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EFFECTS OF FLUORIDES, CORTICO-STEROIDS AND TETRACYCLINES ON EXTRACTION WOUND HEALING IN RATS

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The effects of locally applied fluorides, corticosteroids and tetracyclines upon the healing of extraction wounds were studied in 77 albino rats. It was found that the fluorides definitely inhibit bone resorption; the cortico-steroids markedly increase osteoclasis and bone resorptions; while the tetracyclines stimulate osteogenesis and bone deposition. These actions were limited from 3 to 10 days after these solutions were incorporated into the blood clot. These findings suggest further studies to explore the application of fluorides and tetracyclines to clinical problems such as tooth replants, tooth transplants and in extraction wound healing.

Previous studies of tooth replantation showed that immersion of freshly extracted molars of rats in fluoride solutions, cortico-steroids and tetracyclines prior to replantation greatly affected root resorption and bone formation (*Bjorvatn & Massler*, 1971; *Bjorvatn & Weiss*, 1971). These studies showed a definite inhibition of root resorptions after brief immersion in sodium and stannous fluoride solutions. In contrast, immersion of the extracted teeth in cortico-steroid solutions greatly increased the amount and rate of root resorptions. Immersion in tetracycline solutions caused a marked stimulation of alveolar bone formation while the root resorption pattern remained unaltered so that ankylosis resulted after this treatment. The present study was designed to verify the effects of these compounds upon osteogenesis and the resorptive process using a different model system — the healing of molar extraction wounds in rats.

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MATERIAL AND METHODS

Albino rats were used for this study because of availability of specimens in larger numbers and because previous studies on the effects of fluorides, cortisone and tetracyclines on replanted molars had used the same species as a model.

One hundred and thirty-seven extraction wound specimens were obtained from 77 rats. The upper first right and left molars were extracted and the sockets treated. The lower molars could not be used in this study because the extraction site was immediately covered by action of the lateral ligament which drew the gingival tissue over the extraction site, so that implantation of the solutions became difficult and inconsistent.

Four groups of 9 different compounds were evaluated: three tetracyclines, 2 cortico-steroids, 3 fluoride solutions and a penicillin-streptomycin solution (Table 1). Controls were treated with saline or not treated at all. Fluoride or tetracycline solution was instilled into one socket and cortico-steroid solution or saline was incorporated into the blood clot of the homolateral socket. Care was taken to avoid cross-contamination.

The animals were anesthetized with ether only and operated on a small table using the same technique and instruments previously described for the molar replantation study (*Bjorvatn & Massler*, 1971). The left upper first molar was always extracted first. One half-minute after the extraction, when bleeding had diminished but before clotting could begin, 0.3 ml of the test solution was slowly and gently agitated into the forming clot over a period of one full minute. If one waited too long when the clot was fully formed (after approximately 3 to 5 minutes), the clot was usually displaced and lost with the test solution. The time from extraction to completion of treatment was approximately 2 minutes. A different 1.0 ml disposable tuberculin syringe and needle were used for each solution to avoid cross-contamination. Also, the animal was allowed to recover after the first treatment and move about for about 5 minutes before being anesthetized with ether again. The upper right molar was then extracted and the socket treated with the next test solution.

All animals were between 40 to 90 days of age, weighing between 120 to 345 grams. All gained weight normally following the extractions. All animals were on a normal diet of fluoride free Tec-Lad pellets and fluoride-free distilled water.

Animals were sacrificed at 24 hours, 2 days, 3 days, 5 days, 7 days, 10 days, 14 days and 21 days. These intervals were selected because normal healing of molar extraction wounds in rats follows the time schedule below according

Table I.

