The effect of tetracyclines on socket healing

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An investigation was made of the effect of tetracycline on socket healing when used as bone marker. The material consisted of 52 white rats, on which the first molars were extracted.
The rats were divided in experimental groups given tetracycline in doses of 1 × 30 mg, 3 × 30 mg, 1 × 120 mg and 3 × 120 mg, respectively, and in a control group. Histologic comparison of socket healing between the experimental animals and their controls after 4, 8 and 16 days, showed only small differences between the parameters studied. The differences were, however, not statistically significant.
The results of the investigation thus show that tetracyclines, administered in doses as here, can be used as bone marker for studies of socket healing in rats without untoward effects on the healing procedure.

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Tetracyclines are fluorescent substances and have an affinity to mineralizing tissues. They have therefore been widely used as bone markers. *Milch, Rall & Tobie* (1957) found that tetracyclines injected intravenously spread rapidly to the tissues and were deposited in areas of bone formation. A comparison of some bone markers by *Harris* (1960) showed tetracycline to be very suitable. Tetracyclines have been used as markers for different purposes, *e.g.* for the study of the rate of bone formation (*Frost*, 1961; *Hansson*, 1967; *Sundén*, 1967) and of bone healing after tooth extractions and surgical procedures (*Boyne & Kruger*, 1962; *Boyne*, 1963; 1966 a, b; *Åstrand & Carlsson*, 1969), and after jaw fractures (*Boyne*, 1967; *Boyne, Hayes & Messer*, 1967). Many investigations have been published on the effects of tetracyclines and several good surveys are available (e.g. *Bevelander*, 1964; *Hansson*, 1967; *Hammarström*, 1968).

An ideal substance for *in vivo* staining of tissues should have no effect on the biological processes to be studied. The tetracyclines, however, have two such effects. First, they have been shown to inhibit bone growth (*Bevelander, Goldberg & Nakahara*, 1960; *Bevelander, Nakahara & Rolle*, 1960), and therefore possibly retard bone healing. Second, tetracyclines are antibiotics and in the doses for bone marking, they may have a therapeutic effect.

Cleall, Perkins & Gilda (1964) demonstrated in the white rat that doses less than 70 mg/kg body weight did not demonstrably inhibit bone growth. *Stahl* (1962) gave penicillin and tetracycline to

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rats and studied the healing of gingival wounds. He found that it had a favourable influence in early stages of bone healing, but that the epithelialization of the wounds was not affected. It appears that socket healing has not been studied histologically or experimentally in this respect. *Alty* (1961) and *Kay* (1966), however, found that the incidence of dry socket after removal of the lower wisdom teeth in man was reduced by administration of penicillin. Even when used in small doses as a bonemarker tetracycline may conceivably have a therapeutic effect. This has, however, not yet been investigated.

Tetracycline-induced fluorescence has proved valuable in the study of socket healing and the remodelling processes of the alveolar ridge after extraction of teeth. In view of the two above-mentioned affects of the tetracyclines it is essential to know whether these drugs have a positive or negative effect on these healing processes.

This paper reports a histological comparison between socket healing in rats given tetracyclines and untreated rats.

MATERIAL AND METHODS

The material consisted of two series. The second was started when preliminary results were obtained of series 1.

Series 1. Lower jaw

28 white rats (60 days old), of the Sprague-Dawley strain were used. The animals were fed a diet of coarse hard bread (mouse bread), which was nutritionally adequate and they were allowed water (ordinary tap water) ad libitum. The 1st lower molar on each side was extracted. As it is difficult to avoid root fractures, the teeth were divided with a dental bur before extraction. The operation was carried out under general anaesthesia by intra-peritoneal injections of mebumal — sodium (2 per cent).

The animals were randomly distributed among three groups, namely two experimental groups (A and B) which were given one and three injections of tetracycline, respectively, and one control group (C). Socket healing was studied in two animals (one male and one female) at a time at different intervals after extraction and with different doses of tetracycline (Table I). The tetracycline was given intraperitoneally in a concentration of 10 mg/ml. After decapitation the mandibles were cut out and placed in 10 per cent solution of neutral formalin. They were demineralized in EDTA (0.5 M, pH 8), embedded in paraffin and sectioned through the socket of the distal root. The sections were stained with haematoxilin-eosin and van Gieson's picrofuchsin.

