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# DEPOSITION OF OXYTETRACYCLINE IN PERICHONDRAL OSSIFICATION IN RABBIT

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The periphery of the growth plate and the metaphysis is lined by the perichondral lamella (Figure 1), which constitutes part of the ossification groove (Lacroix 1951, 1961). This lamella is often called the perichondral ring (Lacroix 1951, 1961, Solomon 1966) and is formed by appositional growth from the ossification groove (Rigal 1962, Hansson 1964, Rang 1969).

The function of the ossification groove and its perichondral lamella is not yet properly understood, but different conceptions exist (Policard 1941, Lacroix 1951, 1961, Kember 1960, Rigal 1962, Solomon 1966, Hansson 1967, Langenskiöld et al. 1967, Rang 1969). Judging from the literature, deposition of the tetracycline during the perichondral ossification process has so far been only briefly described (Hansson 1964, 1967). A more detailed description appears justified, since this deposition can give information on the mineralisation as well as on the growth of the perichondral lamella.

## MATERIAL AND METHOD

Forty-eight white rabbits of both sexes and 30 days of age at the beginning of the experiment were used. The animals were given oxytetracycline (OTC-Terramycin®) i.v. in a dose of 10 mg/kg body weight. The animals were killed at the end of the experimental period by mebumal sodium i.v. The animals were killed in groups of 8 after 10 min, 12 h, 1, 2, 4 and 8 days (Table 1). The rabbits were treated in the way described previously (Hansson 1967).

Deposition of OTC was examined under the fluorescence microscope in manually cut sections of material fixed in absolute ethyl alcohol (Hansson 1964, 1967). The following growth regions were studied: proximal tibia and proximal and distal radius.

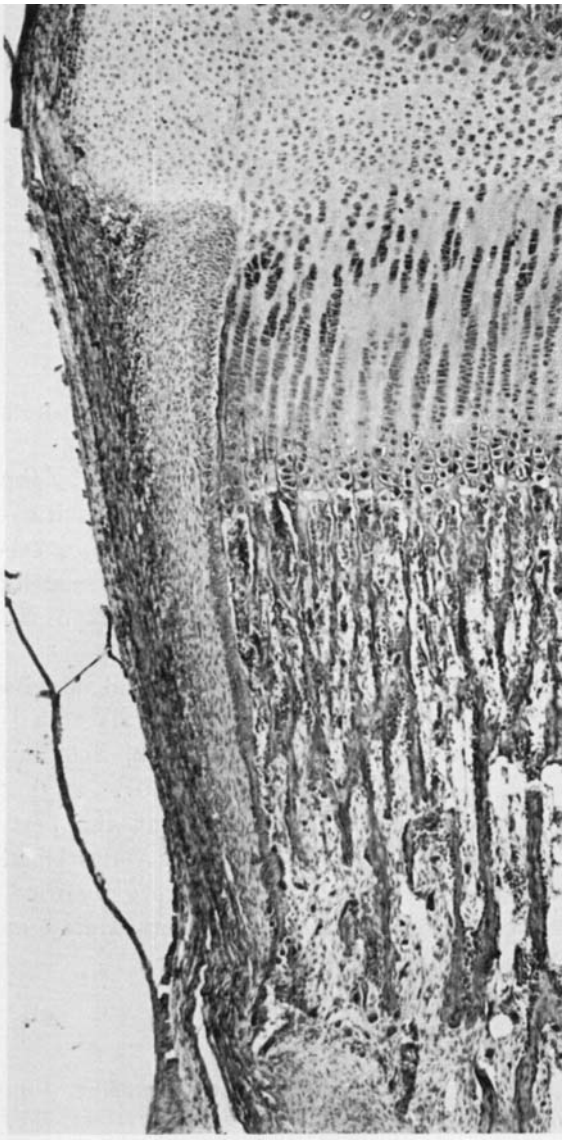

*Figure 1. The ossification groove with the perichondral lamella in distal radius of 30-day-old rabbit. Paraffin section (10 μ) from decalcified material fixed in Bouin's solution. Ehrlich's haematoxylin-cosin (60 ×).*

## RESULTS

Examination in the fluorescence microscope (Figures 2 and 3) revealed that yellow fluorescent OTC was deposited within 10 minutes of the injection of OTC in the perichondral lamella.

