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Morphometric study of cartilage dynamics in the chick embryo tibia
II. Dexamethasone-treated embryos*

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INTRODUCTION

The effects of glucocorticoids on cartilage growth have been widely studied in birds (Karnofsksy, 1950; Karnofsky, Ridgway & Patterson, 1951; Huble, 1957; Barrett, Sledge & Dingle, 1966; Reynolds, 1966; Calcagno, Goyena, Arràmbide & Arruti, 1970; Goel & Jurand, 1976) and in mammals (Rath & Reddi, 1979; Bonucci, Dearden & Mosier, 1982; Silbermann, 1982). In spite of the large number of papers dealing with this subject, the effects of corticosteroids on cartilage dynamics and their physiological role in normal development remain unclear (Takano, Takigawa & Suzuki, 1985). Different factors, such as the specific drug, doses, animal species and physiological status can influence the results (Lebovitz & Eisenbarth, 1975).

In the preceding paper (Gaytan, Ranz & Aceitero, 1987) we have studied the normal development of the chick embryo tibia from Day 11 to Day 14 when the cartilaginous model is being resorbed and focusing on the quantitative changes that happen at this time. The aim of the present study is to quantify the changes induced by dexamethasone treatment on this system, mainly in the cartilage formation and resorption.

MATERIALS AND METHODS

Fertile White Leghorn eggs were incubated at 38 °C for 11 to 14 days. On Day 10, treated embryos were injected with 8 μg of dexamethasone (21-disodium phosphate-dexamethasone) in 25 μl of distilled water into the chorioallantoic membrane. After decapitation on Days 11, 12, 13 and 14 (five embryos per group) the tibiae were carefully removed, fixed in Carnoy's fluid and embedded in paraffin. The contralateral tibiae were diced to form small blocks and fixed for 24 hours in 2% phosphate-buffered glutaraldehyde (pH 7·3) and afterwards embedded in Araldite. Sections 1 μm thick were stained with toluidine blue and observed by light microscopy.

Sections 8 μm thick from the paraffin-embedded pieces were stained with haematoxylin and eosin and used for the morphometric study. It was carried out as described in the preceding paper. The equation of the regression line for the mid-diaphyseal zone was $y = 0{\cdot}05x + 0{\cdot}29$ and the volume of the two truncated cones was given as:

$$V = 0{\cdot}005x^3 + 0{\cdot}0091x^2 + 0{\cdot}528x.$$

The statistical analysis was performed by the one-way analysis of variance and the LSD test for multiple comparisons among means (Bancroft, 1968; Ruiz-Maya, 1977).

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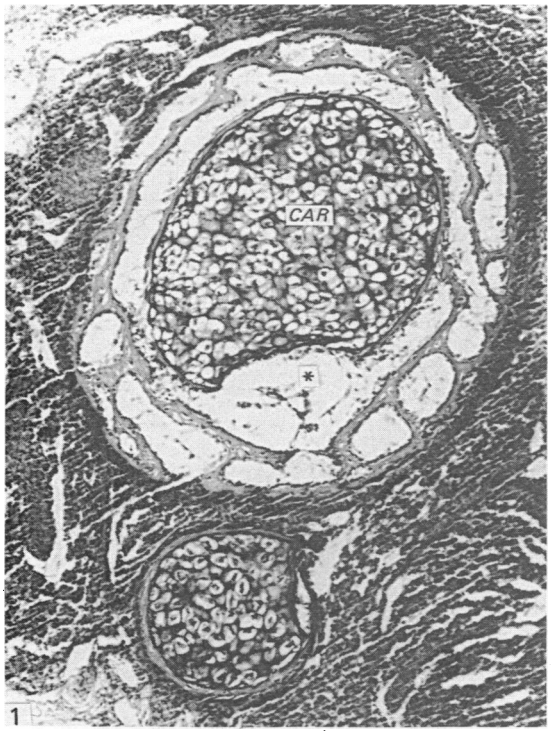

