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CHANGES IN THE ALVEOLAR PROCESS AFTER EXTRACTIONS IN THE WHITE RAT

A HISTOLOGIC AND FLUORESCENCE MICROSCOPIC STUDY

by

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INTRODUCTION

Healing after extraction has been the subject of histologic study in several investigations, most of them in laboratory animals (for review, see *Carlsson, Thilander & Hedegård*, 1967). Interest has been centred primarily on the course of healing in the sockets, while changes in the surrounding bone would seem not to have received close examination. The study by the above authors, however, was concerned with the changes in the labial bony plate in man; it was found that extensive reconstructive processes had taken place as a result of which, 40 days after extraction, practically the whole of the original bony plate had been resorbed and partly replaced by new bone.

The usual histologic technique has recently been supplemented by other methods in the study of bone. In an examination of various agents for *in vivo* staining of bone *Harris* (1960) found that tetracycline was suitable for this purpose. In a comparative study in which *Harris, Jackson & Jowsey* (1962) used fluorescence microscopy after injection of tetracycline, microradiography and autoradiography with 45Ca, it was found that the tetracycline had been taken up at all sites where there had been active deposition of bone. A close agreement between the distributions of tetracycline and45Ca

Received for publication, September 6, 1968.

was found. They considered that the administration of tetracycline followed by fluorescence microscopy is a useful method for examining the process of bone formation and the rate at which it occurs. That tetracycline is incorporated at the site where bone (and dentine) has been formed has been demonstrated also by, among others, *Milch, Rall & Tobie* (1957, 1958), *Bevelander, Nakahara & Rolle* (1960), *Bevelander, Goldberg & Nakahara* (1960), *Bevelander, Rolle & Cohlan* (1961), and *Owen* (1961).

For fluorescence microscopy most authors have used ground sections prepared after imbedding the specimen in methacrylate, but in a study of the mechanism of tetracycline binding *Hammarström* (1967) used a frozen-section technique.

In a study of the possible influence of tetracycline on the increase in weight and bone growth in the rat *Cleall, Perkins & Gilda* (1964) found that these factors were not affected adversely by doses of up to about 70 mg/kg of body weight.

In studies of healing after extraction carried out in the dog and man where administration of tetracycline was followed by fluorescence microscopy *Boyne & Kruger* (1962) and *Boyne* (1966) observed extraalveolar changes such as ossification along trabeculae in neighbouring marrow spaces; it was inferred that the healing process includes a series of complex osseous phenomena involving not only the socket itself but other important anatomic areas as well.

In the present study micromorphologic changes in the jaws occurring after extraction were examined in the white rat by the usual histologic technique and by tetracycline-induced fluorescence; the object was to compare the methods in the study of intra- and extra-alveolar changes in healing after extraction.

MATERIAL AND METHOD

The study was performed on 27 Sprague-Dawley male rats divided into 4 experimental groups of 6, and 3 controls.

After inducing general anaesthesia with 2 per cent mebumal sodium solution, administered intraperitoneally, the first molars in both upper quadrants were extracted. Intraperitoneal injections of tetracycline* — 35 mg/kg of body weight — were given to one rat of each group 2, 4, 7, 11, 16 and 22 days after extraction. The animals were sacrificed as follows:

* Tetradecin (Astra). A solution containing 10 mg/ml was prepared immediately before each injection.

Group A: 2 hours after administration of tetracycline, injected on 2d, 4th, 7th, 11th, 16th and 22d day, respectively, after extraction.

Group B: 24 days after extraction; tetracycline injected (together with Group A) on 2d, 4th, 7th, 11th, 16th and 22d day, respectively, after extraction.

Group C: 6 hours after administration of tetracycline, injected on 2d, 4th, 7th, 11th, 16th and 22d day, respectively, after extraction.

Group D: 24 days after extraction; tetracycline injected (together with Group C) on 2d, 4th, 7th, 11th, 16th and 22d day, respectively, after extraction.

At the time the extractions were performed the rats composing groups A and B were 1/2—1 year old and weighed 325—433 g, and those in groups C and D were about 50 days old and weighed 204—238 g. The experiments were performed first with groups A and B simultaneously and then with groups C and D simultaneously after preliminary inspection of the specimens from the former 2 groups.

After the rats had been sacrificed the alveolar processes in the upper molar segments, together with the adjacent areas of each quadrant, the right lower incisor and the molar segment of the lower left quadrant were removed and placed for 8—10 days in 10 per cent neutral buffered formalin for fixation.