The intensity of the yellow fluorescence was highest in the most

*Table 1. Deposition of OTC in perichondral ossification—results.*

| | Time after injection | Number of animals | Length of perichondral lamella ($\mu$) Epiphyseal autofluorescent part M ± s | Fluorescent part M ± s | Diaphyseal autofluorescent part M ± s | Total M ± s | Extension of perichondral lamella in epiphyseal direction from erosion line ($\mu$) M ± s |
|---|---|---|---|---|---|---|---|
| Proximal tibia | 10 min | 8 | 0 | 366 59 | 981 173 | 1348 175 | 188 18 |
| (561 ± 30 $\mu$/day)* | 12 h | 8 | 258 28 | 381 28 | 633 76 | 1272 58 | |
| | 1 day | 8 | 492 31 | 398 23 | 248 125 | 1138 148 | |
| | 2 days | 8 | 1048 55 | 377 36 | 86 57 | 1511 114 | |
| | 4 days | 8 | 1460 113 | 0 | 0 | 1460 113 | |
| | 8 days | 8 | 1455 78 | 0 | 0 | 1455 78 | |
| Proximal radius | 10 min | 8 | 0 | 87 12 | 448 82 | 535 92 | 107 10 |
| (145 ± 14 $\mu$/day)* | 12 h | 8 | 55 11 | 108 8 | 580 115 | 743 128 | |
| | 1 day | 8 | 120 22 | 107 13 | 463 65 | 690 95 | |
| | 2 days | 8 | 234 23 | 102 11 | 310 27 | 646 18 | |
| | 4 days | 8 | 490 22 | 104 11 | 154 45 | 748 43 | |
| | 8 days | 8 | 645 58 | 0 | 0 | 645 58 | |
| Distal radius | 10 min | 8 | 0 | 373 40 | 718 59 | 1091 96 | 172 13 |
| (486 ± 30 $\mu$/day)* | 12 h | 8 | 235 21 | 362 19 | 527 54 | 1124 51 | |
| | 1 day | 8 | 456 49 | 364 32 | 334 81 | 1154 56 | |
| | 2 days | 8 | 933 59 | 288 44 | 34 53 | 1255 93 | |
| | 4 days | 8 | 1192 72 | 0 | 0 | 1192 72 | |
| | 8 days | 8 | 1161 70 | 0 | 0 | 1161 70 | |

* Rate of longitudinal bone growth from growth plate from Hansson (1967).

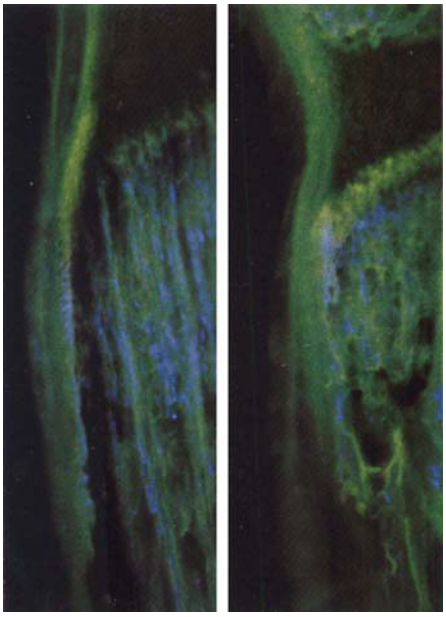

*Figure 2. Deposition of OTC in perichondral lamella in distal radius of 30-day-old rabbit 10 min after intravenous injection of 10 mg OTC per kg body weight. Fluorescence microphotography (60 ×).*

*Figure 3. Deposition of OTC in perichondral lamella in proximal radius of 30-day-old rabbit 10 min after intravenous injection of 10 mg OTC per kg body weight. Fluorescence microphotography (60 ×).*

2. 3.

epiphyseal part of the perichondral lamella, where the latter terminated in a more or less tapering tip. In diaphyseal direction the intensity of the fluorescence decreased gradually and merges diffusely with the autofluorescent area, which was of uniform width. The yellow fluorescence occurred in the form of a network of OTC-containing intercellular substance with autofluorescent osteocytes.

The border between the fluorescent part of the perichondral lamella, on the one hand, and the growth plate and the metaphysis, on the other, was regular and even, while its border to the covering periosteal cells in the ossification groove was irregularly indented by the cellular elements.

In the diaphyseal and mostly autofluorescent part there were areas with yellow fluorescence corresponding to the areas undergoing lacunar resorption. There were often small deposits of OTC in the superficial region of the diaphyseal part of the perichondral lamella, just underneath the periosteum.

The length of the fluorescent part of the perichondral lamella (Table 1) varied widely from one growth region to another, from about 90 $\mu$ in the slowest growing region in the proximal radius to about 370 $\mu$