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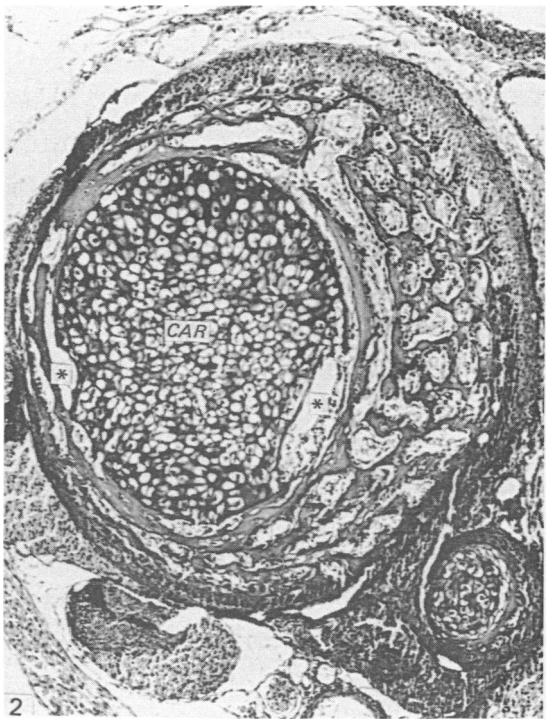

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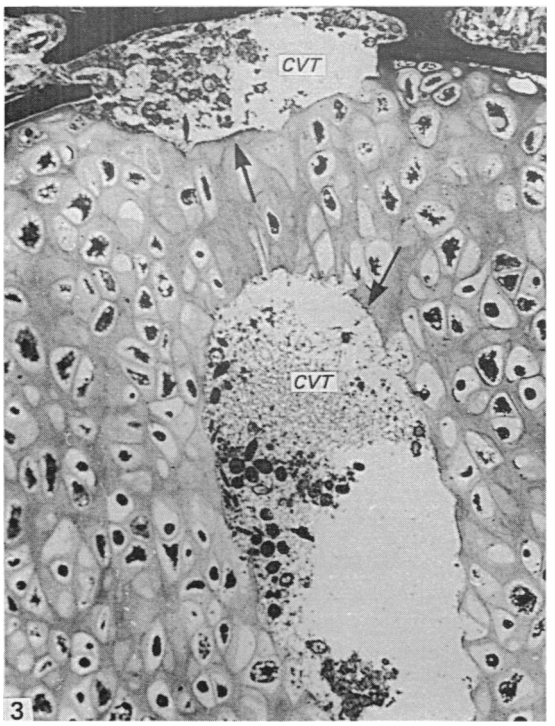

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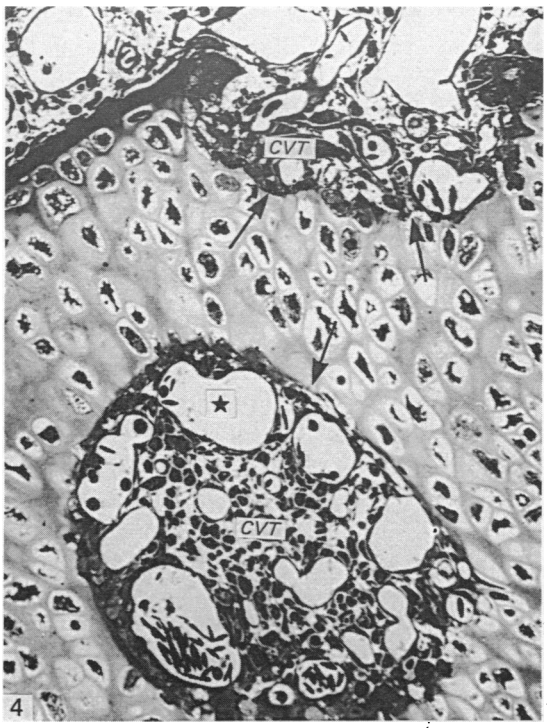

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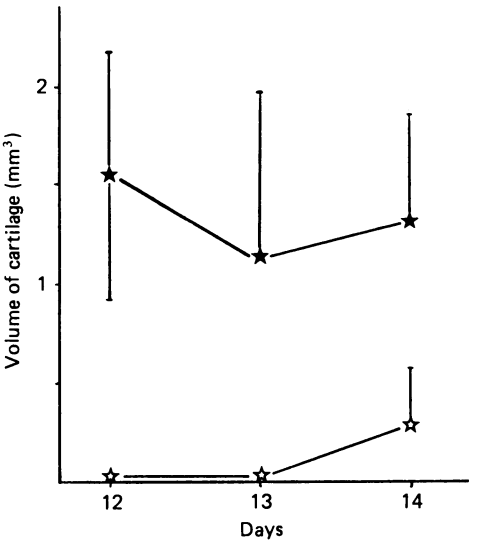

Fig. 5. Cartilage formation (★) and resorption (☆) rates in the dexamethasone-treated embryos. Data are presented as mean ± S.E.M.