Demineralized and ground sections were prepared from the specimens of both upper quadrants; the choice of quadrant was made by drawing lots. For the histologic examination 5-micron longitudinal sections through teeth and sockets were prepared after demineralization. The sections were stained by the following methods: Bock, van Gieson and Gomori's silver impregnation (*Romeis*, 1948).

From the mandibular specimens and the remaining maxillary specimens 60—90-micron ground sections were prepared as described by *Ericsson* (1965). These were examined by fluorescence microscopy.

From the maxillas of the untreated control rats (about 6 months old) histologic sections were prepared after demineralization; in 2 cases longitudinal sections and in the third transverse sections through the teeth and alveolar processes.

RESULTS

In all the groups there was interference with the healing of the sockets by root fragments, sequestra, foreign bodies, etc. (Fig. 1). The following account summarizes observations in normal healing.

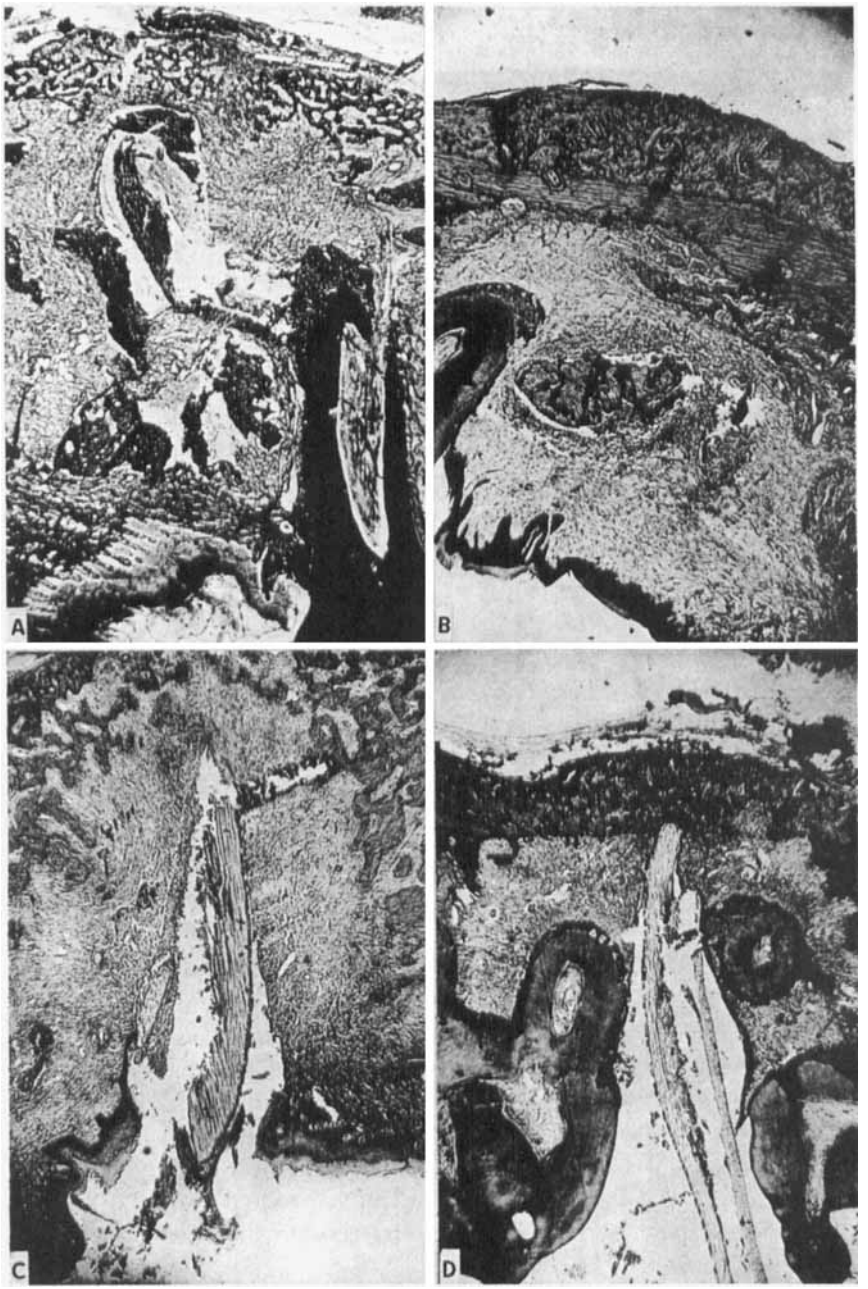

Fig. 1.