Number and distribution of specimens

Controls		= 18
No treatment	= 12	
Saline treated	= 6	
Fluoride Treated Sockets		= 40
NaF 2 %	= 11	
SnF_2 1 %	= 13	
SnF_2 10 %	= 16	
Cortico-Steroid Treated Sockets		= 32
Cortisone (I.M.) 50 mg	= 16	
Prednisolone Sodium Succinate 50 mg (I.V.)	= 16	
Tetracycline Treated Sockets		= 40
Oxytetracycline HCl (I.M.) 250 mg	= 12	
Oxytetracycline HCl (Oral) 250 mg	= 12	
Chlortetracycline 250 mg	= 16	
Penicillin-Streptomycin (Procaine Penicillin G, 2000,000 units & Dihydrostreptomycin 0.250 grams)		= 7
Total		137

to *Boyne* (1966); *Pietrokovski* and *Massler* (1967); *Todo* (1968); *Aarstrand* and *Carlson* (1969); and *Johansen* (1970):

2nd day — wound covered with a layer of immature epithelial cells
3rd day — total organization of the clot
7th day — epithelial covering complete and mature bone formation in fundus of socket complete
11th day — socket completely filled with new remodelling bone
22nd day — remodelling of alveolar ridge complete with dense cortical bone formation within the former socket.

The animals were sacrificed by ether overdose, decapitated, skinned and hemisected. The maxillae were then removed and fixed in 10 % buffered formalin; decalcified in formic acid-sodium citrate solution, washed, double embedded in thin celloidin and paraffin and sectioned searially at 7 microns. Specimens were cut in mesio-distal as well as bucco-lingual directions and stained routinely with H. and E. Special stains were used only occasionally.

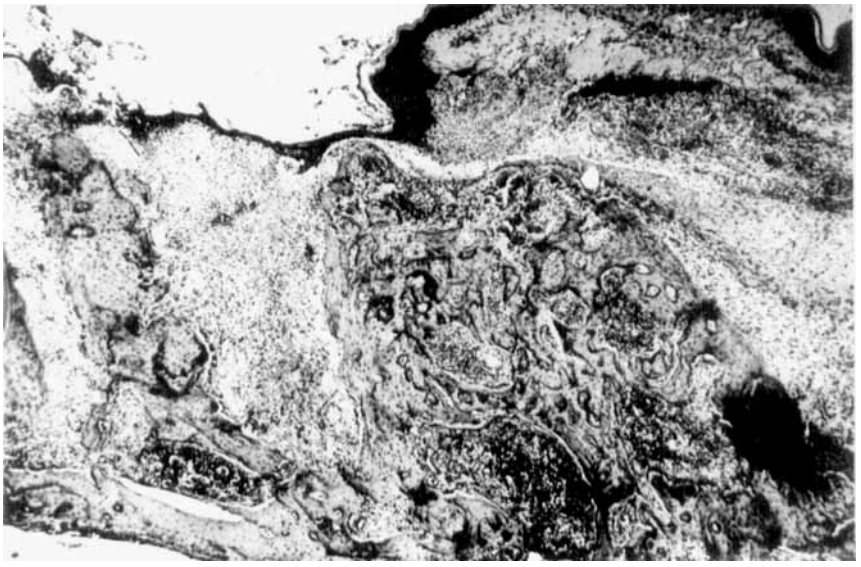

Figure 1. Control specimen 5 days after extraction of the upper first molar. Note that the epithelial ingrowth from the edges completely covers the wound. Trabecular bone formation has begun at the center of the socket, proceeding peripherally towards the fundus and the rim of the socket as well as into the adjacent marrow spaces. The total picture is that of rapid remodelling with filling in the socket. The connective tissue in the oral portion of the socket contains many engorged vessels and many more active connective tissue cells than in the fundic portion. Bone formation and remodelling at the oral aspect of the socket is also more rapid than at the fundic side. Note that a few of the adjacent marrow spaces are filled with hemopoetic tissue instead of the original reticular marrow. (M-D section, H. and E., 50 ×).

RESULTS

The untreated and saline treated extraction wound sockets showed a normal healing pattern and schedule very similar to that described by *Aarstrand* and *Carlson* (1969); and by *Boyne* (1966) (Fig. 1). The wound surface was covered by ingrowth of immature epithelial cells from the periphery, by the 2nd or 3rd day after extraction. By the 7th day, this epithelial covering was complete and consisted of a thick keratinized layer with mature cells and rete pegs. The walls of the fundic portion of the sockets began to fill in with trabecular bone at the third day and the entire socket was completely filled with spongy bone by the 7th to 10th day.