The animals were weighed after the extractions 8 days after the extractions (16 day group) and again before sacrifice. At the histological evaluation socket healing was studied with special reference to epithelialization, organisation of the blood clot, bone formation and inflammation. Comparisons were made between the sockets in one animal from an experimental group and the ipsilateral socket in a sexmatched control that had been operated upon the same day. Healing of the socket in the experimental animal, as judged from the four histological parameters used, was classified as superior or inferior to that in the control animal. At the time of inspection of the specimens the examiner was not aware of the group to which the animal belonged.

Table I. *Animals, treatment and survival times*

Animal number	Number of tetracycline injections	Time of the injections (days after extr.)	Dose (mg/kg body weight)	Survival time (days after extr.)
		Series 1		
Group A				
1 — 2	1	3	30	4
3 — 4	1	3	120	4
5 — 6	1	3	30	8
7 — 8	1	3	120	8
9 —10	1	3	30	16
11—12	1	3	120	16
Group B				
13—14	3	3, 5, 7	30	8
15—16	3	3, 5, 7	120	8
17—18	3	3, 5, 7	30	16
19—20	3	3, 5, 7	120	16
Group C				
21—22	—	—	—	4
23—24	—	—	—	8
25—28	—	—	—	16
		Series 2		
Group A				
29—30	1	3	30	8
31—32	1	3	120	8
33—34	1	3	30	16
35—36	1	3	120	16
Group B				
37—38	3	3, 5, 7	30	8
39—40	3	3, 5, 7	120	8
41—42	3	3, 5, 7	30	16
43—44	3	3, 5, 7	120	16
Group C				
45—48	—	—	—	8
49—52	—	—	—	16

Series 2. Upper jaw

24 white rats (50 days old) were used. They were fed the same diet as the animals in series 1, except that the food was ground to a fluor-like powder. The 1st upper molar on each side was extracted under general anaesthesia (as in series 1). The animals were divided into two experimental groups (A and B) and one control group (C) and treated as in series 1, with the exception that only two observation times were used (8 and 16 days) (Table I).

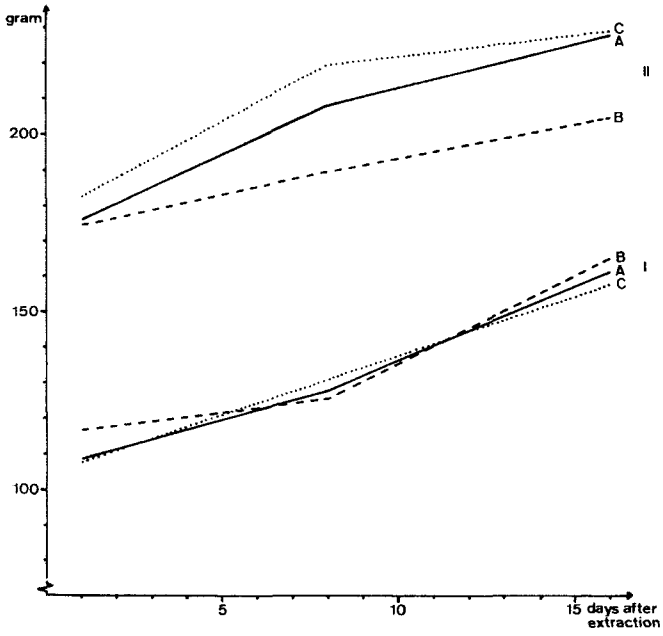

Fig. 1. Weight increase of animals in series 1 and 2 during the experimental periods. For explanation see text.

RESULTS

Gross observations

During the time covered by the experiments no clinical complications were observed in the animals. They appeared to tolerate the experimental procedures well. The main increase in body weight was about the same in the different groups (Fig. 1).

Histologic observations

In order to obtain a general impression of the course of socket healing as judged from the observations at different intervals after the operation, it might be convenient first to give a brief outline of healing as judged from the control specimens in the two series. Complications in the form of root fragments and foreign bodies were fairly common, but this description refers to cases with undisturbed healing. Four days after the extractions (Fig. 2 c—e) the socket was filled with young cellular tissue often poor in fibrils. Only small focal areas could be observed where the blood clot still remained. The entire area of the socket was devoid of newly formed bone, and contained no osteoid tissue. Remnants of the periodontal membrane were visible. Osteoclast resorption of the alveolar bone was evident. Epithelial healing had begun, but in no specimen was it complete. Scattered areas of inflammatory reaction were generally seen.