A. Specimen taken 7 days after extraction. Interference with healing by root fragments. New bone is seen basally, and the original basal bone layer, which forms the upper limit of the maxillary alveolar process, is resorbed. (*Gomori*) × 75.

B. Specimen taken 24 days after extraction. Interference with healing by root fragments. There is marked deposition of bone basally. (*Bock*). × 75.

C. Interference with healing by foreign bodies impacted into the socket (24 days after extraction) (*van Gieson*). × 75.

D. Basal new bone next to a foreign body impacted into the parodontium between the second and third molar. The original basal bone plate has been partly resorbed (*Bock*). × 75.

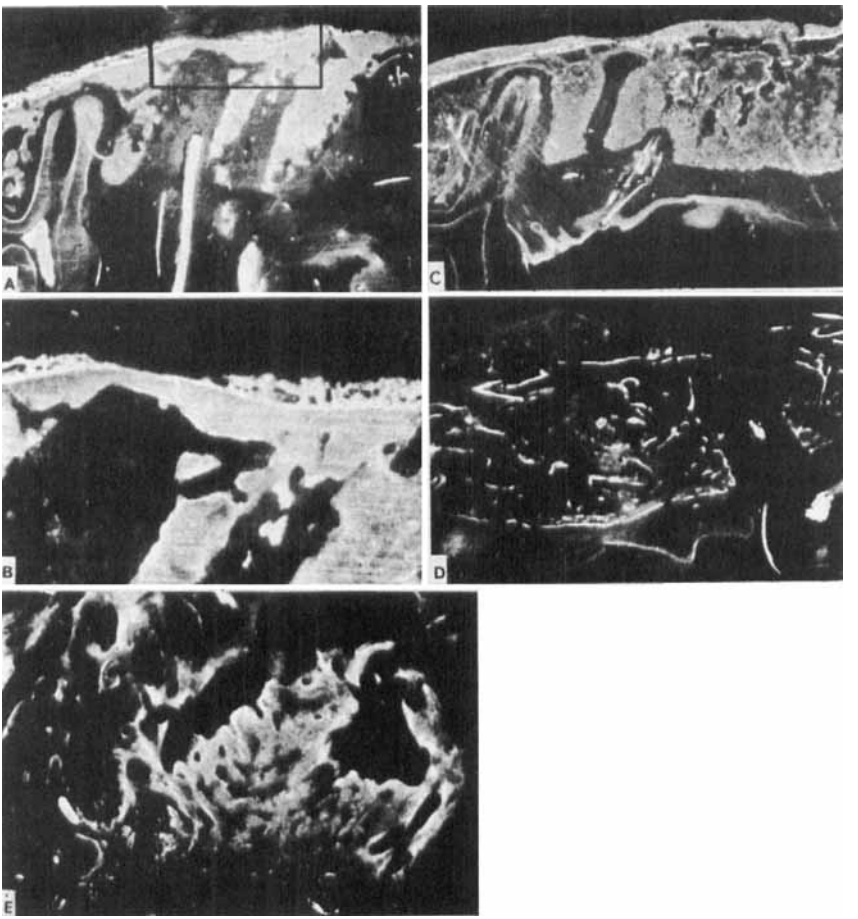

Fig. 2. Ground sections photographed in the fluorescence microscope.

A. Survey of specimen taken 4 days after extraction of the first molar. Tetracycline injected 6 hours before sacrifice. × 25.

B. Detail of framed area in A, showing the superior parts of the 2 distal sockets. Bone formation basally, with evidence of tetracycline activity. × 65.

C. Survey of specimen taken 24 days after extraction of the first molar. Tetracycline injected 4 days after extraction. Foreign body in the distal alveolus has prevented complete healing. Tetracycline activity basally under the first and second molars and in the area between the original sockets, but not within them. × 25.

D. Survey of specimen taken 24 days after extraction of first molar. Tetracycline injected 16 days after extraction. Tetracycline activity observed in the extraction area but also elsewhere in the alveolar process, though not basally. × 25.

E. Detail of specimen taken 11 days after extraction. Injection of tetracycline 2 hours before sacrifice. The figure shows the apical part of the mesial alveolus filled with bone, with marked tetracycline activity. × 60.

Fluorescence microscopy of ground sections Groups A and C (2, 4, 7, 11, 16 *and* 22 *days after extraction*)

Two days after extraction — The tetracycline-induced fluorescence was sparse. It was usually located in the bone around the remaining teeth. No fluorescence was seen in the sockets or along the superior surface of the basal compact bone that delimits the upper alveolar process.