Remodelling bone resorptions and apposition were evident along the walls of the entire socket by the third day and were seen throughout the trabeculae filling the socket by the 7th day, after which more apposition than

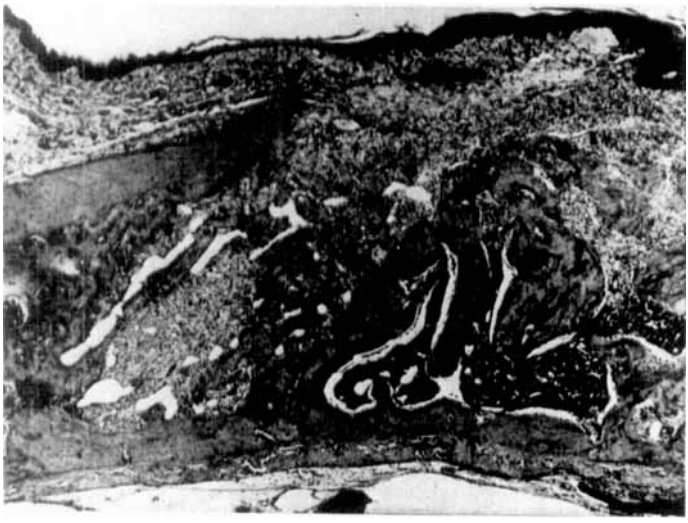

Figure 2. Extraction socket treated by incorporating 0.3 ml of NaF 2 % water solution into the blood clot. Five days later bone formation in the center of the socket is similar to the control with higher activity towards the rim and lesser activity towards the fundus. The major difference is the absence of osteoclasts and resorption bays along the walls of the socket near the rim. However, osteoclastic activity outside the socket area and within the adjacent marrow spaces is normal. (M-D section, H. and E., 50×).

resorption occurred with increased density and compacta of the intra-alveolar bone.

The effects of the test solutions within the socket were seen clearly by the 5th day and were prominently evident at the 7th day. By the 14th day, the effects of the test solutions began to fade, so that by 21 days no differences were evident between the treated sockets and the controls. The healing of the epithelium, the subjacent connective tissue, and the bone outside the socket area were not affected by the test solutions except by the stronger 10 % stannous fluoride solution.

Effects of fluorides. The fluoride solutions significantly inhibited the resorption process along the walls of the socket by the 5th day (Fig. 2). The resorption remodelling of the trabecular bone within the socket was much less affected, since these began by the 10th day, after the effect of the fluoride had diminished.

Bone formation appeared unaffected by the fluorides. The trabecular pattern and amount of bone deposition seemed quite normal and similar to the controls.

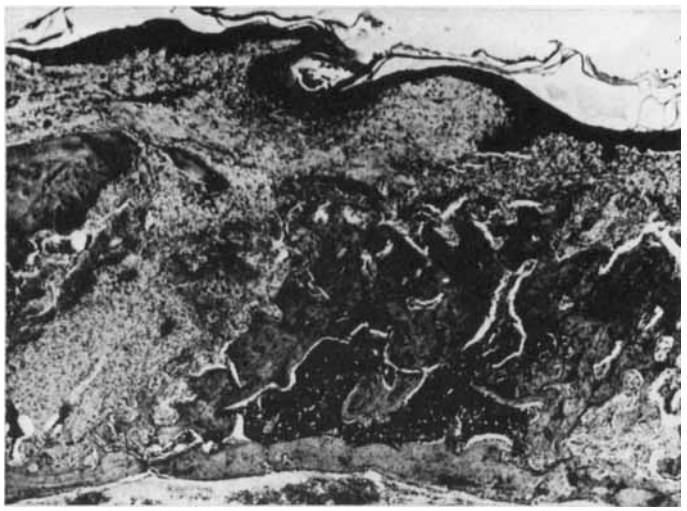

Figure 3. Extraction wound treated with SnF_2 10 % solution. Five days later, osteoclastic action within the socket is almost entirely absent. Bone apposition is slightly delayed. The connective tissue between the rim of the socket and the epithelium is degenerated as compared to the adjacent connective tissue on either side of the socket. Remodelling of the adjacent bone and marrow spaces is normal. Note the hemopoetic tissue within one of the adjacent marrow spaces. (M-D section, H. and E., 50×).

