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OXYTETRACYCLINE BONE LABELLING OF EXPERIMENTAL AFFECTIONS OF THE HIP JOINT

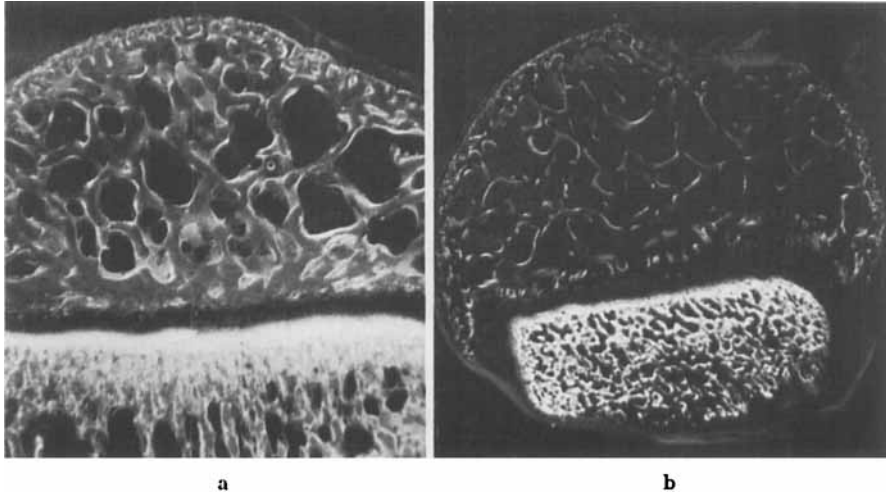
By

P. ROKKANEN, P. SLÄTIS and H. LAINE

Milch, Rall & Tobie's (1958) observations on the affinity of tetracycline for bone, and *Frost, Villaneuva & Roth's* (1960) investigations on the use of tetracycline in bone physiology constitute the basis for a new research method by means of which the formation of new bone can be followed. The tetracyclines produce a fluorescence observable both macroscopically and microscopically under ultraviolet light and demonstrable as much as six years after tetracycline administration (*Frost 1963*). Since tetracyclines in the bone tissue follow ionized calcium (*Finerman & Milch 1963*), they may, to some extent, supersede the radioactive isotopes. By administering tetracycline at certain intervals, the growth of the long bones during the corresponding period can be quantitatively determined (*Vanderhoeft, Kelly & Petersen 1962*, and *Frost 1963*).

Using tetracycline, *Woodhouse* (1962) demonstrated that an avascularized femoral head did not fluoresce. *Milch* (1963) showed that, as a result of osteoarthritis, the bone tissue became unevenly labelled with tetracyclines. In agreement with these observation, it could be demonstrated in a recently published study that necrotic bone does not fluoresce and that in regenerating bone areas, such as osteophytes, oxytetracycline produces strong fluorescence (*Rokkanen, Slätis & Laine 1963*).

In the following, our experience of this fluorescence technique in rabbits on which surgical interference with the femoral neck had been



Figs. 1 a and b.

- a. Fluorescence micrograph of a normal femoral head ground to $100\ \mu$ thickness according to Frost's method. The OTC—fluorescence is evenly distributed in the epiphysis and heavily deposited in the metaphysis. $\times 30$.
- b. Fluorescence micrograph of a normal femoral head. The specimen was embedded in methylmetachrylate and ground to $60\ \mu$ thickness. $\times 30$.

carried out will be related. The aim was to compare the fluorescence pattern of the femoral head with the histological and radiological findings.