Four days — There was sparse tetracycline-induced fluorescence along the border of the alveoli, but no more than in the bone around the residual teeth. On the superior surface of the basal compact bone there was a layer of spongiosa tissue with marked tetracycline activity (Figs. 2 A and B).

Seven days — Tetracycline activity was recorded in new bone located at the bottom and along the walls of the sockets and in the bone around the remaining teeth. There was a faint tetracycline-induced fluorescence along the bone trabeculae in the spongiosa throughout the alveolar process. In specimens from group A the fluorescence was observed basally under the distal alveoli of the first molar and under the second molar.

Eleven days — The sockets were practically filled with bone (group A), and the bone in the area displayed pronounced tetracycline activity (Fig. 2 E). On the superior aspect of the basal bone layer above the first molar (group C) there was weak tetracycline activity.

Sixteen days — The sockets were almost filled with bone that displayed tetracycline activity (group C). In specimens from group A there was new bone both in the bottom of the sockets and along the walls, and this showed fluorescence induced by tetracycline. The basal compact substance was irregular in structure and thickened, but almost invariably devoid of tetracycline activity.

Twenty-two days — The sockets were not distinguishable. The alveolar process appeared to have diminished in height, and throughout the extraction area there was marked tetracycline-induced fluorescence.

Groups B and D (24 *days after extraction*)

Healing was in general complete; the sockets were filled with bone, and as a rule indistinguishable. The alveolar process was diminished in height compared with the mesial and distal areas. The basal layer of bone was usually thickened and the structure more reminiscent of spongiosa than compact bone beneath the first molar and sometimes the mesial root of the second molar.

The following observations were made on the tetracycline-induced fluorescence.

Injection 2 days after extraction — No tetracycline activity was found in the extraction region.

Injection 4 days after extraction — Tetracycline activity was observed in the area between the sockets and in the bone along the remaining teeth. There was also fluorescence basally — most marked above the extraction area (Fig. 2 C).

Injection 7 days after extraction — In both specimens tetracycline activity was observed in the extraction area. Basally, there was fluorescence in the specimen from group B.

Injection 11, 16 and 22 days, respectively, after extraction — In the area of the sockets there was definite tetracycline activity. In the extreme basal area the activity was generally not so marked as in the specimens from 4 and 7 days after extraction (Fig. 2 D).

Tetracycline activity in corresponding mandibles (group A)

In all the specimens (2—22 days after extraction of the upper first molar) there was tetracycline-induced fluorescence in the alveolar process. The fluorescence was most marked in the bone along the roots, and especially in the bifurcations. In some of the specimens the activity appeared to be stronger at the first molar than at the others.

Histologic examination of demineralized sections

Two days after extraction — Practically the whole of the wound was covered by new epithelium. The content of the sockets consisted of a central blood clot, while peripherally there was organization to granulation tissue and connective tissue (Fig. 3 A). There was osteoclastic activity along the marginal parts of the alveoli (Fig. 4 A). Above the basal bone layer was a dense accumulation of cells of the scleroblastic type along the bone margin opposite the distal socket of the first molar (Fig. 3 B).

Four days — The contents of the sockets had been organized into connective tissue, and in places new bone had formed. (Figs. 3 C, 4 B—D). Outside the sockets there had been a pronounced formation of bone above the basal bone plate, corresponding to the area of the first molar up to the mesial root of the second molar (Figs. 4 C and D).

Seven days — In both specimens the epithelial healing was complete. New bone had formed and filled large parts of the sockets. There was marked osteoclasis around the tops of the septa and residual root fragments. Pronounced bone formation was seen basally opposite the first molar and the mesial root of the second molar.