RESULTS

On Day 11 the mid-diaphyseal zone of the dexamethasone-treated embryos presented similar features to those found in control embryos at the same age. The perichondral osseous collar had been eroded and the cartilage resorption had just started (Fig. 1). No significant progression of the invading and vascular tissue was observed in the following days (Fig. 2). By Day 14, some animals presented a greater amount of connective and vascular tissue whereas in others the aspect of the mid-diaphyseal zone was similar to that observed on Day 11. The cavities formed after cartilage resorption were practically empty and the cells in the cartilage-marrow interface were sparse (Fig. 3) when compared to the control embryos (Fig. 4).

The longitudinal growth of the tibiae was retarded in dexamethasone-treated embryos by 2, 16, 30 and 24% from Day 11 to Day 14 respectively, by comparison with the control tibiae.

The results of the morphometric study are shown in Table 1 and Figure 5. The volume density of the cartilage remained nearly constant, decreasing slightly (by a factor of $\times 0{\cdot}88$) from Day 13 onwards. The volume density of the connective and vascular tissue did not change during the whole period studied but the volume density of the external space increased significantly from Day 12 to Day 14 (by a factor of $\times 1{\cdot}24$).

Fig. 1. Eleven days old dexamethasone-treated embryo. The connective and vascular tissue (asterisk) is invading the cartilaginous model (*CAR*). H.E. × 70.

Fig. 2. Thirteen days old dexamethasone-treated embryo. The invading connective and vascular tissue (asterisks) is poorly developed and a wide cartilaginous core (*CAR*) remains unresorbed. H.E. × 70.

Fig. 3. Semithin section from a dexamethasone-treated embryo. The cells in the connective and vascular tissue (*CVT*) and in the cartilage surface (arrows) are scarce. Toluidine blue. × 250.

Fig. 4. Semithin section from a control embryo. Blood vessels (star) and cells apposed to the cartilage surface (arrows) can be observed in the connective and vascular tissue (*CVT*). Toluidine blue. × 250.

Table 1. *Morphometric data for the tibia from dexamethasone-treated chick embryos: values are given as mean ± SEM*

Parameters	Days of age			
	11	12	13	14
Volume density of the cartilage (Vv_{CAR}, %)	56·83 ± 1·09	55·52 ± 1·97	51·58 ± 1·61	45·40 ± 2·53[a, b]
Volume density of the connective and vascular tissue (Vv_{CVT}, %)	3·56 ± 1·08	2·05 ± 1·24	1·00 ± 0·74	2·10 ± 0·98
Volume density of the external space (Vv_{EXT}, %)	39·61 ± 0·83	42·43 ± 0·92	47·43 ± 1·19[a, b]	52·50 ± 1·59[a, b, c]
Volume enclosed by the perichondrium-periosteum (V_{PER}, mm³)	5·94 ± 0·54	8·52 ± 1·21	11·05 ± 1·76[a]	18·97 ± 0·89[a, b, c]
Volume of cartilage (V_{CAR}, mm³)	3·78 ± 0·36	5·29 ± 0·63	6·43 ± 0·87[a]	9·27 ± 0·54[a, b, c]
Volume of the connective and vascular tissue (V_{CVT}, mm³)	0·11 ± 0·03	0·10 ± 0·07	0·09 ± 0·07	0·37 ± 0·15
Volume of the external space (V_{EXT}, mm³)	2·06 ± 0·19	3·12 ± 0·53	4·53 ± 0·82[a]	9·33 ± 0·57[a, b, c]

a, *b* and *c* represent significant differences ($P < 0·05$) versus the values found for Day 11, 12 and 13, respectively.

The total volume enclosed by the perichondrium-periosteum increased significantly from Day 11 ($5{\cdot}94 \pm 0{\cdot}54$ mm^3) to Day 14 ($18{\cdot}97 \pm 0{\cdot}87$ mm^3), showing the most rapid growth from Day 13 onwards (by a factor of $\times 2$). On the contrary, no significant changes were observed for the volume of the connective and vascular tissue, which remained unchanged during the whole period under investigation. However, by Day 14, some embryos presented an increase in the volume of this compartment, although the variability within the group was very high.

The cartilage formation rate (volume of cartilage formed per day) was constant from Day 12 to Day 14 (Fig. 5). The cartilage resorption rate (volume of cartilage resorbed per day) was practically zero on Days 12 and 13 and showed a non-significant increase on Day 14.

