bony substance, i.e., of intensification of reconstruction of the bone tissue. In the rats of this series, unlike the remaining groups, signs of lacunar resorption of the trabeculae in the spongiosa and moderate proliferation of fibrocellular tissue near them were found in the epiphyseal parts of the bones also. After 4-4.5 months of RI, besides changes of parathyroid osteodystrophy type, deposits of osteoid of varied thickness were present on the bony structures in some places, indicating the addition of bony changes of osteomalacia type.

In rats treated with DTS changes similar to those described above were found but they were less marked (Fig. 1E) and much less frequent. After 1-1.5 months of RI visible changes in the bones, of varied degrees of severity, were observed in fewer than half of all cases (Fig. 1E).

Among rats receiving DTS for 2-2.5 months changes in the bones as a whole also were less marked than in rats not receiving DTS; in nine of 13 rats the effect of RI on the bone tissue in general either could not be detected or was extremely slight.

After administration of DTS for 4-4.5 months visible changes in bones were observed in only two of 11 rats: moderate lacunar resorption of bony trabeculae was found in the region of the metaphysis with widening of the vascular canals of the cortical lamina, which were filled with fibrocellular tissue. Foci of proliferation of this tissue, of varied thickness, were found here and there along bony trabeculae of the metaphysis (Fig. 1F).

In experimental RI histological changes in bones were thus found more frequently and were more marked with an increase in the duration of RI, and in some cases the picture resembled that observed in parathyroid osteodystrophy. This suggests that one of the factors responsible for these changes is secondary (nephrogenic) hyperfunction of the parathyroid glands. Treatment with DTS reduced the frequency of occurrence of the bone changes (Table 1): comparison of the treated and untreated groups of rats (by the chi-square test) showed that these differences were on the borderline of statistical significance at the level generally used in biology (P about 0.05) after 1-1.5 months of RI, and were statistically significant ($P < 0.02$) after RI for 4-4.5 months and at all stages of the experiment as a whole ($P < 0.01$). Morphometric investigation of transverse sections through the diaphysis of the bone showed that the degree of rarefaction of the cortical lamina in the untreated rats was greater than in rats treated with DTS. These differences were statistically significant ($P < 0.5$, Wilcoxon–Mann–Whitney U criterion).

It should be noted that in rats receiving DTS, in some cases distinct changes were absent or were very slight even if the blood nonprotein nitrogen level was relatively high (82.5-180 mg %). Similar changes in rats with RI not treated with DTS occurred at a lower blood nitrogen level.

The results are evidence that treatment with DTS can prevent the development of bone lesions even in the presence of marked RI, but not in all cases.

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria to Assess Differences between Two Groups of Observations in Medico-Biological Research [in Russian], Moscow (1969), pp. 17-19.
3. V. E. Predtechenskii, Textbook of Clinical Laboratory Investigations [in Russian], Moscow (1964), pp. 203-207.
4. A. V. Rusakov, in: Textbook of Pathological Anatomy in Several Volumes [in Russian], Vol. 5, Moscow (1959).
5. M. Kaye, G. Chatterjee, G. Cohen, et al., Ann. Intern. Med., 73, 225 (1970).
6. A. Morrison, Lab. Invest., 11, 321 (1962).
7. H. Pogglitsch, H. Schmidberger, and G. Gell, Müench. Med. Wochenschr., 118, 131 (1976).