Eight days after the extractions (Figs. 3 a and b) the remnants of the blood clot had disappeared and bone formation had begun along the walls of the alveolus. In the specimens where an inflammatory reaction could be detected it was associated with a foreign body or a sequestrum. In most specimens epithelial healing was complete.

Sixteen days after the extractions (Figs. 3 c and d) large parts of the socket were

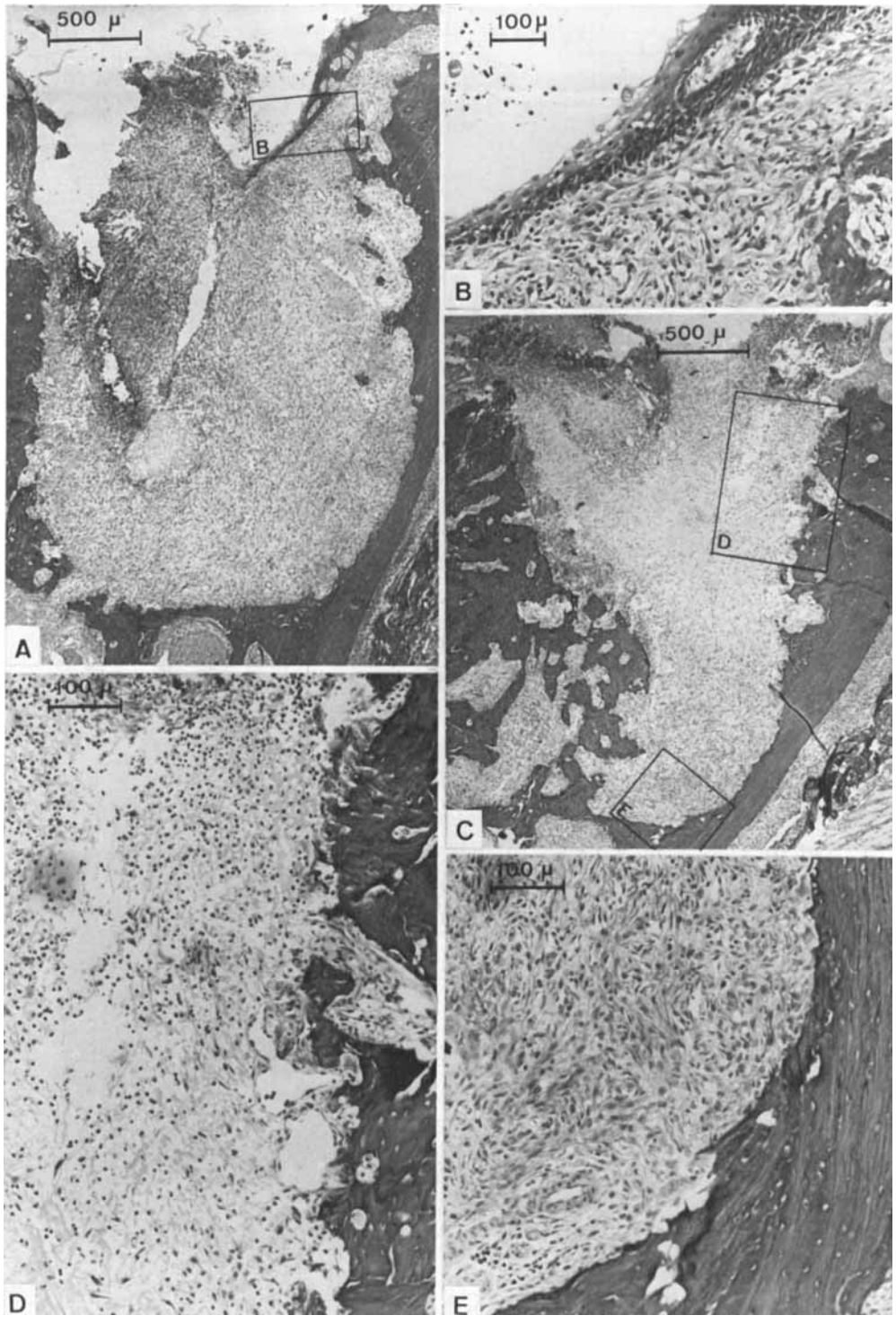

Fig. 2. a and c) Survey of sockets 4 days after extraction. *a.* Tetracycline group 1 × 30 mg. *c.* control group. No clear differences in healing.
b) Higher magnification of framed area in *a.* The epithelium has begun to migrate over the socket content.
d and e) Higher magnification of framed area in *a.* showing different degrees of organisation of the content. In *d.* newly formed connective tissue sparse in fibrils. An inflammatory reaction is clearly seen. In *e.* the connective tissue is more organised and is rich in fibrils.