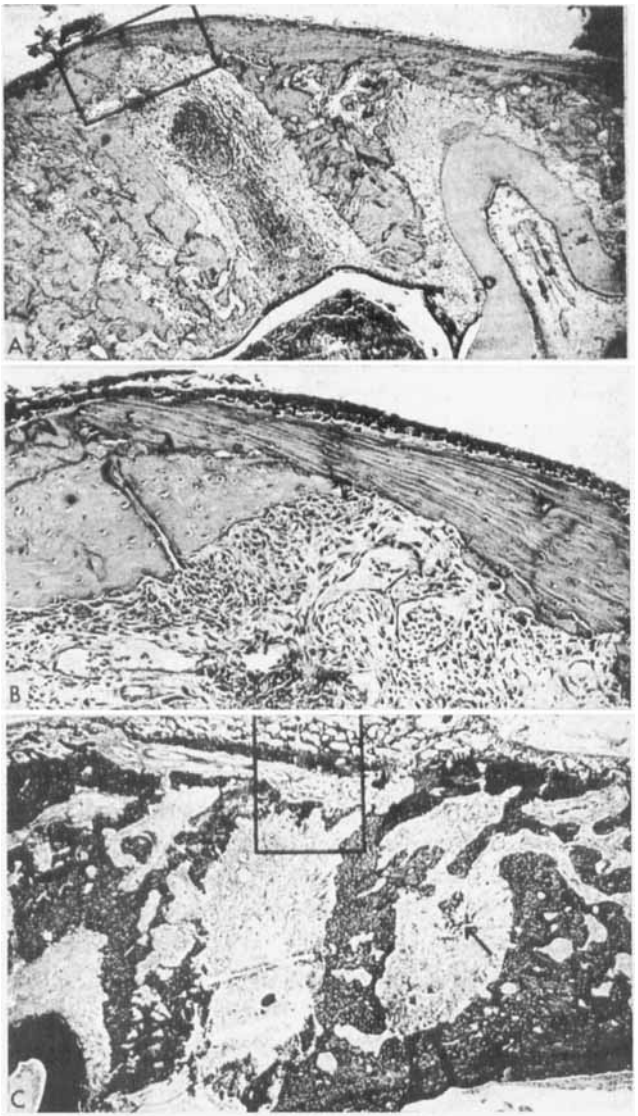

Fig. 3.

A. Survey of specimen taken 2 days after extraction. Central blood clot in the socket, with granulation tissue and connective tissue peripherally. The epithelium covers the opening of the socket. (*Bock*). × 92.

B. Detail of framed part of A. Along the superior surface of the basal bone layer there is a dense accumulation of cells. Highly vascularized tissue in the deepest part of the alveolus (*Bock*). × 385.

C. Survey of specimen taken 4 days after extraction. Bone formation basally over the first and second molars and centrally in the mesial socket (*arrow*). (*Gomori*). × 92.