DISCUSSION

Dexamethasone-treated embryos presented a retarded growth in all the stuctures enclosed by the perichondrium-periosteum as well as in the longitudinal growth of the whole bone anlage from Day 12 onwards. A delay in the longitudinal growth after corticosteroid administration has been reported by several authors (Sobel & Freund, 1958; Reynolds, 1966; Hall & Kalliecharan, 1975). It has been established that corticosteroids act on this system by inhibiting certain processes such as the proliferation of mesenchymatous cells (Reynolds, 1966; Rath & Reddi, 1979), the maturation and hypertrophy of chondrocytes (Silberberg & Silberberg, 1972; Silbermann, Kleinhaus, Livne & Kadar, 1976; Bonucci *et al.* 1982), the synthesis of mucopolysaccharides (Silberman, 1982) and the synthesis of collagen (Kivirikko, 1963; Ebert & Prockop, 1967; Blumenkrantz & Asboe-Hansen, 1976; Cutroneo, Rokowski & Counts, 1981).

Although dexamethasone-treated embryos presented a retarded cartilage growth when compared to control embryos of the same age, a complete lack of cartilage growth was not found in these animals. The cartilage formation rate remained above 1 mm^3/day from Day 12 to Day 14 though the variability among animals was very high.

On the contrary, dexamethasone-treated embryos presented a total inhibition of cartilage resorption during the same time. The volume of cartilage resorbed did not change during the whole period studied and the cartilage resorption rate was practically zero up to Day 13. This might be related to the scarcity of resorptive cells in the cartilage-marrow interface observed in the same embryos. A diminution in chondrolysis after corticosteroid treatments has been reported in several conditions and sites such as in rheumatoid arthritis (Krane, 1982), in the cartilage growth plate of the rat (Silberberg & Silberberg, 1972; Silbermann & Kadar, 1977; Rath & Reddi, 1979), in tibial cell cultures (Schryver, 1966) and after treatment *in ovo* by Moscona & Karnofsky (1960).

In the present study the most important effect of dexamethasone occurred at the level of cartilage resorption. We cannot establish from the present data whether this effect was due to the existence of cartilage alterations or to the effect on the invading connective and vascular tissue, or both. In this respect, a slower proliferation of connective and vascular tissue has been found after corticosteroid treatment (Laron & Boss, 1962; Simmons & Kunin, 1967) while Moscona & Karnofsky (1960) found that cartilage resorption was delayed after the administration of corticosteroids *in ovo* before Day 9. This absence of cartilage resorption could be due to the lack of invasion of the cartilage by connective and vascular tissue by inhibiting the erosion of the

perichondral osseous collar, since inhibition of osseous resorption after corticosteroid treatment has been reported by several authors (Tapp, 1966; Jee, Park, Roberts & Kenner, 1970). In the present study, the drug was administered on Day 10, because at this age the erosion of the perichondral osseous collar had already started, along with cartilage resorption at the mid-diaphyseal zone. Therefore the absence of cartilage resorption found in these animals cannot be attributed to a lack of invasion by connective and vascular tissue, since this process had already been completed when the drug was administered.

The inflection found in 13 day control embryos in the cartilage formation rate (Gaytan *et al.* 1987) was not present in the dexamethasone-treated ones and this may be due to the changes produced by corticosteroid treatment in the normal growth pattern. Furthermore, a certain growth recovery was observed from Day 13 to Day 14, although the variability on this day was very high. All the structures enclosed by the perichondrium-periosteum showed their most rapid growth at this age. This might be due to a decrease in the levels of dexamethasone and/or to a change in the response of tissues to the drug. It is in agreement with the results of other authors who reported that the effects of corticosteroids on growing bone are transient and reversible (Hulth & Olerud, 1963; Dearden & Mosier, 1972; Rath & Reddi, 1979).

SUMMARY

The cartilage dynamics in the tibia of dexamethasone-treated chick embryos has been studied by means of morphometric methods. Treated embryos showed a delay in the longitudinal growth of the tibia, as well as in the growth of all structures enclosed by the perichondrium-periosteum. The cartilage formation rate remained nearly unchanged (above 1 mm^3/day) from Day 12 to Day 14, whereas the cartilage resorption rate was zero up to Day 13, and showed a non-significant increase from Day 13 onwards. This might be related to the scarcity of resorptive cells found in the cartilage-marrow interface. By Day 14 a certain recovery of the growth rhythm was observed. These results indicate that the greatest effect of dexamethasone occurs at the level of cartilage resorption.

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