MATERIAL AND METHODS

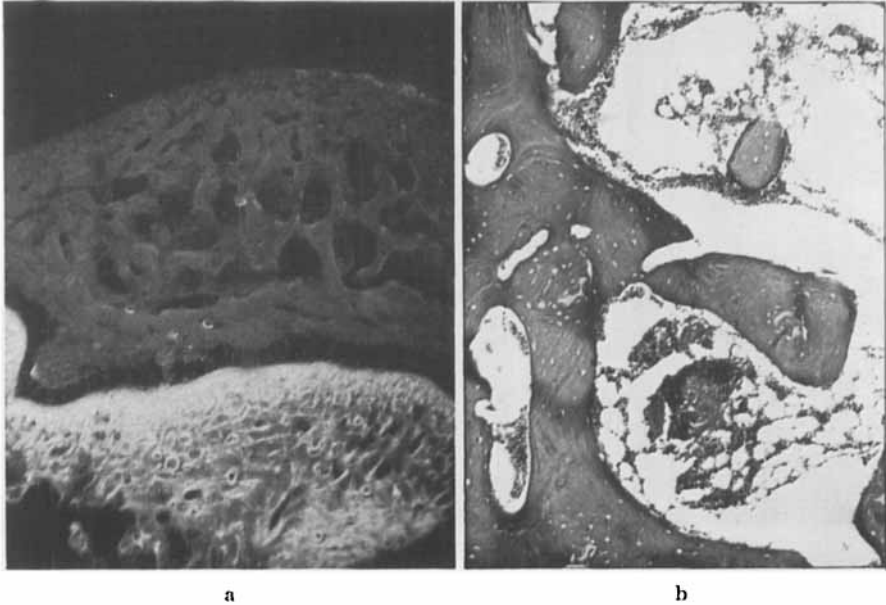
The series comprises 39 white rabbits of varying ages and of both sexes, on the left hip of which the following measures had been carried out, while the right hip acted as a control:

- 1) On 15 rabbits the neck of the femur was tightly ligated with steel wire. The follow-up time for 8 young (8 week old) rabbits was from 1 to 28 weeks and for 7 adult (12 month old) rabbits 22 months.

- 2) On 24 young (8 week old) rabbits total osteotomy of the femoral neck was carried out. In 12 of these animals the ligamentum teres was simultaneously divided. The animals were killed 1 to 20 weeks after the osteotomy.

Oxytetracycline labelling. Three days prior to sacrifice, each animal was injected with 50 mg oxytetracycline (OTC)¹ per kilogram of body weight. The animals were killed with Intraval®, the hip joint was exarticulated and the head of the femur was sectioned in the frontal plane.

¹ Terramycin® intramuscular Pfizer.



Figs. 2 a and b.

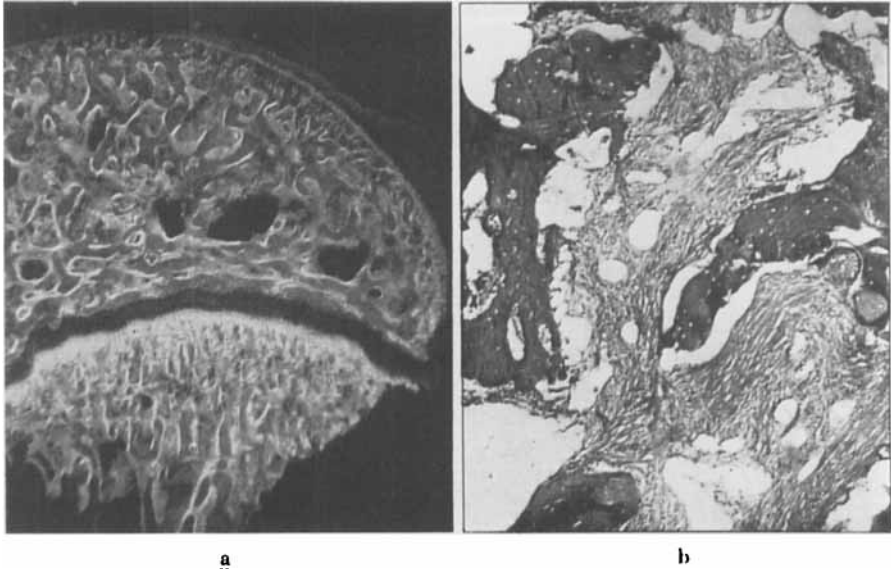
- a. Fluorescence micrograph 3 weeks after exarticulation, tight ligation of the femoral neck and severance of the lig. teres. Oxytetracyclinefluorescence is seen in the femoral metaphysis but is lacking in the femoral epiphysis above the epiphyseal cartilage. $\times 30$.
- b. Micrograph of the central area of the same epiphysis shows predominantly nucleated bone areas. The trabecular structure is normal. Loss of nuclei in some osteons and the scanty quantity of marrow tissue in the cancellous spaces give some evidence of nutritional disturbances of the bone. Histologically the femoral epiphysis is partially necrotic. $\times 100$.