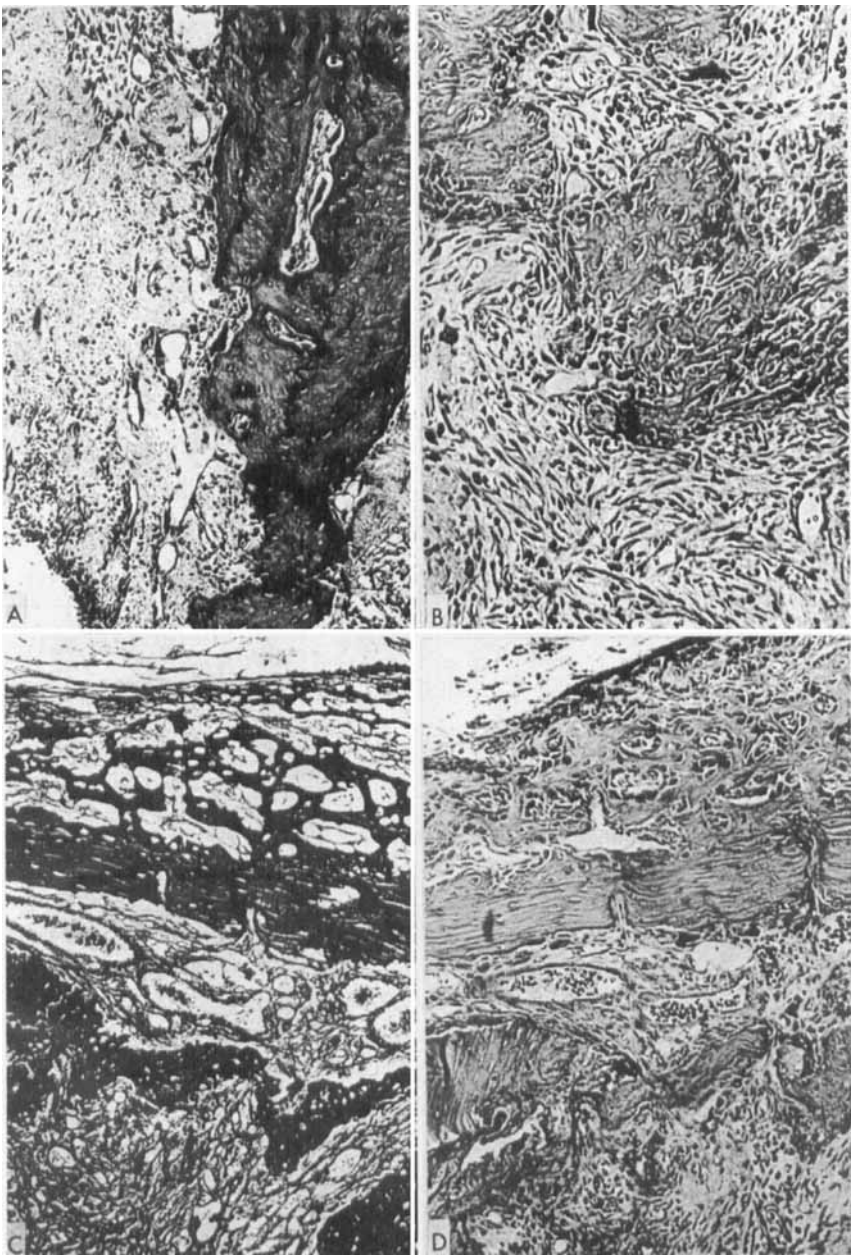

Fig. 4.

A. Detail of alveolar septa from specimen taken 2 days after extraction. (Another section from the specimen in Figures 3 A and B.) Osteoclasts and highly vascularized tissue along the alveolar border. (*Bock*). × 330.

B. Detail of central part of the mesial socket in specimen taken 4 days after extraction. Incipient formation of bone (*van Gieson; cf. arrow* in figure 3 C). × 455.

C, D. Details from framed area in figure 3 C. Bone formation above the basal bone layer and in the deepest part of the socket. (*Gomori and van Gieson*). × 330.