Sodium fluoride 2 % and stannous fluoride 1 % showed similar results upon bone resorptions, and no toxic effects were evident in terms of cellular morphology or bone structure. However, the 10 % stannous fluoride solution showed a decided toxic effect upon the connective tissue in addition to marked inhibition of resorption. The connective tissue at the entrance to the socket just below the epithelial covering, showed hyalization of the collagen fibers, shrinkage of connective tissue cells (fibrocytes), and diminished numbers of fibroblasts (Fig. 3). Round cell infiltration was quite prominent, but hyperemia was minimal or absent. The histologic picture suggested toxic rather than merely irritant action by the 10 % stannous fluoride solution. It was interesting to note that 10 % SnF_2 showed very little effects upon the contents of the socket, as if this toxic material had been pushed towards the surface of the endosseous granulating tissue. By the 10th day, the connective tissue immediately over the socket was normal, with only residual toxic effects evident in the connective tissue immediately under the epithelium (Fig. 3).

Effects of cortico-steroids. The local application of cortico-steroids produced a dramatic increase in the resorptive process with the appearance of many osteoclasts within the socket and adjacent bone. Large numbers of resorption bays were evident along the walls of the socket by the 3rd day, reaching a peak by the 5th to 7th day (Fig. 4A). The effects diminished from the 10th day and were no longer evident by the 14th day. Giant cells of the osteoclastic type appeared in enourmous numbers in the young connective tissue within the healing socket by the 3rd day, in addition to the large numbers of osteoclasts within resorption bays in the bone lining the socket and in the bone adjacent to the socket (Fig. 4 B).

Filling in of the socket with trabecular bone was significantly delayed, new bone formation in the fundic portion of the socket being delayed until after the 10th day (Fig. 4 B).

There were no effects evident upon the epithelium and subepithelial connective tissue.

Effects of tetracyclines. The tetracyclines consistently and prominently accelerated the bone deposition within the socket without depressing the osteoclastic activity. At the 3rd day, one-third of the socket treated with tetracycline was already filled with young boney trabeculae in contrast to the controls in which bone deposition was only beginning. New bone formation began in the middle of the socket and in the fundus (Fig. 5A). The accelerated rate of bone deposition reached a peak by the 5th day, so that the entire socket was filled with bone, in contrast to the controls in which only one-third of the socket was filled. In addition, bone maturation and remodelling was definitely farther accelerated in the tetracycline treated specimens; the trabeculae being thicker, more dense, and with fewer but larger marrow spaces than in the controls. This was evident by the 10th day and very prominent by the 14th day (Fig. 5B).

The entire osteogenic process, remodelling resorption as well as apposition, appeared to be very exuberant in the tetracycline treated specimens. It was quite clear that the tetracyclines acted as a stimulant to new bone formation under the conditions of these experiments.

Effects of penicillin-streptomycin. Treatment of the blood clot with a penicillin-streptomycin solution did not produce any observable effects upon the healing of the extraction wound. At times, there appeared to be a slight increase in the number of foreign body giant cells within the endosseous reparative tissue. There were no significant changes in the vascular pattern

Figure 4A. Extraction socket treated with a cortico-steroid solution (Cortisone-Upjohn). At 5 to 7 days there is a marked increase in the number of osteoclasts within resorption bays throughout the bony walls and trabeculae within the socket. Giant cells are widespread within the connective tissue of the healing socket. Bone filling into the socket is delayed because new bone spicules are quickly resorbed. (M-D section, H. and E., 170×).

Figure 4B. Seven days after extraction many giant cells appear in the connective tissue of the socket treated with cortico-steroids (Prednisolone). These cells are no longer visible at 10 days so that socket healing is equivalent to that of the controls by day 14. (M-D section, H. and E., 170×).