Histological examination. After EDTA decalcification histological preparations of all samples were made, using Weigert-van Gieson haematoxylin and periodic-acid-Schiff (PAS) staining.

Radiological examination. The specimens were radiographed immediately after the animals had been killed. On the adult animals a radiographic examination was also made while they were still alive.

Preparations of the undecalcified bone sections were made in two different ways. Following the procedure of Frost (1958), slices of bone were ground with waterproof adhesive carborundum abrasive paper to a thickness of ca. 100μ (Fig. 1 a). From other specimens taken at the same time 60 to 80μ thick methylmetachrylate slices were made (Fig. 1 b). These were examined microscopically under ultraviolet light and were photographed.

In ultraviolet microscopy an HBO 220 (Osram) UV lamp and a BG-12/6 mm primary filter emitting 3660 Angström UV rays were used.



Figs. 3 a and b.

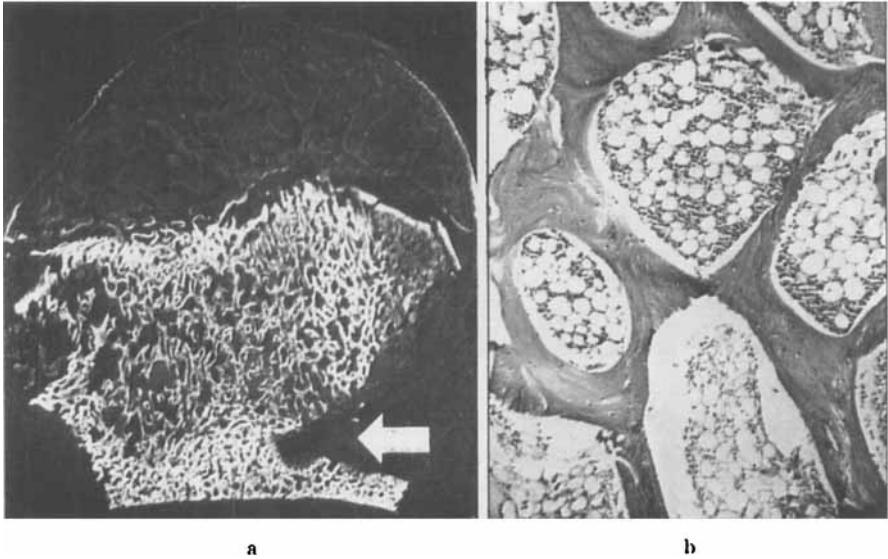
- a. Fluorescence micrograph 8 weeks after exarticulation, tight ligation of the femoral neck and severance of the lig. teres shows derangement of the trabecular structure, especially in the central parts, combined with unevenly distributed fluorescence. $\times 30$.
- b. Micrograph of the central part of the same femoral head shows regenerative changes among the dead trabeculae. The cancellous spaces are filled with granulation tissue invading the femoral head from the metaphysis. $\times 100$.

RESULTS

Tight Ligation of the Neck of the Femur

1 to 3 weeks after the operations, both nucleated and non-nucleated areas of cancellous bone were seen in the epiphysis of all the young animals, being an indication of degenerative changes. It could not, however, be concluded on histological grounds whether the epiphysis was wholly necrotic. During this period no noteworthy radiological changes were observed. Fluorescence micrographs showed OTC uptake on the operated side in the femoral neck but not at all in the epiphysis (Fig. 2a, b).

6 to 28 weeks after the operation strong regeneration was observed histologically among the dead trabeculae in the epiphysis. Radiologically, a flattening of the femoral head was seen, while there were no changes in density. Fluorescence micrographs showed a more marked



Figs. 4 a and b.