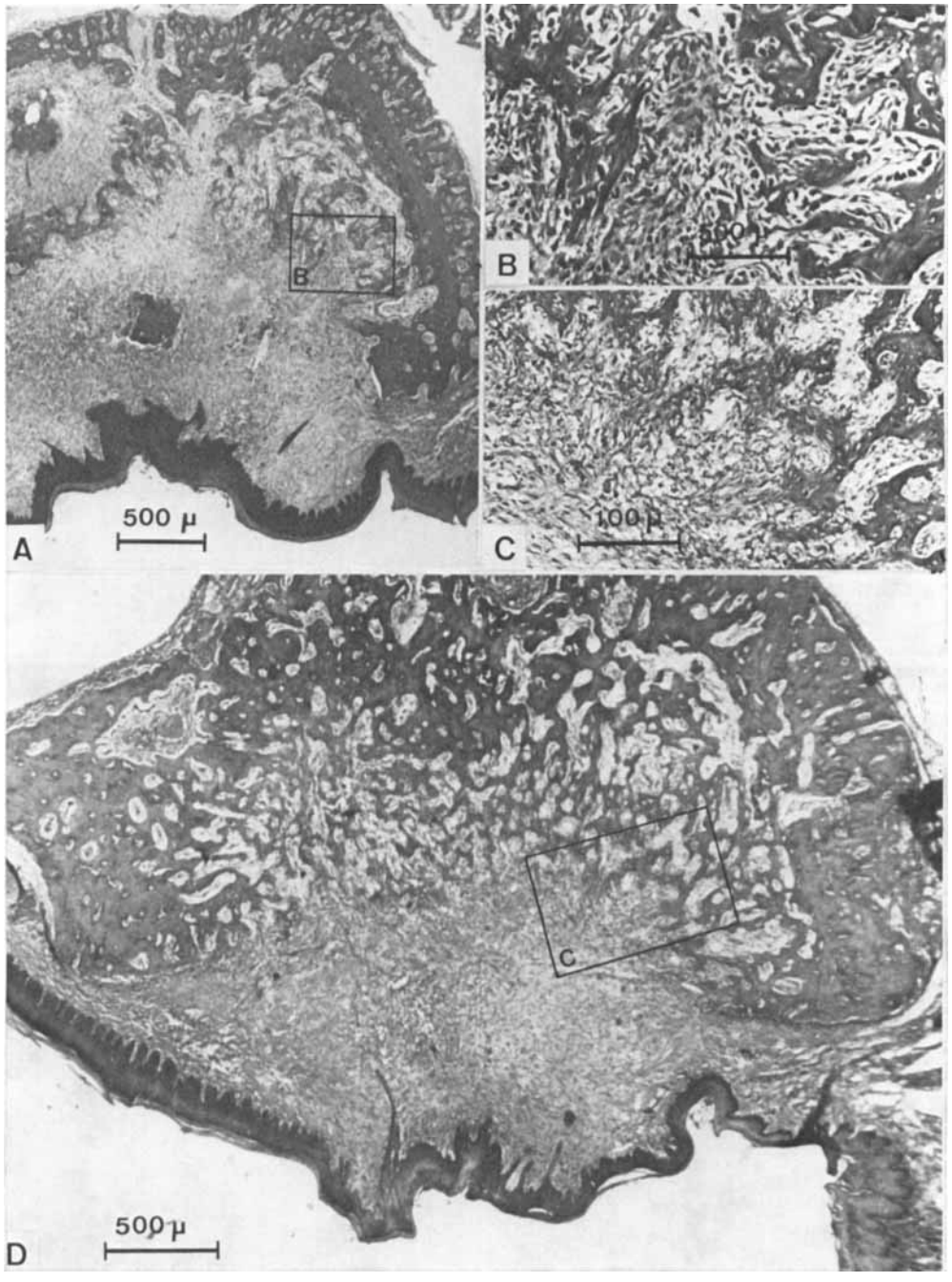

Fig. 3. a) Survey of maxillary socket 8 days after extraction. Control grpup. Newly formed bone restricted to peripheral parts of socket. In central part a bone sequestrum surrounded by inflammatory cells. The new-formed bone is seen in higher magnification in *b*.
d) Survey of maxillary socket 16 days after extraction. Control group. Large part of the socket filled with new-formed bone, illustrated in higher magnification in *c*.