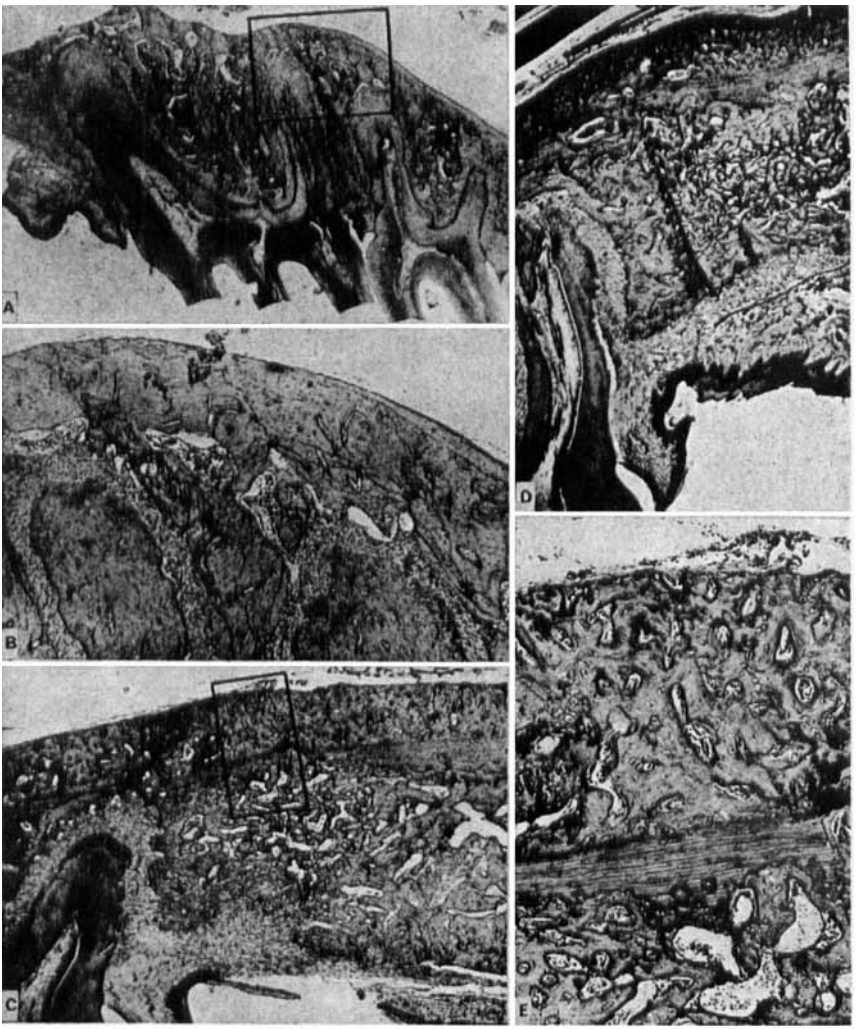

Fig. 5.
A. Survey of specimen from control rat (*Bock*) × 50.
B. Detail of area above the distal root of the first molar and the mesial root of the second molar. The basal bone layer is chiefly of the lamellar type. × 260.
C, D. Survey of 2 specimens taken 16 days after extraction (*Bock*). Newly-formed basal bone, which differs distinctly in structure from the residual parts of the lamellar plate. × 62.
E. Detail (framed area in Figure C) of the thick bone layer above the basal, lamellar compact substance. × 260.

Eleven days — The contents of the alveoli were for the most part filled with new bone. Above the basal compact substance there was a layer of spongiosa, which in one specimen (group A) was extremely thick and in the other (group C) quite thin.

Sixteen days — In normal healing the sockets were completely filled with new bone. Above the first molar the basal layer of bone had been thickened by deposition of new bone (Figs. 5 C—E).

Twenty-two and 24 days — The original sockets could not be distinguished. The bone level appeared to be lower on the mesial aspect of the second molar than on the distal side of the third molar. The basal bone layer above the first molar had thickened by the formation of new bone with no lamellar structure, as was also observed after 16 days.

DISCUSSION

The normal intra-alveolar healing after extraction in this experimental series bears a close resemblance as regards both structure and time to the results obtained by other authors in the white rat (*Huebsch et al.*, 1952). The healing would thus seem not to be affected appreciably by the administered tetracycline; nor did the dose given appear to affect the increase in body weight and thus probably not the general status (*Cleall et al.*, 1964). Nonetheless, for further use of tetracycline experimentally as a bone marker a more thorough examination of its possible influence on the healing process is required.

The healing can be considerably affected by the presence of residual fragments of root or root cementum, sequestra due to extraction trauma, and foreign bodies pressed into the sockets, as is illustrated in figure 1 and earlier reported by *Frandsen* (1963).

Extra-alveolar changes after extraction have been noted by *Huebsch et al.* (1952) in the mandible of the rat, and detailed descriptions have been given by *Boyne & Kruger* (1962) and *Boyne* (1966), who studied them in the dog and man. In the present series it is obvious that extensive bone formation took place first of all outside the sockets, and chiefly along the basal compact substance that forms the superior border of the maxillary alveolar process. This activity would seem to have begun about 3 days after extraction and to have continued for a further week. It would appear normally to be restricted to the area opposite the 2 distal sockets of the extracted tooth and the mesial root of the remaining second molar. This extra-alveolar formation of bone is possibly a compensatory reaction to the change in the loading

conditions, or is intended to reinforce the alveolar process after loss of the tooth. A similar formation of bone along the basal compact substance occurred also opposite an area affected by a traumatically evoked periodontal lesion between the second and third molars (Fig. 1 D). In this case, too, there would appear to have been a compensatory, reparative process. This occasional observation in one of the rats thus provides support for the view that the observed extraalveolar bone formation may be a compensatory, reparative process. A similar observation was made by *Hertz* (1936) in experimental studies of fractures. He found that the bone formation takes place most rapidly in those parts of the callus that are farthest away from the fracture line; he called it a »distant periosteal reaction». The hypothesis that the basal bone formation is intended to reinforce the jaw after the tooth loss is also supported by observations made by *Arwill & Ågren* (1968), who found formation of new bone on the opposite side of experimentally produced bone cavities in epiphyseal bones in the rabbit.

In the control rats where no extractions were performed there was no such thickening of the basal bone layer (Figs. 5 A and B).

The fluorescence observed in the mandibles is evidence of progressive reconstruction of the alveolar process, probably as a result of the slow eruption of the rat molars, which is regarded as compensatory to the abrasion (*Farris & Griffith*, 1963). Whether, as a consequence of the extraction of the antagonist, the first molar erupts more rapidly than the other molars could not be established with certainty, although some of the specimens displayed more marked activity along the roots of these teeth than of the second and third molars.