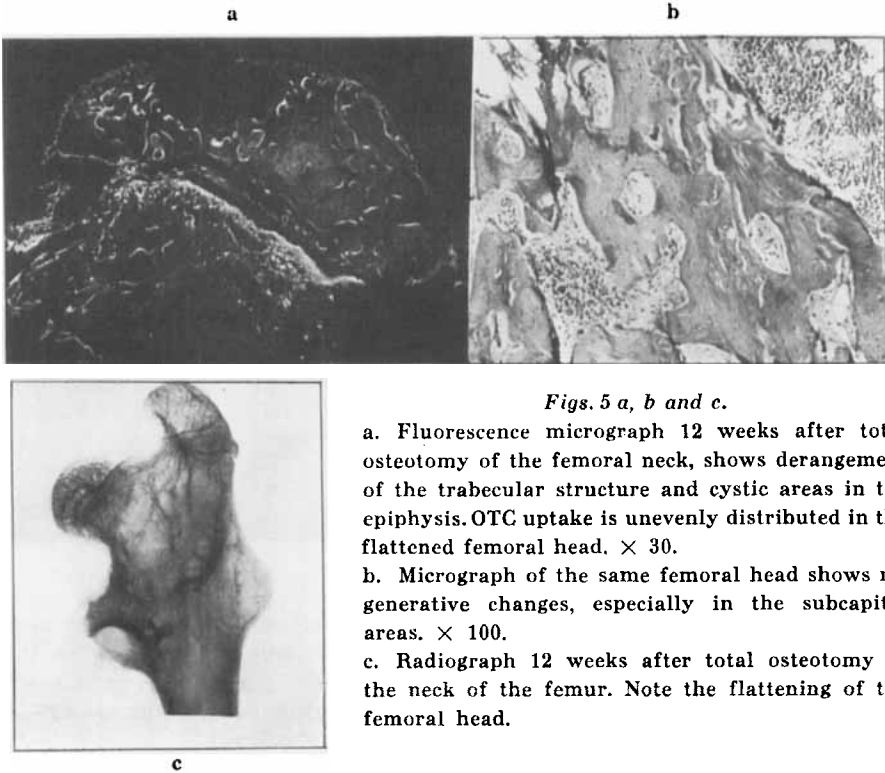
- a. Fluorescence micrograph 3 weeks after total osteotomy of the femoral neck shows an entirely non-fluorescent epiphysis and strong fluorescence in the metaphysis and the femoral neck. The line at the osteotomy site is seen (arrow). Note the fluorescence in the area between the osteotomy and the epiphyseal cartilage. $\times 30$.
- b. Micrograph of the same femoral head shows both nucleated and non-nucleated bone. Histologically not decisive necrosis. $\times 100$.

OTC uptake on the operated side. Intensely fluorescent areas were often seen (Fig. 3a, b).

22 months after ligation of the femoral neck in adult animals, histologically and radiologically demonstrable regenerative changes were observed in one out of seven animals only. In all there was more marked fluorescence in the femoral head on the operated than on the intact side.

Total Osteotomy of the Neck of the Femur

1 to 3 weeks after osteotomy of the femoral neck, histological examination revealed that the epiphyseal cartilage in young animals was completely without nuclei. In the epiphysis the cancellous bone had preserved its structure, but here and there non-nucleated bone areas were observed. No changes could be demonstrated radiographically. Fluorescence micrographs showed an entirely non-fluorescent epi-



Figs. 5 a, b and c.

a. Fluorescence micrograph 12 weeks after total osteotomy of the femoral neck, shows derangement of the trabecular structure and cystic areas in the epiphysis. OTC uptake is unevenly distributed in the flattened femoral head. $\times 30$.

b. Micrograph of the same femoral head shows regenerative changes, especially in the subcapital areas. $\times 100$.

c. Radiograph 12 weeks after total osteotomy of the neck of the femur. Note the flattening of the femoral head.

physis in five out of six animals. In one animal clear fluorescence was seen in the medial border of the epiphysis but in no specimen was it possible to demonstrate uptake in the area of attachment of the ligamentum teres (Fig. 4a, b).

After 6 to 20 weeks there were histologically demonstrable regenerative changes, especially in the area of the neck. The epiphyseal cartilage often fused more quickly on the operated than on the intact side. Degenerative changes were often seen near the epiphyseal cartilage, and less frequently in the area of the neck. In five out of eight animals the head of the femur was radiologically flattened, while there was no clear change in density (Fig. 5c). The fluorescence was regularly more intense on the operated than on the healthy side. Fluorescence of varying intensity and frequently in a patchy pattern, appeared in the area of the epiphysis in all animals 6 weeks or more after the osteotomy.