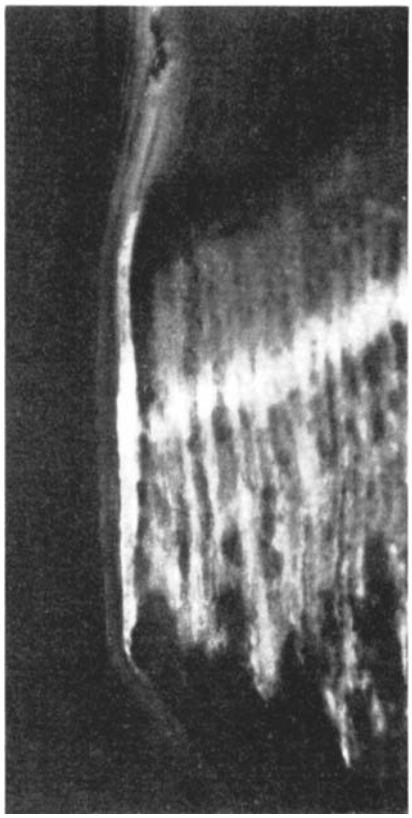

*Figure 4. Deposition of OTC in perichondral lamella in distal radius of 30-day-old rabbit 1 day after intravenous injection of 10 mg OTC per kg body weight. Fluorescence microphotography (60 ×).*

in the fastest growing region in the proximal tibia. The tip of the fluorescent lamella in the proximal tibia and proximal and distal radius extended in epiphyseal direction to the level of the zone of hypertrophic cells of the growth plate.

During the first hours after the injection of OTC the length of the fluorescent part of the lamella increased somewhat. The demonstrable deposition of OTC ceased within 12 hours.

The diaphyseal autofluorescent part gradually became shorter and was finally resorbed some time between the first and fourth day in the proximal tibia and distal radius and between the fourth and eighth day in the proximal radius. At the same time the autofluorescent part epiphyseally to the OTC-containing part increased in length.

The fluorescent part of the lamella thus shifted in diaphyseal direction (Figure 4). This occurred at the same rate as the migration of

the fluorescent band in the metaphysis, which appears in association with the endochondral calcification process within the growth plate.

The fluorescent part in the proximal tibia as well as in the distal radius was usually completely resorbed within 4 days after the injection of OTC, and within 8 days in the proximal radius.

## DISCUSSION

The OTC-containing and yellow-fluorescent region in the perichondral lamella corresponds to the area that is undergoing active calcification at the time of the injection and the subsequent period as long as there is OTC in the circulation. The rate of growth in the growth region influences the length of the fluorescent lamella, with the result that the lamella becomes much longer in the proximal tibia and distal radius, where the rate of growth is high, than in the proximal radius, where the rate of growth is much lower (Hansson 1967). Thus the length of the fluorescent lamella varies in a way similar to the endochondral calcification process (Hansson 1967).

In an investigation Solomon (1966) found that the perichondral lamella within the distal femur extended further in epiphyseal direction in quickly growing infants than in slowly growing older children. In addition Lacroix (1951) and Solomon (1966) found that the perichondral lamella extended in epiphyseal direction at the level of the zone of hypertrophic cells within the growth plate. Similar findings were shown in this investigation. Thus in a growth region with a high rate of growth the perichondral lamella extended further in epiphyseal direction than in a region with a low rate of growth, but the perichondral lamella extended to the level of the zone of hypertrophic cells of the growth plate regardless of the growth rate of the region. The zone of perichondral calcification extended further in metaphyseal direction in a growth region with a high rate of growth than in a growth region with a low rate of growth. The perichondral calcification process thus varies in a way similar to the endochondral calcification process (Hansson 1967). This relation between the sites of the endochondral and the perichondral calcification process must result in good stability of the growth region and protection of the fragile metaphyseal part of the growth plate adjacent to the line of erosion (Salter & Harris 1963, Morscher et al. 1965), and thereby counteract epiphyseolysis.

The faint green yellow fluorescence often observed in the epiphyseal

part of the perichondral lamella (Figure 4), despite an elapse of several days after the injection of OTC, probably corresponds to post-vitally deposited OTC (Hansson 1967). It might also be due to part of the OTC released in connection with resorption being redeposited (Lacroix 1962).

The fluorescent part of the perichondral lamella appeared to have shifted in diaphyseal direction with time. This shift, however, was not true but only apparent, and was due to the continuous appositional growth of the perichondral lamella in epiphyseal direction from the ossification groove. This growth within the epiphyseal part proceeded at the same rate as resorption of the diaphyseal part and at the same rate as the endochondral growth from the growth plate. This rate of growth of the perichondral lamella varied from one growth region to another in the same way as the rate of endochondral growth (Hansson 1967).

## SUMMARY

The deposition of oxytetracycline in connection with the perichondral ossification process in young rabbits was examined with the aid of the fluorescence microscopy.

It was found that during the first hours after injection the fluorophore was deposited in the most epiphyseal part of the perichondral lamella in the ossification groove. During the following days the fluorescent part was displaced in diaphyseal direction at roughly the same rate as the fluorescent band appearing in association with the endochondral calcification owing to the longitudinal growth process. The fluorescent part of the perichondral lamella was resorbed some days after the injection varying from one growth region to another.

The relationship between the perichondral growth from the ossification groove and the endochondral growth from the growth plate is discussed. It is proposed that the perichondral lamella has a stabilising function on the growth region.

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