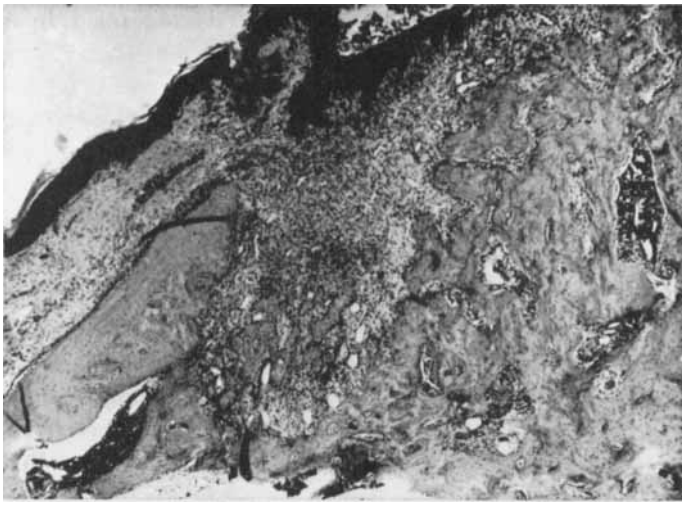

Figure 5A. Extraction socket 3 days after treatment with a tetracycline (oxytetracycline IM). Bone is already filling in the fundic portion of the socket in contrast to the controls at 3 days which showed only organization of the clot and no bone formation. (M-D section, H. and E., 50×).

Figure 5B. At 5 days after treatment with tetracycline (oxytetracycline HCL^{IM}) the socket is half filled with thick and dense bone trabeculae. Note remodelling at the lip and rim of the socket. (B-L section, 50×).

or the type or amount of endosseous connective tissue formed. In summary, any effects were minor or absent.

DISCUSSION

Effects of extraction upon adjacent marrow tissue. The tooth extraction affected also the adjacent larger marrow spaces. Blood infiltrated from the socket into the adjacent marrow spaces (Fig. 1). By the 5th day, when blood clot organization was completed and bone formation was begun within the socket, hemopoetic tissue appeared in the otherwise resting large marrow spaces adjacent to the socket (Figs. 4 and 5). The hemopoetic tissue persisted until approximately the 10th day as the remodelling process proceeded, and was no longer visible by the 21st day when remodelling of the alveolar ridge was completed.

Fluorides. The results of this study substantiate previous studies indicating the depressing effect of fluorides upon bone and root resorptions. The effect of systemic fluorides in inhibiting osteoporotic processes is well known (*Hudson,* 1961; *Epker,* 1966; *Baylink & Bernstein,* 1967). This implies an inhibition of osteoclastic action. *Goldhaber* (1967) showed a distinct inhibition of osteoclasis by fluorides in tissue culture. *Shulman, Kalis* and *Goldhaber* (1968) demonstrated inhibition of root resorption by fluoride treatment of replanted teeth in monkeys — a finding which was confirmed and extended in rats by *Bjorvatn* and *Massler* (1971). *Bernick* and *Zipkin* (1967) showed by histochemical means that systemic fluorides improved the crystal texture of the apatite in bone and reduced its chemical reactivity in rats.

Cortico-steroids. The finding of markedly increased numbers of osteoclasts and resorptions after topical application of cortico-steroids was unexpected. Whether this action was the result of the very high concentrations achieved by the topical application and whether the action could result also from systemic cortico-steroids which produce much lower tissue concentrations, remains to be seen (*Simmons & Kunin,* 1967). One might review the pattern and degree of root and alveolar bone resorptions in patients receiving therapeutic doses of cortico-steroids to test this hypothesis. Or, systemic cortico-steroids could be given to rats during experimentally induced tooth movements to see whether systemic cortico-steroids increase the amount of root and alveolar bone resorptions (*Singer, Furstman & Bernick,* 1967).

Tetracyclines. This study showed that high concentrations of locally applied tetracyclines might have an osteogenic action, in addition to its known antibiotic action. The fact that the tetracyclines are bone-seeking substances and therefore useful in vital labelling of newly forming and calcifying bone is consistent with a concomitant osteogenic stimulating potential. This property might be explored further, since high local concentrations of tetracyclines are possible during surgical procedures. Systemic tetracyclines, up to 75 mg./kilo body weight, apparently do not affect osteogenic activity (*Boyne,* 1968; *Likins, Pakis & McClure,* 1963; *Nordenram & Edelund,* 1968).

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