DISCUSSION

In the present investigation the blood circulation in the area of the femoral head was impaired by ligation or complete osteotomy of the femoral neck, combined in most animals with severance of the lig. teres. These procedures resulted in nutritive disturbances in the femoral head histologically demonstrable as anuclear areas of bone and later as invasion of granulation tissue in the cancellous spaces. The structural derangement of the femoral head was also evident from radiological flattening of the femoral head.

Comparison of the histological and radiological findings with the fluorescence pattern induced by OTC labelling revealed that bone rendered avascular by surgical interference was non-fluorescent. The lack of fluorescence was most evident in the early stages and the borderlines with living, fluorescent bone easily recognized. OTC fluorescence recurred, however, at a later stage and corresponded to the histologically visible regeneration of the necrotic area.

Milch (1963) states that tetracycline labelling is a specific and suitable method for investigating the blood supply of the bone. Correspondingly, the lack of fluorescence in bone from which the blood supply is cut off seems to provide a simple and accurate method of early recognition of ischaemic disturbances of the bone.

SUMMARY

In 39 rabbits the degenerative and regenerative changes in the femoral head after various procedures directed at the neck of the femur has been investigated. Oxytetracycline labelling of the bone and histological and radiological methods were used in parallel in the investigation. Particular attention was attached to a comparison of the fluorescence phenomenon and the histological picture.

Avascularized bone in the femoral head and neck showed no fluorescence and was early distinguishable from the surrounding living bone. Histologically, the degenerative changes in the corresponding area did not become evident until 2-3 weeks after the interference.

Absence of fluorescence preceded the histologically demonstrable necrotic changes.

Regenerative changes were clearly noticeable with both histological and fluorescence techniques. Intense fluorescence followed upon for-

mation of new bone. For many months after the interference the operated femoral head had a more intensive OTC fluorescence than the intact side.

RESUME

Chez 39 lapins, les modifications dégénératives et régénératives de la tête fémorale après différents procédés appliqués au col du fémur ont été examinés. Un label de l'os à l'oxytétracycline et des méthodes histologiques et radiologiques ont été utilisées parallèlement dans cette enquête. Il a été attaché une attention particulière à la comparaison du phénomène de la fluorescence dans le tableau histologique.

Un os avascularisé de la tête et du col fémoral ne présente pas de fluorescence et s'est montré très tôt discernable de l'os vivant environnant. Au point de vue histologique, les modifications dégénératives de la surface correspondante n'ont été apparentes que 2 à 3 semaines après l'intervention.

L'absence de fluorescence précède les altérations nécrotiques histologiquement décelables.

Les modifications régénératives ont été clairement observables aussi bien avec la technique histologique qu'avec celle de la fluorescence. Une fluorescence intense suit la nouvelle formation osseuse. Durant de nombreux mois après l'intervention, la tête fémorale opérée présentait une fluorescence OTC plus intense que le côté intact.

ZUSAMMENFASSUNG

Bei 39 Kaninchen wurden die degenerativen und regenerativen Veränderungen im Femurkopfe nach verschiedenen Eingriffen am Femurhalse untersucht. Oxytetracyclinmarkierung des Knochens, sowie histologische und röntgenologische Methoden wurden bei der Untersuchung parallel miteinander verwendet. Besondere Aufmerksamkeit wurde dem Vergleich des Fluoreszenzphänomens mit dem histologischen Bilde zugewendet.

Gefäßloser Knochen im Femurkopf und -hals zeigte keine Fluoreszenz und konnte frühzeitig vom umgebenden, lebenden Knochen unterschieden werden. Histologisch wurden die degenerativen Veränderungen in einem entsprechenden Gebiete nicht vor der 2.-3. Woche dem Eingriff sichtbar.

Das Fehlen der Fluoreszenz ging den histologisch nachweisbaren nekrotischen Veränderungen voraus.

Regenerative Veränderungen waren mit histologischer und Fluoreszenztechnik deutlich wahrnehmbar. Intensive Fluoreszenz folgte der Bildung neuen Knochens. Während vieler Monate nach dem Eingriff zeigte der operierte Femurkopf eine intensivere OTC Fluoreszenz als die intakte Seite.

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