It is difficult to evaluate the two methods used with respect to their differences and their respective merits and shortcomings. Fluorescence microscopy of ground sections provides a good survey of the bone healing and a possibility of judging where and when the mineralization phase in the process of ossification occurs. The preparation of ground sections, however, is considerably more time-consuming than that of ordinary histologic sections, and the resolution is not so great for the thicknesses used in this study; with a refined method it is possible to obtain considerably thinner ground sections, however, (*Ericsson*, 1965). A combination of tetracycline-induced fluorescence, examined in ground sections, and the ordinary histologic technique with demineralized specimens has advantages over either method used on its own. Other micromorphologic methods — for instance, those involving the application of bone-labelling isotopes — have important advantages, but unlike the methods used in this investigation, they probably cannot be used in studies on man.

SUMMARY

Changes in the alveolar process after extractions were studied in 24 white rats, the first upper molars of which were extracted. The healing process was examined by fluorescence microscopy after administration of tetracycline, and also by the usual histologic technique. The observation periods ranged from 2 to 24 days.

The course of healing in the sockets appears to have been largely similar to that described by other authors. The formation of bone, however, was also seen outside the sockets; it was localized to the basal bone layer, which forms the upper limit of the maxillary alveolar process in the rat. This ossification appeared to begin after about 3 days and to continue for about a week. It was usually restricted to the area opposite the socket of the extracted tooth and might be a compensatory process, whereby the alveolar process is reinforced after loss of the tooth.

The described extra-alveolar changes were clearly observed in ground sections by means of tetracycline-induced fluorescence and were confirmed is sections from the demineralized specimens.

RÉSUMÉ

MODIFICATIONS DANS LE PROCÈS ALVÉOLAIRE APRES EXTRACTIONS CHEZ LE RAT BLANC. ÉTUDE HISTOLOGIQUE ET PAR MICROFLUOROSCOPIE

Les modifications prenant place dans le procès alvéolaire après extractions ont été étudiées sur 24 rats blancs chez lesquels on a extrait les premières molaires supérieures. Le cours de la cicatrisation a été suivi par microfluoroscopie après administration de tétracycline, ainsi que par la technique histologique habituelle. Les périodes d'observation allaient de 2 à 24 jours.

Le cours de la cicatrisation parait être dans une large mesure similaire à ce qu'ont décrit d'autres auteurs. La formation osseuse, cependant, a aussi été constatée en dehors des alvéoles; elle était localisée au niveau de la couche osseuse basale formant la limite supérieure du procès alvéolaire de la mâchoire supérieure chez le rat. Cette ossification semblait commencer au bout d'environ 3 jours et continuer pendant environ une semaine. Elle se limitait en général à la zone correspondant à l'alvéole de la dent extraite et constituait peut-être un processus compensatoire par lequel le procès alvéolaire se trouvait renforcé après la perte dentaire.

Les modifications extra-alvéolaires décrites ont été nettement observées sur des coupes par usure en utilisant la fluorescence provoquée par la tétracycline, et ont été confirmées sur des coupes de spécimens déminéralisés.

ZUSAMMENFASSUNG

VERÄNDERUNGEN IM ALVEOLARFORTSATZ NACH ZAHNENTFERNUNG EINE HISTOLOGISCHE UND FLUORESZENZ-MIKROSKOPISCHE UNTERSUCHUNG AUF WEISSEN RATTEN

Die ersten oberen Molaren 24 weisser Ratten wurden entfernt, und die folgenden Veränderungen des Alveolarfortsatzes wurden studiert. Die Heilungsvorgänge wurden teils mit Fluoreszenz mikroskopie nach Zufuhr von Tetracycline, teils mit gewöhnlicher histologischer Technik untersucht. Die Observationszeiten haben zwischen 2 und 24 Tagen variiert.

Die Heilung der Extraktionswunden scheint in gleicher weise geschehen zu sein, wie andere Verfasser sie früher beschrieben haben. Knochenbildung ist aber auch ausserhalb der Alveolen beobachtet worden (»extra-alveoläre Knochenbildung»). Sie ist zu der basalen Knochenschicht lokalisiert, die aufwärts den oberen Alveolarfortsatz der Ratte abgrenzt.

Diese Knochenbildung fängt ca 3 Tage nach der Extraktion an und scheint ungefähr eine Woche anzudauern. Sie ist am grössten gerade gegenüber dem entfernten Zahne und möchte als ein kompensatorischer Prozess erklärt werden, womit der Alveolarfortsatz nach dem Zahnverlust verstärkt wird.

Die beschribenen »extra-alveolären» Veränderungen konnten deutlich auf Schleifschnitten mit Tetracycline-induzierter Fluoreszenz beobachtet werden, und sie konnten auf Schnitten von den demineralizierten Präparaten verifiziert werden.

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