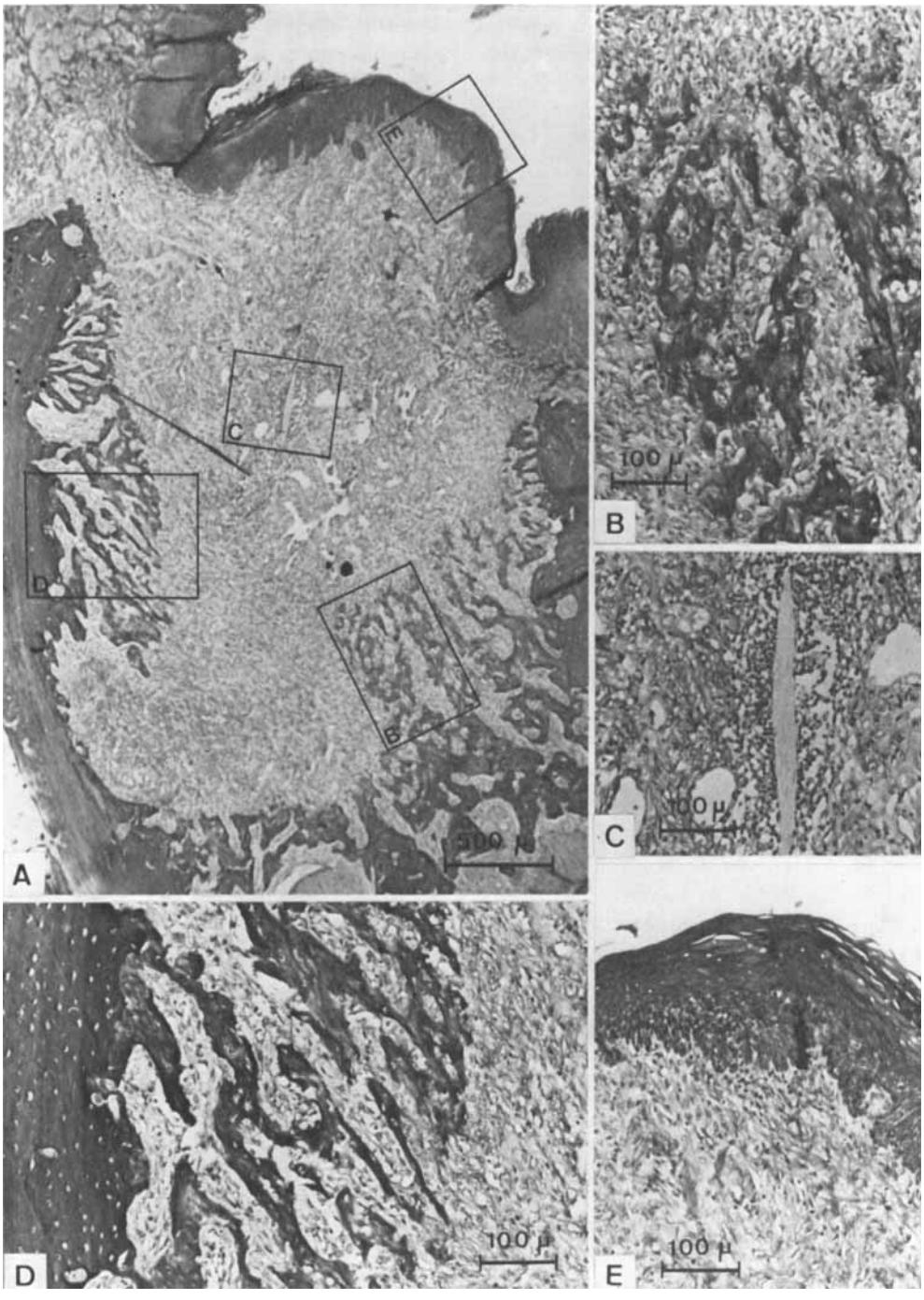

Fig. 4. a) Survey of a socket 16 days after extraction. Tetracycline group. No clear differences compared with control group (Figs 3 c and d).
b and d) Higher magnification of *a.* showing new-formed bone lingually (b) and buccally (d).
c) Higher magnification of *a.* Cellular infiltration around a foreign body (missed during technical procedure).
e) Higher magnification of *a.* The epithelial healing is complete. The epithelium of normal appearance. Mild subepithelial inflammation.

filled with immature bone. In most cases the lining epithelium was wider than in the eight-day-specimens.

Comparison between control and experimental animals

The results will be given first for each series separately and then for both combined.

Series 1. After *four days*, no difference could be observed, after a dose of 1 × 30 mg. In the group given 1 × 120 mg tetracycline two sockets out of four showed less inflammation than the corresponding sockets in the control animals. No differences were found for the other parameters (Figs. 2 a and b). A comparison between controls and experimental animals after an observation time of *eight days* revealed no clear difference between the groups that had received tetracyclines in a dose of 1 × 30 mg, 3 × 30 mg or 3 × 120 mg. After a dose of 1 × 120 mg the socket content was more organised, epithelial healing was better and bone formation was more intense in two of four sockets in the tetracycline group. After an observation time of *sixteen days* two sockets out of four showed better epithelial healing than their controls after a dose of 1 × 30 mg. No difference between the groups could be observed after a dose of 1 × 120 mg, 3 × 30 mg and 3 × 120 mg (Fig. 4).

Series 2. In the *eight days* specimens no difference could be found after a dose of 1 × 30 mg and 3 × 30 mg. Three sockets ot of four in the animals given 1 × 120 mg and two sockets of four of each group in those given 3 × 120 mg showed more inflammation and less advanced organisation of the blood clot than corresponding sockets in the control animals. At *16 days* two sockets of four in the group given 1 × 30 mg tetracycline showed better epithelial healing than the controls. No other differences were seen.

Series 1 and 2. Minor divergences between tetracycline treated animals and controls in the two series have been described above.

A statistical analysis (sign test) of the findings in each of the two series separately and taken together showed no differences between the experimental groups and control groups.

DISCUSSION

It was originally intended to confine the investigation to the lower jaw. Examination of the specimens from series 1, revealed that healing of the sockets had often been disturbed mainly by the presence of foreign bodies. In the belief that this complication would be less common in sockets of the upper jaw, a new series (series 2) was started in which the upper jaw was used. In order to reduce the risk of socket healing being disturbed by large pieces of bread, the bread was ground to a fine powder before it was given to the animals.

The increase in weight was about the same in both series (Fig. 1). The main difference in weight between the animals of the two series, may be explained by the fact that they were obtained from two different colonies. In both series the histological differences between the groups and their controls were small and not statistically significant.

As mentioned in the introduction, the use of tetracycline might be expected to have two different effects — a negative one due to its inhibitory effect on bone growth and a positive one due to its anti-infectious effect. These effects may counter-balance one another or one may be

superior to the other. Judging from the results, it would appear that these two effects counter-balance one another or that tetracycline, as used here, had no effect on socket healing in rats.

Besides the possible effect of tetracycline on socket healing root remnants and foreign bodies may interfere with healing. The frequencies of such disturbances were roughly equal in all the groups and probably had no effect on the results of the intergroup comparisons. To check this point, however, sockets with undisturbed healing in experimental groups were compared with sockets with undisturbed healing in the corresponding controls irrespective of side. A corresponding comparison was made with sockets with disturbed healing. The results of this new comparison, however, were the same as those of the original comparison. The presence of sockets with disturbed healing thus appear not to interfere with the results obtained.

The results of this investigation indicate that tetracyclines administered as here, can be used as bone markers for studies of socket healing in rats without untoward effects on the healing procedure.

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